REVISED

NUFFIELD ADVANCED SCIENCE

BIOLOGY

TEACHERS' GUIDE I

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BIOLOGY

TEACHERS' GUIDE I

Science Learning Centres
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BIOLOGY
TEACHERS' GUIDE I

PART ONE MAINTENANCE OF THE ORGANISM

PART TWO CONTROL AND CO-ORDINATION IN ORGANISMS

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The answers included in this *Teacher's guide* are, of course, provided by the author and not by the Board.

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FOREWORD

When the Nuffield Advanced Science series first appeared on the market in 1970, they were rapidly accepted as a notable contribution to the choices for the sixth form science curriculum. Devised by experienced teachers working in consultation with the universities and examination boards, and subjected to extensive trials in schools before publication, they introduced a new element of intellectual excitement into the work of A-level students. Though the period since publication has seen many debates on the sixth form curriculum, it is now clear that the Advanced Level framework of education will be with us for some years in its established form. That period saw various proposals for change in structure which were not accepted but the debate to which we contributed encouraged us to start looking at the scope and aims of our A-level courses and at the ways they were being used in schools. Much of value was learned during those investigations and has been extremely useful in the planning of the present revision. The time since first publication has also seen a remarkable expansion in the number of candidates taking A-level biology and it is encouraging to us to know that we helped in this development.

The revision of the biology series under the general editorship of Grace Monger has been conducted with the help of a committee under the chairmanship of Arthur Lucas, Professor of Curriculum Studies, CSME, Chelsea College, University of London. We are grateful to him and to the committee. We also owe a considerable debt to the Joint Matriculation Board which for many years has been responsible for the special Nuffield examinations in biology, and to the representatives of the Board who sat on the advisory committee and who have given help in many other ways.

The Nuffield–Chelsea Curriculum Trust is also grateful for the advice and recommendations received from its Advisory Committee, a body containing representatives from the teaching profession, the Association for Science Education, Her Majesty's Inspectorate, universities, and local authority advisers; the committee is under the chairmanship of Professor P. J. Black, academic adviser to the Trust.

Our appreciation also goes to the editors and authors of the first edition of Nuffield Advanced Biological Science, who worked under the joint direction of W. H. Dowdeswell and P. J. Kelly, the project organizers. Their team of editors and writers included John A. Barker, John H. Gray, Margaret K. Sands, and C. F. Stoneman. The present revision has only been possible because of their original work.

I particularly wish to record our gratitude to Grace Monger, the General Editor of the revision. This is the second occasion on which we have asked her to undertake the revision of one of our biology series, as she was responsible for the highly successful O-level Biology revision. We are therefore doubly grateful to Miss C. M. Holland, Headmistress of the Holt School, Wokingham and the Berkshire Education Authority for agreeing to her secondment. Grace Monger has had a particularly onerous task because the many topics that biology covers have been subject to an exceptional number of changes and new discoveries in recent years. She and her team of editors have been fortunate in being able to draw on the help, as writers and consultants, of experts in their fields in universities, teaching hospitals, and other institutions of learning. To Grace Monger and her editors, John A. Barker, T. J. King, M. B. V. Roberts, Ianto Stevens, Tim Turvey, and Colin Wood-Robinson, and to the many contributors, we offer our most sincere thanks.

I would also like to acknowledge the work of William Anderson, publications manager to the Trust, his colleagues, and our publishers, the Longman Group, for their assistance in the publication of these books. The editorial and publishing skills they contribute are essential to effective curriculum development.

K. W. Keohane
Chairman, Nuffield–Chelsea Curriculum Trust

PREFACE TO THE FIRST EDITION

The materials produced by the Nuffield Advanced Biological Science Project do not represent a rigid syllabus. They have been devised after careful evaluation of the results of extensive school trials so they can be used in a variety of ways related to the different circumstances found in schools and the varied abilities, backgrounds, and aspirations of students.

The work has three major objectives:

To develop in students the intellectual and practical abilities which are fundamental to the understanding of biological science.

To introduce students to a body of biological knowledge relevant to modern requirements, through investigating living things and studying the work of scientists. In doing so, students will consider the processes of research and the implications of science for society.

To develop in students the facility for independent study, especially how to learn through critical evaluation rather than memorizing by rote.

These aims have been central not only to the design of the publications and other materials but also to the complementary examinations that have been prepared.

Abilities

It is intended to develop abilities in the following kinds of work which are assumed to provide the basis for learning biological science.

1 Acquiring a knowledge of living things and an understanding of the techniques used to study them.
2 Making observations and asking relevant questions about them.
3 Analysing biological data and synthesizing them into conclusions and principles.
4 Handling quantitative information and assessing error and degree of significance.
5 Critical judgment of hypothetical statements in the light of their origin and application.
6 Making use of acquired knowledge for identifying and investigating problems with unfamiliar materials.
7 Evaluating the implications of biological knowledge for human society.
8 Communicating biological knowledge both coherently and with relevance.

Subject matter

The subject matter covers four units, each of approximately 90 periods (of 40 minutes) of class work and parallel homework or preparation. The units can be taken in various sequences and there are opportunities for a flexible treatment within each unit. The outlines of the units are as follows:

Maintenance of the organism
Interaction and exchange between organisms and their environment
Gas exchange systems
Transport inside organisms
Transport media
Digestion and absorption
Enzymes and organisms
Photosynthesis
Metabolism and the environment

The developing organism
Sexual reproduction
Early development
Cell development and differentiation
The nature of genetic material
Gene action
Development and the internal environment
Development and the external environment

Organisms and populations
Variation in a community
Inheritance and the origin of variation
The cell nucleus and inheritance
Population genetics and selection
Population dynamics
Organisms and their physical environment
Organisms and their biotic environment
The community as an ecosystem
Evolution and the origin of species

Control and co-ordination in organisms
The organism and water
The cell and water
Control by the organism
Stimuli and their influence
Nerves and movement
Structure and function in the nervous system
Social behaviour

We have attempted to provide a comprehensive, balanced, and integrated coverage of the main fields of biological science, both pure and applied (including biological technology). Where it is relevant, we have considered aspects of the physical sciences and mathematics in a biological context. Also, under a number of topics, we consider the nature of biological investigation and the social implications of the subject.

The units cover all levels of biological organization, that is, molecular levels, cellular levels, organ and tissue, organism and population. However, the focus of each is on the whole organism. They also illustrate many of the major themes or concepts of biology, such as variety and adaptation; structure in relation to function; organisms in relation to their environment; the similarity of many processes of physiology and behaviour; the genetic and evolutionary continuity of life; matter and energy cycles; homeostasis; development; and the uniqueness of the individual.

We hope that students will also be encouraged to appreciate the aesthetic and humanitarian aspects of the subject, although this is clearly something for which the teacher must be primarily responsible.

Approach

We suggest that investigations should play a major part in the work. These can involve either practical work or exercises of an investigatory nature based on secondhand data, mainly in the form of excerpts from published literature, the results of experiments carried out by the project, or visual aids.

The practical work is described in the *Laboratory guides*, non-practical work in the *Study guide*.

The work in the *Laboratory guides* and *Study guide* is complementary and, in some cases, alternative. This makes it possible for students to do practical and non-practical work in varying proportions at the discretion of the teacher. At the same time, through the use of cross-references, questions, and bibliographies (which mention the specially prepared Topic Reviews), it is possible to make discussion, reading, demonstration, and written work an integral part of the investigations.

The work in the *Laboratory guides* and *Study guide* is of three types. *Preliminary work* aims to cover possible deficiencies in the students' background and provides reinforcement material for the less able student. *Main work* is within the capacity of the majority of A-level students. The amount of practical work has been carefully limited to fit into a reasonable allowance of time.

Extension work contains broader and more rigorous treatments of some of the topics included as main work. It is intended for the more able and advanced student and consists of a range of exercises from which a choice can be made.

The materials have been devised so that they can be used for work with a class or small group or by individual students. However, we recommend that students should be encouraged gradually to rely more and more on their own resources. This is particularly important for Extension work and for projects. The latter are small-scale, open-ended, individual investigations described in *Projects in biological science.*

In short, this is not a specialized course, nor is it harnessed only to the needs of future biologists. It is an attempt to provide a way of presenting biological science as an interesting and important subject, relevant to scientist and non-scientist alike. We also hope that it will help those who undertake it to cultivate the abilities and attitudes which are necessary if they are to understand and evaluate the contribution that biological science makes to our society.

THE REVISED EDITION

The Nuffield Advanced Biological Science materials were first published in the 1970s. Ten years later it was obviously necessary that a decision about their future should be taken. A working party representing various interests was set up and it is as a result of its conclusions that this revision has come about.

It was quite clear that there was much in the original materials that was worth preserving although a revision must be forward-looking. At the same time it appeared that the original intention of the books may have been misunderstood and that the biological ideas were not being seen clearly enough. In addition practical work had become the dominant form of student activity and had tended to overburden the scheme. When the working party reported to the Nuffield–Chelsea Curriculum Trust it recommended that a revision of the materials should be undertaken. It stated that the original aims of the scheme should be kept and given even more emphasis; however, it did suggest that different types of books were needed.

All schools which entered candidates for the Nuffield Advanced level biology examination, which is administered by the Joint Matriculation Board, were written to and teachers were asked for their views. The teachers who sent their comments, many of them very detailed, made a greatly valued contribution to the form this revision has taken.

The major feature of this revision, then, is a change in the form and content of the books. The *Study guide* is in two volumes and contains a substantial amount of descriptive text in addition to a collection of biological data presented in Study items. There is no specified extension work although in some areas many teachers may think the detail goes beyond that required for Advanced level. This has been done deliberately where the subject is of such interest as to justify it and for the benefit of the more able students who may appreciate a greater depth of knowledge.

The subject matter still covers four areas arranged as follows:

Volume I
Maintenance of the organism
Control and co-ordination in organisms

Volume II
Inheritance and development
Organisms and the environment

The outline of the areas covered is as follows:

Maintenance of the organism
Gas exchange
Breathing and gas exchange in Man
The circulatory systems of animals and plants

Blood and the transport of oxygen
Cells and chemical reactions
Heterotrophic nutrition
Photosynthesis

Control and co-ordination in organisms
The plant and water
The cell and water
Control by the organism
Co-ordination and communication
The response to stimuli
Behaviour
The human brain and the mind

Inheritance and development
Cell development and differentiation
The cell nucleus and inheritance
Variation and its causes
The nature of genetic material
Gene action
Population genetics and selection
Principles and applications of biotechnology
Methods of reproduction
Patterns of development
Control and integration through the internal environment
Development and the external environment

Organisms and the environment
Organisms and their physical environment
Organisms and their biotic environment
Population dynamics
The community as an ecosystem
Evolution

The *Study guide* therefore contains the core of the work of the revised scheme. The recommendations made by the working party, which gained support from the teachers too, have also been implemented in the revision. These are:

1 That the process of biology, rather than the content, should be emphasized (the lack of content has been criticized in the past but the new form of the *Study guide* should remedy the deficiency of factual material).
2 That the revised material must be appropriate to, and appeal to, those students who will not continue with an academic study after A-level, as well as to those who will do so.
3 That the central theme should be the whole organism, although there are several subsidiary themes.
4 That there would be a modest development of learning through historical aspects so that students will appreciate how present day knowledge and ideas have been reached.

The working party proposed few changes in content, but as a result of teachers' comments it was realized that certain areas needed more attention. In the revised books, more detail about cells, for instance, is included and there is a separate booklet, *Systematics and classification.* There is also a new chapter on biotechnology, and one on human behaviour and the mind. Other areas have been expanded and brought up to date. These include photosynthesis, water relations of cells, immunology, and control by hormones. The chapters on genetics and development have been extensively rewritten and the ecology chapters have a new look. Evolution is presented in a novel way. There is a second supporting booklet, *Mathematics for biologists.*

The practical investigations are now contained in seven separate *Practical guides* and are fully cross-referenced to the *Study guide.* The parallel *Teachers' guides* contain, as before, principles, assumptions, and answers to questions. In addition there is a second part to each chapter which is the guide to the *Practical guide.*

The Nuffield Advanced level biology examination will continue to be administered by the Joint Matriculation Board. It will, however, be based on a syllabus which will be prepared by (and available from) the Joint Matriculation Board.

Although we have used modern nomenclature based on the International System of Units for laboratory chemicals, we have not attempted to do so for biochemical pathways, because of the confusion it would cause.

Attention is drawn to the joint statement of the Association for Science Education, the Institute of Biology, and the Universities' Federation for Animal Welfare, entitled 'The use of animals and plants school science'. This is reproduced in the Appendix.

We would also emphasize that students should not be required to carry out dissections against their wishes. There may be instances where students would prefer to watch the investigation being carried out on video.

Obviously a major revision such as this is has involved a lot of hard work by a great many people. I should also like to thank the editors mentioned in the Foreword and the authors whose names appear on the books. This revision has relied very heavily on the large number of authors who have written material for us and who have shown such an interest in the whole exercise. But it is the editors who have carried the main responsibility for the final version of the text. They have been a most professional team and it has been a pleasure to work with them. The contribution made by the staff of the Publications department of the Nuffield–Chelsea Curriculum Trust cannot be overestimated and the final result owes a great deal to their skill and dedication.

I should also like to thank personally Joyce Moate who has done all my typing and finally I should like to record my appreciation of the two people who have made it possible for me to continue with this task after the one year full-time secondment ended. Firstly, to Miss C. M. Holland, headmistress of the Holt School, Wokingham, who has supported me in

many ways, and has provided me with facilities which have made the task so much easier. Secondly to Mary Sangster, who has shared a teaching timetable with me on a regular basis and on whom I have always been able to rely to give extra time. It would have been unthinkable to complete the task in the time available but for the calm and efficient way in which she has assisted me.

Many people therefore have cooperated to produce these revised materials. We hope the result will be seen as an interesting and exciting approach to studying biology at Advanced level whether it is the intention to enter for the Nuffield A-level examination or not.

Grace Monger
General editor

PART ONE MAINTENANCE OF THE ORGANISM

In references to figures and tables, **'S'** denotes the **Study guide**.
'P' refers to the **Practical guide**.
Example: 'figure (S)2'.

□ denotes the end of a Study item.

CHAPTER 1 GAS EXCHANGE

A review of the chapter's aims and contents

1 The principle of homeostasis is established and built on throughout this and the subsequent chapters.
2 The adaptation of structure to function is a recurring theme.
3 A variety of organisms is introduced and used to illustrate the requirements of gas exchange.
4 Insects and mammals are investigated in depth in order to illustrate the principles that have been established.
5 A variety of types of data is examined and hypotheses are put forward.

PART I The *Study guide*

1.1 Gas exchange in plants

Assumptions

1 Understanding that aerobic respiration occurs in all living cells of terrestrial angiosperms and requires oxygen.
2 Awareness that tissues such as phloem and cambium are living.
3 Knowledge that the central xylem of woody stems is dead and does not require oxygen.
4 Knowledge that the outer cell layer of angiosperms is the epidermis, which is usually one cell thick.

Principles

1 Gas exchange is a fundamental activity of organisms and tissues.
2 The structure of an organ reflects its function.
3 The distribution and density of stomata in the epidermis of an angiosperm are related to the species of the plant and the position of the epidermis.
4 Diffusion gradients must be maintained in order to permit continuous gas exchange.

Question and answer

a ***To what extent are these expectations met by the structure of the stem and leaves of a typical plant?***

Leaves have vast networks of air spaces in their interiors; in stems, especially thicker, older ones, the deeper tissues are dead xylem elements, whereas the active phloem and cambium are superficial, being relatively close to the epidermis.

STUDY ITEM
1.11 The diffusion of a gas through holes of different diameters

Practical investigation. *Practical guide 1*, investigation 1A, 'Gas exchange in leaves'.

Section 8.5 discusses in greater detail the role of stomata in the control of water vapour diffusion.

Questions and answers

a ***Compare the figures in columns 1 and 2 of table [S]1 and also those in columns 1 and 3, by plotting two graphs with the diameter of the hole on the horizontal axis*** **(abscissa).**

The graphs should illustrate the principle expressed in answer **b**.

b ***Express in words the relation between the hole diameter and***
1 the volume of gas diffusing per hour
2 the volume of gas diffusing per unit area of hole per hour.

1 As the hole diameter increases, the volume of carbon dioxide diffusing through the hole increases. The relation is more or less linear.
2 As the hole diameter increases, the volume of carbon dioxide diffusing per unit area of hole decreases in an exponential fashion.

c ***Calculate the ratio of circumference :area for the hole diameters given in table [S]2.***

Hole diameter (mm)	*Circumference:area* (mm^{-1})
22.70	0.176
12.06	0.332
6.03	0.662
3.23	1.233
2.00	2.006

d ***From your calculations are you able to suggest the main physical characteristic of stomata that accounts for column 3 in table [S]1?***

The ratio of circumference:area increases as the hole diameter decreases; so does the volume of gas diffusing per unit area of hole. This suggests that the presence of the 'edge' of the stoma somehow influences the rate of diffusion through the hole. This assistance given to diffusion through the pore is sometimes called the 'edge' effect.

e ***Comment upon the densities of stomata in table [S]3 in relation to the type of plant.***

These data represent a very small sample, but the comments that could be made are:
most leaves have more stomata on their under surfaces;
monocotyledons have similar densities of stomata on both upper and lower surfaces, which is understandable since their leaves are often more or less vertical;
floating leaves have none on the lower surface, since there will be no air in contact with that surface;
the tree leaves have a very high density of stomata on the underside and none on the top. □

STUDY ITEM

1.12 (Essay) *(J.M.B.)*

Questions and answers

a ***How is a typical mesophyte leaf adapted for gaseous exchange?***

Students might be expected to make mention of the role of guard cells and the size of the stoma, and the way in which the latter influences air flow through the stoma. The nature of the cells which constitute the mesophyll is important, as is their arrangement. The size and moistness of the internal surface of the leaf play a vital part in assisting gaseous exchange.

b ***How do internal and external factors influence the rate of gaseous exchange in such a leaf?***

The factors which could be considered, and whose action might be explained, include: turgor and the operation of stomata; the influence of light through photosynthesis; the influence of temperature on the steepness of the diffusion gradients; the effect of wind and of atmospheric humidity; even the influence of the soil water availability, since the passage of water vapour out of a leaf is gaseous exchange. □

1.2 Gas exchange in animals

Assumptions

1 That students know that the movement of gases across surfaces such as cell membranes occurs in solution.
2 Some understanding of the concept of osmosis.

Principles

1 Gas exchange in terrestrial organisms means inevitable water loss; structural or physiological mechanisms may be employed to reduce this loss.
2 Active pumping of oxygen-bearing medium across gas exchange surfaces is achieved in different ways in different organisms, but is essential in large organisms in order to meet their oxygen demands.
3 Structure is adapted to function.

STUDY ITEM

1.21 Gas exchange in fish

Principles

1 Gas exchange occurs across the gill lamellae of teleost fish.
2 A concentration gradient of oxygen from water to blood is maintained across the gills.
3 Water and blood flow in opposite directions across the gill lamellae – this is counter-flow.
4 In the gills a relatively large surface area of thin, vascularized tissue is brought into close contact with water.

Questions and answers

a ***Suggest how the fish might maintain a continuous flow of water across the gill lamellae in the direction shown in the diagram.***

Students will suggest that some pumping is performed by an appropriate part of the organism: some may suggest the mouth and floor of the buccal cavity; others may suggest that the muscular operculum is able to draw water through the gill cavity. In either case a valve (the closing of the mouth) is vital if flow is to be achieved. Students should also see that, without some sort of double pumping action (mouth and operculum), continuous flow is impossible. In fact it has been shown that the movements of the floor of the buccal cavity and of the operculum constitute two linked pumps and the mouth and opercular edge act as the valves. The two parts of this double pump act together, but out of phase. A more or less continuous water flow is maintained.

b ***Apart from the direction of blood flow in the lamella and its very large surface area, what structural feature would you expect the lamellae to possess?***

They would be expected to have epithelium which was very permeable, either by its chemical or physical properties or by virtue of being very thin.

c ***In what way does the evidence in the graphs enable you to suggest what advantage the fish gains from employing a counter-flow system in its gill?***

With the counter-flow system, the blood flowing into the lamella is meeting water that has not yet lost any oxygen, all the time; hence oxygen will be extracted from the water until the blood is as saturated as the incoming water. In the parallel system, the water and blood are effectively stagnant, so diffusion occurs from the water into the blood until an equilibrium is achieved. This equilibrium is likely to represent an oxygen concentration midway between that of the incoming water and the incoming blood. Counter-flow is a much more efficient system for the oxygenation of the blood.

d ***What have you assumed about the flow rate of water and blood in order to answer question c?***

The assumption is made that the flow rate is equal in the parallel flow system. If the blood were to flow less quickly than the water, the equilibrium reached would be at an oxygen concentration greater than that of the mean value for the two media.

e ***From the information given here can you suggest reasons why, when a fish is taken from water (which has a relatively low oxygen concentration) and put into cool air (which has a relatively high oxygen concentration), it very quickly dies from lack of oxygen?***

The problem is not one of oxygen availability but one of maintenance of the surface area of the gills for exchange. Out of water there are no skeletal supports for the filaments or lamellae, so the gills collapse and a huge reduction in exchange occurs. This deprives the fish of oxygen. □

STUDY ITEM
1.22 Gas exchange in locusts

> **Practical investigations. *Practical guide 1*, investigation 1B, 'Dissection of the ventilation system of a locust', and investigation 1C, 'The fine structure of the ventilation system of a locust'.**

Principle

1 Air penetrates via the tracheal system direct to the respiring tissue; there is no intermediate, internal, oxygen-transporting medium.

Questions and answers

a ***Explain the function that might be served by the thickened rings of cuticle in the tracheae.***

These will prevent the collapse of the tracheae under the negative pressure which will exist from time to time during ventilation. Attention may be drawn to the existence of similar strengthening in mammalian tracheae and even in the xylem of plants.

b ***How do you think sufficient oxygen supply is maintained in small insects when there is no ventilation of the tracheal system?***

Diffusion of gases through the tracheae is sufficient to supply the oxygen demands of the smallest insects. This relation with size contrasts with the position in mammals, which underlines one of the important consequences of homoiothermy. □

STUDY ITEM
1.23 (Short answer question) *(J.M.B.)*

Principles

1 The ventilation movements of a locust are influenced strongly by the gaseous composition of the air.
2 Interpretation of data often demands the resolution of two or more contrasting influences.

> **Practical investigation. *Practical guide 1*, investigation 1D, 'The effect of gas changes on locusts' breathing'.**

Questions and answers

a ***State the effects on abdominal pumping movements of an increase in the concentration of* (1) *oxygen and* (2) *carbon dioxide.***

The trend in the first five sets of readings shows that if the oxygen concentration is increased the pumping rate falls; as the carbon dioxide concentration rises the pumping rate increases.

b ***From these results is it possible to explain the effect of human exhaled air on abdominal pumping movements in terms of its gaseous composition? Explain your decision.***

Not entirely, since the increase in pumping rate could be ascribed to a number of factors – the exhaled air contains less oxygen, more carbon dioxide, but also more water vapour, and it is at a higher temperature, than atmospheric air.

c ***1 What was the purpose of the flushing technique in this investigation?***
2 Comment upon its effectiveness.

1 This was to remove the gas used in the previous treatment which, in any event, had been altered in composition by the activities of the locust, and to re-establish some sort of control for comparison.
2 After severe treatments (the pure oxygen and carbon dioxide) the flushing period is probably not long enough for the ventilation to equilibrate to atmospheric air again.

d ***State a possible explanation for the changes in abdominal pumping movements***
1 from readings 16 to 18;
2 from readings 19 to 21.

1 The stimulatory effect of carbon dioxide on ventilation is very marked and occurs rapidly. Prolonged exposure to the gas, however, has a quite different effect and acts as an anaesthetic.
2 After exposure to carbon dioxide, during which the spiracles remain closed, that gas will have accumulated within the tracheal system. On exposure to atmospheric air, abdominal pumping rate increases, as does spiracle 'beating'; the high carbon dioxide levels in the system keep the rate high. The effect of this is to flush the network once more with atmospheric air.

e ***State a physiological advantage for the insect which may be related to the spiracle activity during readings 16 to 18.***

Keeping the spiracles closed during this period means that although the movements of the abdomen are rapid they do not result in the filling of the tracheal network with carbon dioxide. Hence, anaesthesia
☐ is slow, being largely the result of accumulation of metabolic CO_2.

1.3 Adaptations for gas exchange in mammals

Principles

1 Mammals have a high oxygen demand related to their homoiothermy.
2 Oxygen consumption per gram of tissue increases as the size of a mammal falls, which reflects the increase in surface area:volume as the overall size decreases.

Practical investigations. *Practical guide 1*, investigation 1E, 'The breathing apparatus of mammals', and investigation 1F, 'The fine structure of the lungs'.

Questions and answers

a ***Explain the shape of the curve in* figure [S]6.**

This negative exponential curve is related to the homoiothermy and heat loss of mammals. As a mammal becomes larger its surface area decreases in relation to its volume. The surface area represents the potential for heat loss, while the volume represents the potential for heat production. Since heat production is metabolic, it requires oxygen. Thus, as the animal gets larger, its oxygen demand per gram of tissue decreases.

b ***Comment upon the structure and operational function of the mammalian lung in relation to each of the above factors.***

This question will require students to have investigated the structure and physiology of the lung either at first hand or by reference to standard texts.

1 Ventilation is aided by the presence of elastic fibres in the lung tissue. Although only 15 per cent or so of the air in the lungs is replaced with each breath, this is quite sufficient for the animal's needs.

2 Alveoli give a vast surface area in contact with the air.

3 Alveolar walls are exceedingly thin, as are those of the lung capillaries.

4 Gases diffuse readily through cytoplasm, and the presence of moisture on the alveolar walls puts the gases into solution; this makes possible their rapid diffusion across the cell membranes.

5 and **6** The gradient is always in the direction from alveolus into the blood and is maintained by the continuous flow of blood through the lung capillaries.

PART II The *Practical guide*

The investigations related to this chapter appear in *Practical guide 1, Gas exchange and transport in plants and animals.*

IMPORTANT. Whenever dissecting equipment has been used, it must be cleaned effectively, preferably by autoclaving or by sterilizing in an approved disinfectant solution (for example, one containing cetrimide with a detergent).

Attention is also drawn to the guidance on the dissection of animals given in the joint statement of the Association for Science Education, the Institute of Biology, and the Universities' Federation for Animal Welfare, entitled 'The use of animals and plants in school science'; this is reproduced in the Appendix.

INVESTIGATION
1A Gas exchange in leaves

ITEMS NEEDED

Fronds of *Dryopteris* sp.
Leaves of *Impatiens* sp., *Cymbalaria muralis*, barley, and other plants, fresh
T.S. of leaf of *Impatiens* sp. or *Ligustrum* sp., prepared slide 1/2

Nail varnish, clear
Water

Beaker, 250 cm^3 1/1
Bunsen burner 1/1
Eyepiece graticule, 0–10 mm × 0.1 mm 1/1
Filter paper
Forceps 1/1
Gauze 1/1
Heat-resistant mat 1/1
Micrometer, stage, 0–1 cm × 0.1 mm 1/group
Microscope 1/1
Microscope slides and coverslips
Petri dish
Tripod 1/1

(*Study guide* 1.1 'Gas exchange in plants'.)

The aim of this investigation is to seek an anatomical basis for the function of gas exchange in leaves.

Students should be warned thát if microscopes are used with natural light the sun must not be focused on the instrument.

Assumptions

1 The knowledge that molecules can diffuse through apparently solid barriers such as cell walls.
2 The ability to use a microscope.

Principles

1 Leaves have a spongy mesophyll and contain air.
2 The escape of air from leaves can be connected with the distribution of stomata as seen under the microscope.

Showing the escape of gas from a leaf is a common 16+ exercise and students need not repeat it if they are already quite familiar with it.

Several alternative methods for examining the distribution of stomata are given. A procedure can be chosen to suit the material available, or the class may be divided into groups, each group trying a different method and comparing the results. Many ferns other than *Dryopteris* are satisfactory for direct observation. It would enhance the investigation if as many different plants as possible were available for study. Certainly the contrast between something like *Impatiens* and barley is striking in all respects and is likely to stimulate enthusiasm. Ivy-leaved toad flax (*Cymbalaria muralis*) is particularly good. For the third part, it is ideal if microtome leaf sections of the same plant as the one used for epidermal investigation are available.

It will not be possible to see air emerging from the leaves unless the water is very nearly boiling. It is vital to emphasize that each objective lens has to be calibrated separately for use with the graticule.

Question and answer

a ***From which surface do the air bubbles come in the leaves that you observed? (Use the terms 'upper' and 'lower' to refer to the natural position of the leaf on the plant.)***

From the lower surface only in some leaves (*e.g. Impatiens* and holly); from both surfaces in other leaves (*e.g.* barley).

There are other methods of observing the distribution of stomata in a leaf epidermis.

Method D: Procedure

1 Remove a leaf from a plant and dip it in propanone.

2 Shake the leaf to remove excess propanone and then quickly place it onto a piece of cellulose acetate film. Apply firm pressure, using the base of the thumb or a cork.

3 Remove the leaf and in a few seconds the propanone will have evaporated, leaving a clear impression of the surface of the leaf in the cellulose acetate film. This can now be examined under the microscope.

Method E: Procedure

1 Apply a little Silcoset 151 to the upper surface of a leaf. There is no need to spread it thinly; it will harden rapidly.

2 Peel off this pad of rubber and put it on a flat surface with the epidermal impression uppermost.

3 Paint on a thin film of clear nail varnish.

4 Allow this to dry (5 to 10 minutes). Then peel off, mount in a drop of water and examine under a microscope.

Questions and answers

b ***How are the stomata distributed between the upper epidermis and the lower epidermis of the leaves which you examined?***

They are on the lower surface only in *Impatiens*; on both surfaces in leaves such as *Tradescantia.*

c ***....From each of two contrasting leaves, make a simple outline drawing of a single stoma and the four or five cells immediately surrounding it.***

–

d ***Estimate the number of stomata to be found in a square millimetre of one of your leaves. Compare your results with those of the rest of the class.***

The stomatal frequency varies from 10 to 400 mm^{-2}. On average there are about 200 mm^{-2} on the lower epidermis. The number varies on different parts of a leaf and on different leaves of the same plant and may be influenced by environmental conditions during development. (See table (S)3.)

e ***How many different kinds of cells, judging by their shape, can you find in the leaf section? Briefly describe their shapes and positions.***

Four or more. Epidermal cells, palisade and spongy mesophyll cells, and vascular units (xylem and phloem).

f ***How much greater than the length of a stoma is the distance between a stoma and the upper epidermis?***

Stomatal lengths may be around 20 μm; the internal thickness of a *Ligustrum* leaf is around 190 μm – around 9 to 10 times greater.

g *Do your investigations of the structure of leaves agree with the experimental results you obtained and does knowledge of the structure of a leaf help you to explain how gaseous exchange with the air around it takes place?*

This is a question for discussion and will provide an opportunity to emphasize the importance of relating structure to function.

INVESTIGATION
1B Dissection of the ventilation system of a locust

ITEMS NEEDED

Locust, freshly killed 1/1
Locust, live, adult 1/1

Ethanol, 70 per cent
Saline, insect (or water)
Sudan black in kerosene and olive oil

Dissection dish, shallow 1/1
Forceps, fine 1/1
Hand lens 1/1
Microscope 1/group
Microscope, stereo 1/group
Pins
Scissors 1/1
Seeker 1/1
Specimen tube, stoppered, large 1/class
Syringe, clear plastic, 20 cm^3, or specimen tube, stoppered 1/1

(*Study guide* 1.2 'Gas exchange in animals'; Study item 1.22 'Gas exchange in locusts'.)

Saline, insect
Sodium chloride, 0.9 g
Water, distilled, 100 cm^3
Sudan black in kerosene and olive oil
Kerosene, 10 cm^3
Olive oil, 10 cm^3
Sudan black, 1 g
Mix the kerosene and olive oil and dissolve the Sudan black in it.

Assumption
1 The ability to use a microscope.

Principles
1 Spiracles allow oxygen to enter and carbon dioxide to escape from the tracheal system of the locust.
2 The co-ordination of opening and closure of spiracles allows the directional flow of gas through the tracheal system.
3 All organs in a locust are richly supplied with tracheal branches.
4 Air sacs are an aid to more effective ventilation of the tracheal system during abdominal 'pumping'.
5 The investigative dissection of dead animals provides information complementary to observations made upon the living animal; thus it allows a fuller understanding of the functioning of the whole organism.

Before starting the dissection students are asked to observe the opening and closing of spiracles. It is suggested that they concentrate on the spiracle on the second thoracic segment; those on the abdominal segments are much smaller. Students should observe that the thoracic spiracles are opened and closed by two chitinous valves which are quite apparent, whereas the abdominal spiracles have no valves. It may be possible for students to observe that the opening is synchronized, and that the anterior spiracles open when the posterior ones close and *vice versa*. This permits a uni-directional passage of air from anterior to posterior in the insect which increases the efficiency of the tracheal system. Students may experience difficulty in seeing the movement of spiracles since, owing to the need for water conservation, these are only opened at intervals whilst the locust is at rest. This may be overcome by passing exhaled breath over the locust as in investigation 1D.

As a demonstration place a freshly killed locust into a saturated solution of Sudan black in a mixture of olive oil and kerosene. Dissect the animal; the fluid displaces air in the tracheae, thus making them easily visible.

Questions and answers

a **_What exactly are the movements made by the thoracic spiracle which you observed, and what do you think they achieve?_**

The valves of this spiracle separate from each other, both moving, and then close again at the midline (see *figure 1*). This action is performed quite rapidly (0.3 s). The movement allows some diffusion of gases into and out of the tracheal adjoining the spiracle. The rate of diffusion will depend upon the difference in concentration of each gas between external and tracheal atmospheres.

b **_Describe the location and appearance of the air sacs. What could their function be?_**

The number and size of air sacs which are visible varies considerably. They appear as pale grey, pearl-like structures and occur throughout the abdomen and thorax. (*Figure 2*.)

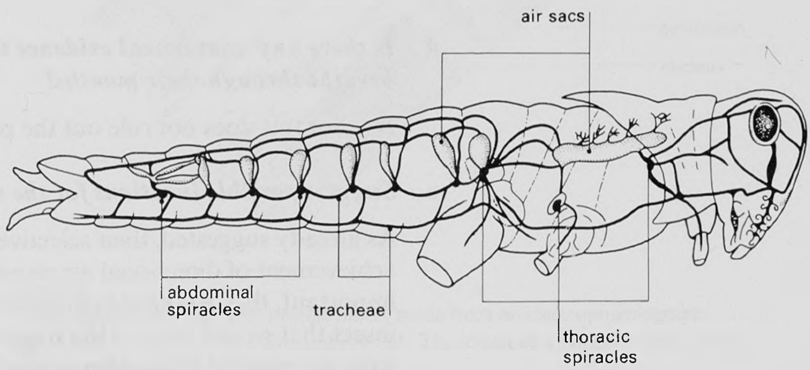

Figure 2
A diagram showing air sacs and tracheae on the right side of the locust.
Based on Borradaile, L. A. and Potts, F. A., The Invertebrata, *4th edn, Cambridge University Press, 1961.*

They are reservoirs of air, not for oxygen supply reserves (which may be suggested), but as an indirect aid to gas exchange. They are not so rigidly supported as the tracheae and collapse readily. When the locust's abdomen is compressed, some of their contained gas will be expelled, helping to 'flush' the tracheal system.

c **_Describe the general arrangement of all the tracheae and air sacs, and their relation with the spiracles in each segment. Record your findings in a diagrammatic form, that is, in a plan which shows the relative positions but does not necessarily make the organs look like the real ones._**

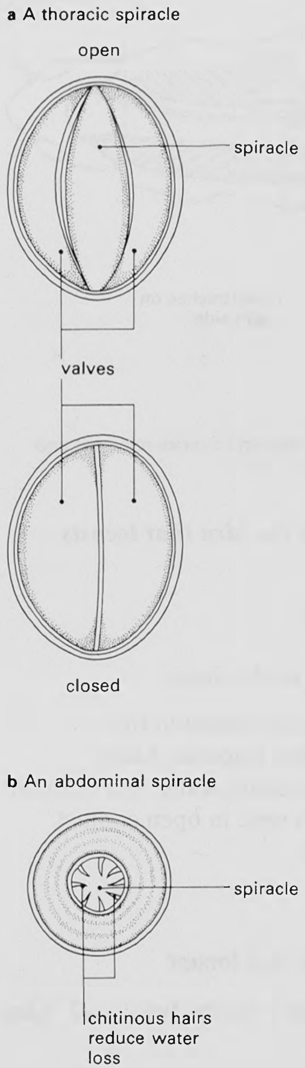

Figure 1
A surface view to show the appearance of abdominal and thoracic spiracles.
a A thoracic spiracle, open and closed.
b An abdominal spiracle.
After Clarke, W. M. and Richards, M. M., The locust as a typical insect, *John Murray, 1976.*

This is a difficult task; students should not be allowed to spend too long trying to work out the system. See *figures 2* and *3*.

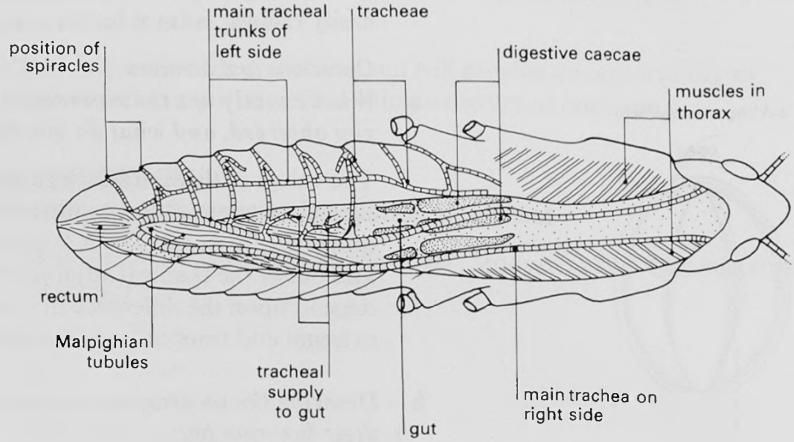

Figure 3
Part of the main tracheae as seen in dissection, with air sacs and ovaries removed and the gut held over to the right.

d ***Is there any anatomical evidence to support the idea that locusts breathe through their mouths?***

No, but this does not rule out the possibility.

e ***Suggest possible functions for the spiracles in this insect.***

As already suggested, their selective opening may assist in the achievement of directional air movement in the tracheae. More important, they are vital in preventing the excessive water loss from the insect that would occur if the tracheal system were in open contact with the external atmosphere the whole time.

INVESTIGATION
1C The fine structure of the ventilation system of a locust

ITEMS NEEDED

Leaf of *Impatiens*, fresh 1/1
Locust, freshly killed adult 1/1

Methylene blue, 0.5 % aqueous
Water (or saline, insect)

Forceps 1/1
Microscope 1/1
Microscope slides and coverslips
Mounted needles 2/1
Pipettes 2/1
Scissors, fine 1/1
Watch-glass 1/1

(*Study guide* 1.2 'Gas exchange in animals'; Study item 1.22 'Gas exchange in locusts'.)

Saline, insect
Sodium chloride, 0.9 g
Water, distilled, 100 cm^3

Assumptions

1 The ability to use a microscope.
2 The ability to recognize cells and their nuclei, using a microscope.

Principles

1 The thread-like tracheae which permeate organs such as the gut are easily seen by using a microscope. Their smallest branches appear to end abruptly.

2 It is the presence of fluid in the tracheae which obscures the finest terminations of the system.

The *Practical guide* does not mention tracheoles because they cannot be seen in temporary, unstained preparations.

Pieces of gut wall immersed in ethanol or ethylene glycol monoethyl ether for a few hours or overnight usually have the appearance shown in figure (P)8.

The preparation of leaf midrib tissues should only take a minute or two and could be delegated to one member of the class.

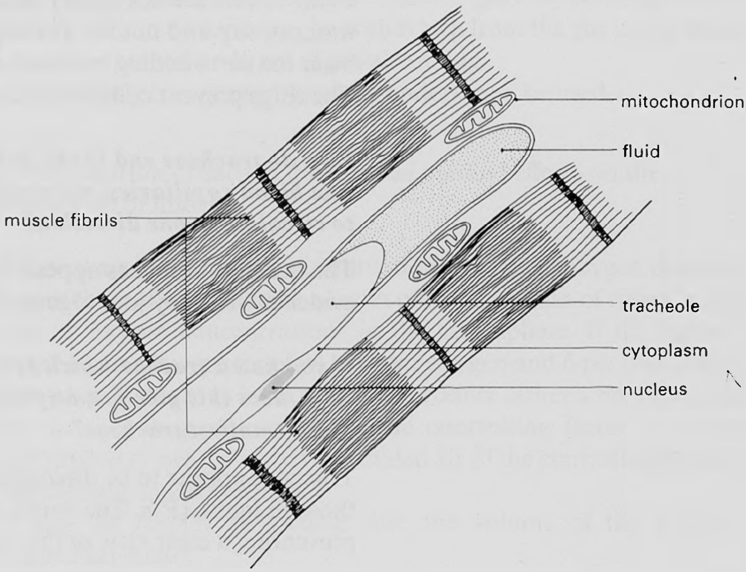

Figure 4
A drawing of tracheoles and muscle fibres, made from an electronmicrograph.
Based on Clarke, W. M. and Richards, M. M., The locust as a typical insect, *John Murray, 1976.*

Tracheae branch into tracheoles, which can eventually penetrate individual cells. With the aid of electron microscopy, it has been shown that in flight muscles the blind ends of tracheoles lie between the muscle fibrils. Between the muscle fibrils and the tracheoles are many large mitochondria. Oxygen diffuses directly from the tracheoles; oxidative phosphorylation occurs in the mitochondria, thus ensuring an efficient supply of ATP to the muscle fibres to provide the energy for contraction. During inactivity there is some fluid – mainly water – in the end of each tracheole, and oxygen must dissolve in this before diffusing out into the tissues. This fluid acts as a diffusion barrier between the cells and the oxygen in the tracheoles. However, during muscular activity this fluid disappears and gaseous oxygen diffuses directly and more rapidly through the cytoplasm to the muscle cells. The fluid is withdrawn from the tracheoles by osmosis because the rapidly metabolizing cells have accumulated osmotically active waste products. (See *figure 4*.)

Questions and answers

a ***What function do you think the rings or hoops in the tracheae perform?***

The rings may prevent the tubes from being squashed flat, and from being blocked by the pressure of the tissues round them.

b ***If there are ringed vessels in the midrib of a leaf too, does this suggest that they do a job similar to that of an insect's tracheae? Do the vessels in the plant appear to contain air?***

Plant vessels are not silvery in appearance and presumably contain water or sap and not air. Perhaps these vessels are subject to pressure from the surrounding cells and might become blocked for this reason. The rings prevent collapse because of low pressure in the vessels.

c ***How do tracheae end in the gut wall? Is there any evidence that they, like blood capillaries, eventually join larger tracheae to enable air to circulate in one direction?***

The smallest branches appear to end blindly. There is no visible evidence that they join together like a capillary bed.

d ***If you find a trachea which appears to be discontinuous, as in* figure [P]8, *does this give you any help in interpreting the 'blind' endings of the smallest tracheae?***

Tracheae appear to be discontinuous after immersion in liquid, like those in figure (P)8. The 'blind' endings may be filled with liquid, thus preventing a clear view of the real endings.

e ***By way of a summary of your findings from the last two investigations, explain briefly the means by which locusts bring about the exchange of gases between their internal organs and the surrounding air.***

During exercise, muscular contractions telescope the abdomen. This change in volume results in internal pressure changes which act on the thin-walled air sacs to move air through the system, with the sacs acting like bellows.
When the locust is at rest the movement of air within the system is mainly by simple diffusion but this must be slow, considering the diameter of the smallest trachea.

f ***Can you relate the method of gaseous exchange to the fact that few insects grow to a size in which their diameter is greater than 15 mm?***

A limit is placed on the size to which insects can grow by the difficulty of gaseous diffusion in narrow tubes through any but the shortest distances, at an adequate rate to supply working tissues. In a larger animal the mass and pressure of other internal organs would tend to crush smaller tracheae.

INVESTIGATION

1D The effect of gas changes on locusts' breathing

ITEMS NEEDED

Locust, live, adult 1/1

Carbon dioxide cylinder or means of making carbon dioxide 1/class
Oxygen cylinder, with regulating valve 1/class

Cottonwool
Plastic tubing to fit syringe, 20 cm^3 1/1
Stopclock or wristwatch with second indicator 1/1
Syringe, 20 cm^3, clear plastic 2/1

(*Study guide* 1.2 'Gas exchange in animals'; Study item 1.22 'Gas exchange in locusts'.)

Carbon dioxide manufacture
Hydrochloric acid, dilute
Marble chips

Add the dilute acid to the marble chips, using a thistle funnel in a conical flask fitted with a delivery tube. Lead the tube to an inverted gas jar full of water on a beehive stand, so that the gas may be collected over water. The carbon dioxide may be withdrawn from the gas jar by means of a syringe attached to 40 cm of plastic tubing.

Alternatively compressed gas in cylinders may be used.

Principle

1 The concentration of carbon dioxide has marked effects on the breathing rate of insects.

Exhaled air increases locust ventilation rate; pure oxygen decreases it. Both of these effects could be a result of the alteration of either carbon dioxide or oxygen concentrations in the atmosphere. If the locust is subjected to an atmosphere of 94 per cent oxygen and 6 per cent carbon dioxide (step 7) this may be expected to produce either a breathing rate similar to that in pure oxygen (if the controlling factor is oxygen concentration) or nearer to that in exhaled air (if the controlling factor is carbon dioxide concentration).

It may be helpful to assume that the volume of the locust is approximately 4 cm^3.

Table 1 gives some typical results for this investigation.

	COMPOSITION OF AIR					
	Atmospheric air (step 2)	*Exhaled air* (step 3)	*Pure oxygen* (step 5)	*Gas mixtures* (step 8)	(step 9)	(step 10)
Approximate percentage oxygen	21.0	16.4	100.0	94.0	88.0	83.0
carbon dioxide	0.1	4.1	0.0	6.0	12.0	17.0
Specimen results	26	52	5	48	41	54
	22	46	12	42	46	49
	31	48	6	46	51	46
Average ventilation ($30\,s^{-1}$)	26.3	48.7	7.7	45.3	46.0	49.7

Table 1
Typical results from investigation 1D. (Figures show the number of ventilation movements in 30 seconds.)

Questions and answers

a **_What conditions around the locust were altered when you breathed into the syringe barrel? How could you modify this stage of the procedure in order to reduce the number of variable factors associated with exhaled air?_**

In comparison with atmospheric air the oxygen concentration is less; the concentration of carbon dioxide and that of water vapour are greater and the temperature is higher. The exhaled air could be passed through soda lime to remove carbon dioxide and through anhydrous calcium chloride to remove water; it could be cooled by being passed through a glass tube immersed in a water bath before being led to the locust.

b **_From your records can you detect what factor seems to have the controlling influence over ventilation, and hence which of the two hypotheses the results support? Explain your answer._**

Following the argument outlined above it becomes clear that relatively large changes in oxygen content have little effect upon ventilation, while relatively small changes in carbon dioxide produce marked effects, supporting hypothesis 2. This phenomenon is also observed in humans.

INVESTIGATION
1E The breathing apparatus of mammals

ITEMS NEEDED

Mouse or rat, freshly killed 1/1
Ethanol, 70 per cent, aqueous
Cotton
Dissecting dish, shallow 1/1
Forceps, fine 1/1
Blunt seeker or wooden dowel
Microscope, stereo 1/group
Pins
Pipette (Pasteur) 1/1
Scalpel 1/1
Scissors 1/1
Seekers 2/1
Specimen tube, stoppered, large 1/group

(*Study guide* 1.3 'Adaptations for gas exchange in mammals'.)

Assumption

1 The ability to recognize muscle in the abdominal wall.

Principles

1 Air goes to the lungs, not directly to every part of the body.
2 Another medium (blood) carries dissolved gases to and from the body tissues.
3 The thorax is airtight, and pressure changes within it can bring about inspiration and expiration.

Mice are suggested for dissection because they are cheaper and may be more readily available than rats. Rats are preferable because they are larger. The dissection of a mammal differs considerably from that of a locust; one aim of this dissection is to extend the experience of students so that they become aware of general, gross differences in organization between insects and mammals.

A collection of hearts should be kept in ethanol for the work of investigation 3E. Students should note the positions of the main abdominal organs for future reference.

To investigate the physical nature of the lungs. If a rat is available the same procedure as for a mouse should be followed. Additionally, after completing step 12, a large syringe barrel should be attached to the pipette which has been inserted into the trachea. The piston is then inserted and pushed home to inflate the lungs. The volume of air used should be noted. If the syringe is then removed the fact that the lungs return to their original size indicates their elasticity.

One rat per class would be sufficient to enable everyone to appreciate the above point.

The dissection of mice requires good illumination and the frequent use of a stereomicroscope. After the thorax has been fully opened it may be helpful to cover the dissected mouse with water; this will help to separate the organs and to remove blood.

Provided that it is done carefully, inserting the pipette tip into the trachea should pose no problems. The trachea is exposed so that the student can see what is happening. The larynx should be grasped with the forceps and held while the pipette tip is wiggled into place. If the tip is briefly flamed before use to blunt the sharp edges, the trachea will not be punctured.

Questions and answers

a ***Living muscle can contract and become taut. At other times it can be relaxed and hardly tense at all. Do the muscles of the dead animal appear to be contracted or relaxed?***

Muscles of a freshly killed mouse or rat are usually relaxed.

b ***Was the muscular diaphragm tight or loose when you first examined it? What is the probable cause of this?***

Tight: this could be due to rigor mortis or to tension induced by something other than active muscular contraction.

c ***Describe the tension of the diaphragm when the thorax has been opened. If the tension has changed how do you account for this?***

The diaphragm is slack, at least on the side that has been effectively punctured. This could be because of a change in pressure as a result of the puncture.

d ***How can you distinguish between the oesophagus and the trachea? Is the difference between them related to their functions?***

The trachea is ventral. It has half hoops of cartilage to keep it permanently open. Movement of food down the oesophagus is by muscular peristalsis and so its lumen need not remain permanently open.

e ***Is there any similarity between the trachea of a mouse or rat and that of a locust?***

Yes; in the many transverse hoops.

f ***If gases pass from the external atmosphere to the lungs, how do they pass on to all the other tissues of the body? Put your answer in the form of a simple hypothesis based on your mammalian dissection rather than upon previous knowledge.***

The only vessels connected to the lungs, other than the trachea and bronchi, are blood vessels. Presumably blood carries gases to and from the lungs.

g ***A rise in the level of fluid in the pipette in step 10 indicates an increase in pressure. When you moved the diaphragm did you cause any changes of pressure in the trachea? What does your answer to this question suggest to you about the mechanism of inspiration?***

Yes; pulling the diaphragm back causes a fall in pressure. In life this would correspond to the domed diaphragm becoming flattened, causing air to enter the lungs.

h ***What happened to the fluid in the pipette during step 11? What does your answer imply or suggest?***

The diaphragm has little or no effect on air pressure in the trachea once the thorax has been punctured. The lungs are not perforated. The action of the diaphragm must be indirect. This is a point at which the part played by the pleural membranes and cavity could be discussed. In the pleural cavity there is a negative pressure which gradually develops from birth and is responsible for keeping the elastic lungs expanded against the chest wall. If the interpleural pressure rises to that of the atmosphere, because of a puncture in the chest wall, then the lung on that side collapses.

INVESTIGATION
1F The fine structure of the lungs

(*Study guide* 1.3 'Adaptations for gas exchange in mammals'.)

ITEMS NEEDED

Lung, prepared slide T.S., azan-stained, or equivalent 1/2
Lung, prepared slide T.S., stained for elastin 1/2

Eyepiece graticule 1/1
Microscope 1/1
Stage micrometer 1/class

Assumptions

1 Ability to use a microscope and to recognize structures such as cell nuclei.
2 Ability to discriminate between different tissues and to recognize distinct structures, such as blood vessels, under a microscope.

Principles

1 The barrier between air and blood is very thin.
2 The structure of an organ or tissue reflects its function.
3 The lung contains tissues and structures that facilitate inflation, diffusion of gases, expiration, and mechanical support of the organ.

This investigation draws attention to the interpretation of sectioned material. Sections of leaf and gut wall are thin but, in a sense, complete structures. The leaf section is transverse, fairly easy to relate to a whole leaf, and probably familiar to many students from diagrams in elementary textbooks. A lung section has no apparent orientation in relation to the whole organ and cuts many structures at various angles. A section never looks like a diagram of a lung; students should not expect this.

It is essential to obtain good preparations and to know the stains used. The teacher can vary the detail and depth of this part of the chapter to suit the needs of each particular class. This is an opportunity to introduce some of the terminology of histology if desired. The topic of slide preparation is introduced to help the student to appreciate that the section being studied is part of a whole, three-dimensional organ and that staining is a necessary aid to interpretation. The availability of a projection microscope or of transparencies and a slide projector would enable instruction in the recognition of tissues to be given.

Questions and answers

a **How can bronchi be distinguished from bronchioles? How can these air passages be distinguished from blood vessels?**

Bronchi are larger and contain glands and cartilage in their walls; bronchioles are smaller and have no glands or cartilage. Air tubes have a folded epithelium of distinct, columnar or cuboid cells, whereas blood vessels have a smooth lining without distinct cells. The blood vessels seen in section will usually contain red blood cells; the air tubes will be empty.

It is worth pointing out the smooth muscle which is very evident in the walls of bronchi and bronchioles. It gives a considerable ability to alter the diameter of these tubes, and this may be used to alter the resistance to air flow. Severe constriction of these tubes occurs during bronchial asthma attacks.

b **Your lungs produce mucus which is usually moved up the trachea. Can you see cells in the bronchial epithelium which might be responsible for secreting mucus or for moving it?**

Goblet cells are usually fairly obvious in the lining epithelium. They are, however, very infrequent in bronchioles. The free surface of the epithelial tissue bears cilia; these may be visible. In life the cilia beat towards the trachea and throat, thus operating a constant upward stream of mucus which carries with it all types of foreign particles, helping to keep the airways clean.

c **Comment on the distribution of elastic fibres, bearing in mind how a whole lung behaves after it has been inflated.**

It may appear that the lung's elasticity could be due to an elastic coat around it, but the slide shows that this is not so. Elastic fibres are distributed generally throughout the lung tissues; they are found in the connective tissue separating capillaries situated between adjacent alveoli. (*Figure 5.*)

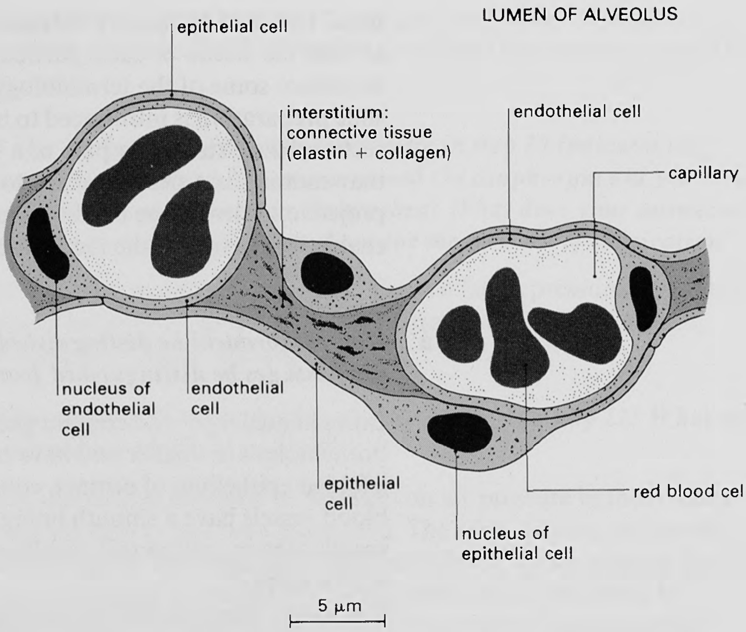

Figure 5
The wall of an alveolus.
Based on Marshall, P. T. and Hughes, G. M., Physiology of mammals and other vertebrates, *2nd edn, Cambridge University Press, 1980.*

d ***From your measurements and estimates work out how far a gas molecule would have to travel from the centre of an average alveolus to the side, and from the side of an alveolus through the wall into a capillary. Draw an alveolus to scale.***

The point of importance here is that the gas space is large compared with the thickness of the wall surrounding it, as diffusion in a gas is some ten thousand times faster than diffusion in water. Between the blood and the lumen of the alveolus are squamous epithelial cells which are very thin. The thickness of the air/blood barrier is only about 0.5 μm in most parts of the alveolar sac.

e ***What problems can you see for some premature babies that are born without this lung surfactant?***

Lack of lung surfactant makes it necessary to work about ten times as hard as normal in order to overcome surface tension. It gives rise to respiratory distress syndrome, in which it is obvious that the baby is having great difficulty in breathing at all and is failing to oxygenate the blood sufficiently.

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CHAPTER 2 BREATHING AND GAS EXCHANGE IN MAN

A review of the chapter's aims and contents

1 The concept of partial pressure is introduced and used in explanations about gas exchange in humans.
2 The way in which human breathing is controlled and the ways in which it may be altered are examined in detail.
3 The principle of homeostasis and feedback control is developed further.
4 Quantitative data are used to investigate the changes in the composition of inspired and expired air, so as to enable students to see the links between ventilation, gas exchange, energy, and work.
5 The introduction of the problems of smoking in a biological context enables students to see the overlap with things that they may consider are social and personal rather than biological and environmental.
6 The data on smoking also enable students to undertake more hypothesis assessment in the light of evidence from a variety of sources.

PART I The *Study guide*

2.1 The control of breathing in humans

Principle

1 Respiration (energy release) is an essential feature of all living organisms.

Detecting change

Assumption

1 Awareness that diffusion to equilibrium occurs across a gas exchange surface.

Principles

1 Gas concentrations are expressed as partial pressures.
2 With increasing altitude the p_{O_2} decreases, although the percentage composition of the atmosphere remains constant.
3 Increasing the p_{CO_2} in inspired air increases ventilation.
4 Alveolar p_{CO_2} is the chief determinant of blood p_{CO_2}.
5 Blood is buffered against pH change.

The reduction of p_{O_2} with altitude receives only passing mention here, but it will be more thoroughly discussed in Chapter 4, when acclimatization is studied in more depth.

Ventilation is introduced and defined. It is important to understand that an increase in ventilation may be the result of an increase in the frequency or depth of breathing or a combination of the two effects. Only practical investigation will ascertain which is producing the increase in ventilation in a subject. Oxygen has a less marked effect on ventilation than does carbon dioxide, although it has been observed that the effect of the appropriate changes in both gases is synergistic – that is, the combined effect of lack of oxygen and increased p_{CO_2} is greater than would be expected from a consideration of their individual influences.

The fact that changes in pH and in p_{CO_2} can be used to indicate a necessity for a change in ventilation means that the pH of blood can alter. However, it is equally important to realize that blood is very well buffered against such changes. All pH changes consistent with health are small and temporary. Chronic or acute kidney disease, chronic vomiting, infantile diarrhoea, starvation, diabetes – all these conditions can produce large and more permanent pH changes which, if not corrected by appropriate therapy, may lead to death.

Blood is buffered in a number of ways. It is not necessary to go into this in depth, but the basic mechanisms should be thoroughly understood. It is haemoglobin that is the most potent contributor to the overall buffering system. The red blood cells are freely permeable to protons, and any excess protons in the plasma may diffuse into one of these cells and be absorbed by one of the thirty-eight histidine components of each haemoglobin molecule. The hydrogen carbonate ions are only of importance because of their relation with carbon dioxide; the plasma proteins absorb protons, but only weakly; and the phosphate ions in the plasma can also buffer to an extent but are in such low concentration that their contribution is insignificant. So it is haemoglobin that gives to the blood its remarkable proton-absorbing capacity and, by so doing, prevents large pH changes.

Questions and answers

a ***What is the relevance of the data in table [S]4 to mountaineers attempting to climb Mount Everest (8848 m) without oxygen?***

Although the percentage of oxygen in the atmosphere at the summit of the mountain is 21 per cent, its partial pressure is less than 8.1 kPa. Since the body is adapted to function in an atmospheric p_{O_2} of 21.2 kPa, this reduction will be enough to diminish effective oxygen supply significantly, and provide an insufficient gradient to supply oxygen to the respiring cells.
This idea will be returned to in the section on acclimatization in Chapter 4 and we shall discuss there the problems associated with living and working in high altitudes, and how such problems may be overcome.

b ***The data in table [S]5 appear to suggest that the body uses nitrogen, and that it absorbs more oxygen than it releases carbon dioxide. Comment upon these observations.***

Certainly there is no evidence to suggest *use* of nitrogen. The figures may appear to suggest absorption of nitrogen but in fact the added water vapour has caused an apparent reduction in the contribution of nitrogen to the total lung gas pressure. The number of moles of nitrogen present is probably the same as it was upon inspiration. The same fact probably also accounts for the apparent discrepancy in the figures for oxygen and carbon dioxide, though it may be that the metabolism is principally using fats, which would lower the RQ and produce an effect such as this.

c ***What values would you expect for the partial pressures of these gases in expired air (air breathed out)?***

Nitrogen should be approximately the same; oxygen will be higher and carbon dioxide lower because of the mixing of alveolar air with that from the dead space where no gas exchange has occurred. The water may remain somewhere near its alveolar value since the whole internal surface of the bronchial system is mucus-covered.

Chemical control of breathing

Assumptions

1 Knowledge of the use of neurones in interoception is assumed; *i.e.* it is assumed that students are familiar with the idea of nerve endings monitoring the 'internal state' of the body.

2 Some knowledge of circulation is assumed – for instance, that the heart pumps blood to the body via the systemic aorta and that this blood is highly oxygenated and has a low p_{CO_2}.

Principle

1 Detection of imbalance by receptors may lead to a change initiated by the brain which serves to restore the balance; *i.e.*, to homeostasis.

Cerebrospinal fluid (CSF) is mentioned and briefly explained. There is little need to go into detail here; it is only necessary that the students understand the significance of the fluid. It is formed by secretion by the choroid plexi and contains very little protein and no cells apart from a few lymphocytes. Its hydrogen carbonate content is low compared with that of blood plasma and the ion diffuses into it relatively slowly from the blood. Carbon dioxide and protons, on the other hand, pass readily into the CSF and within a few seconds of the plasma concentrations rising, the increases will be detectable in it. CSF is reabsorbed into the venous return from the brain, allowing a continuous cycle of secretion and reabsorption to occur. The cerebral blood supply is copious and well maintained, and provides for the main metabolic requirements of the brain. The CSF performs a less vital nutritive function and is more concerned with support and protection from mechanical damage.

Question and answer

a **_Why do you think that there is no direct monitoring of blood pH, p_{CO_2}, or p_{O_2} in the pulmonary blood vessels?_**

There is no point in monitoring blood in the pulmonary artery since the gas exchange that will occur in the alveoli will alter all three of the characteristics mentioned. The monitoring of blood in the systemic aorta in effect monitors blood from the pulmonary vein as this has had no opportunity for gas exchange since leaving the lungs.

Nervous control of ventilation

Assumptions

1 Knowledge that intercostal muscles are used to produce movements of the rib cage.

2 Knowledge that the bronchi and bronchioles contain smooth muscle in their walls.

Principle

1 Elasticity of the lung tissue accounts largely for expiration in humans.

The fact that nervous control of ventilation is centred in the medulla is without doubt. However, there is some uncertainty about the terminology that is appropriate for the various components of the ventilation control centre in that part of the brain. Thus a descriptive scheme has been adopted which avoids use of the terms 'respiratory centre' and 'pneumotaxic centre'. It is not the terminology which is important, but rather the way in which the control operates.

A self-controlled and oscillating system operates but the existence and importance of voluntary override by the cerebral cortex should be stressed.

The vagus nerve is heavily implicated as an afferent nerve in the context of ventilation. Vagotomy leads to apneustic breathing and it would seem that proprioceptive input to the ventilation control centre comes via the vagus, as does most other interoception. This may be worth mentioning, especially if it draws attention to another role for this nerve, which is often only mentioned in connection with cardiac control and is probably associated in some minds with that function only.

The term proprioception is not used as such in the text but could be introduced here.

Homeostasis

Principle

1 Homeostasis is demonstrated by the maintenance of a constant blood p_{CO_2} through the adjustment of ventilation.

Homeostasis and feedback and the idea of the maintenance of steady state are discussed. The difference between negative and positive feedback is of fundamental importance. The vital point to realize is that negative feedback is essentially stabilizing, whereas positive feedback is essentially destabilizing. Hence the latter is seldom encountered except in circumstances where a sudden change or adjustment is required.

Cheyne-Stokes breathing is referred to. In this condition there are periods of hyperpnoea (characterized by increasing depth of ventilation) alternating with periods of apnoea. This may occur at advanced stages of some serious illnesses (such as chronic kidney disease), when it is an indication of the disease condition. It is also occasionally noticed in very young children during sleep and in hibernating animals; in such cases it is of little significance.

Somewhere in this section the topic of exhaled air resuscitation may arise. This could prove to be a fruitful illustration of some of the points mentioned so far. The two most potent stimuli that could effect the restarting of an individual's own ventilation control are the stretching of the spindles in the patient's intercostal muscles when air is forced into his lungs and the raised p_{CO_2} that the presence of expired air in the lungs will produce. The former will result in stimuli passing to the ventilation control centre from the receptors in the muscles; the latter will cause the chemoreceptors to be stimulated and thus will stimulate the ventilation control centre.

Questions and answers

a ***Describe one other example of a negative feedback system in operation in biology, and explain briefly how it operates.***

The example should give an explanation of the system chosen, not merely a statement of what occurs. It is not acceptable simply to say that the blood sugar level is controlled by insulin from the pancreas. The student should explain, for example, that the blood sugar level rises as result of a meal; this rise results in the stimulation of the islets of Langerhans (either directly by blood glucose or indirectly via the vagus nerve) so that their β-cells increase their secretion of insulin into the blood stream; insulin stimulates target cells in muscle, fatty tissue, and the liver, causing them to take up glucose from the blood stream; this action lowers the blood sugar level; the reduction in blood sugar level, in turn, reduces the stimulation of the islets of Langerhans so that their output of insulin falls once more. The blood sugar level is the operator of this feedback system.

b ***Why do you think that there are very few examples of positive feedback systems in biology? Can you suggest one positive system and explain how it operates?***

Positive feedback is a destabilizing system rather than a homeostatic one. In positive feedback an event is self-stimulatory, leading, in effect, to a 'catastrophe' of some sort. A simple example is that of the thyroxine control of amphibian metamorphosis. In this case the secretion of thyroxine by the developing thyroid gland stimulates the hypothalamus and thereby causes an increase in its own rate of secretion, through the action of thyrotrophic hormone (TTH). This is released from the anterior pituitary and causes increased activity of the thyroid gland. Thus, the greater the concentration of thyroxin in the blood stream of a tadpole, the faster the hormone is secreted. Hence there is a sudden and dramatic surge in blood thyroxin level, which is in part responsible for initiating metamorphosis. (See also *Study Guide II*, Chapter 24.)

Coughing, sneezing, and swallowing

Principle

1 The reflex control of ventilation may be overcome and inhibited completely in order that functions such as coughing, sneezing, and swallowing may occur.

Coughing performs a protective function. The functions of chewing and swallowing illustrate the dual use of the oropharynx, and the intersection of airway and food passage at the back of the buccal cavity.

2.2 Analysis of human breathing

Practical investigations. *Practical guide 1,* investigation 2A, 'The capacity of the human lungs', and investigation 2B, 'Human consumption of oxygen'.

Principles

1 Basal metabolic rate (BMR) is a measure of respiratory activity in an organism.
2 As the intensity of human activity increases so does the BMR.

BMR is measured by the rate of oxygen consumption and so, in humans, may be determined by spirometry.

STUDY ITEM

2.21 (Multiple choice) (*J.M.B.*)

Question and answer

Basal metabolic rate in a mammal decreases with an increase in

A body size
B muscular activity
C food intake
D thyroid activity.

A – as body size increases the ratio of surface area:volume decreases. Hence the loss of heat in relation to heat production will decrease. Since thermostasis is important, the BMR of a larger animal will be less than that of a smaller animal, with the result that there is less heat production.
The other three options all increase the BMR as they increase; muscular activity directly influences the oxygen usage, and in any case would not permit the measurement of *basal* metabolism while it was continuing; thyroid activity increases metabolic rate directly through

the increase in thyroxin production; and food intake causes an indirect increase in metabolic rate because of the associated digestive enzyme syntheses, active absorption, and peristaltic movements. □

The use of the Douglas bag provides an opportunity to collect expired air for later analysis during strenuous or mobile exercise.

The concept of the respiratory quotient is introduced. RQ, as measured by the ratio of carbon dioxide output to oxygen uptake, is an indicator of the chemical nature of respiratory activity, and may be assumed to give some indication of what substrate is currently being respired. However, it may be pointed out that it is really rather an unsatisfactory measure because of the many different possible interpretations of a particular value. Nonetheless, it is included since it allows a rather different analysis of one aspect of breathing and also because it is still widely referred to by exercise physiologists.

STUDY ITEM

2.22 (Multiple choice) *(J.M.B.)*

The graph shows the respiratory quotient of germinating sunflower 'seeds' **[figure (S)14].** ***The table indicates the predominant type of respiratory activity at the points X, Y, and Z on the graph. Which one of the alternatives A to D gives the correct sequence of activities?***

	X	Y	Z
A	anaerobic respiration	aerobic respiration of fats	aerobic respiration of carbohydrates
B	aerobic respiration of carbohydrates	anaerobic respiration	aerobic respiration of fats
C	aerobic respiration of carbohydrates	aerobic respiration of fats	anaerobic respiration
D	anaerobic respiration	aerobic respiration of carbohydrates	aerobic respiration of fats

An RQ at X of 1.3 suggests anaerobic respiration since the volume of carbon dioxide evolved is more than the volume of oxygen taken in. The RQ at Y is 0.7 which is typical of fat respiration, while that at Z (1.0) is typical of the respiration of carbohydrates. So option A is the correct choice. □

STUDY ITEM

2.23 The use of the Douglas bag

This exercise is simply to test the students' understanding of the principles on which the use of the Douglas bag is based. The values derived have no intrinsic significance.

	dm^3
Total volume of air expired	29.27
Percentage of oxygen in inspired air	20.50
Percentage of oxygen in expired air	15.93
Percentage of carbon dioxide in inspired air	0.04
Percentage of carbon dioxide in expired air	3.98

Use these figures to calculate:

a ***The subject's $\dot{V}_{O_2}$ (oxygen consumption) in cm^3 kg^{-1} minute^{-1}.***

In 29.27 dm^3 of expired air there are $\frac{15.93}{100} \times 29.27$ dm^3 of oxygen

$= 4.66$ dm^3 oxygen.
In the same volume of inspired air there would have been

$\frac{20.50}{100} \times 29.27 = 6.00$ dm^3 of oxygen.

Hence $\dot{V}_{O_2} = 6.00 - 4.66 = 1.34$ dm^3 in 5 minutes.

This is equivalent to $\frac{1.34}{5} \times 1000$ cm^3 minute^{-1} = 268 cm^3 minute^{-1}

$= \frac{268}{65} = 4.12$ cm^3 kg^{-1} minute^{-1}.

b ***The subject's carbon dioxide output in cm^3 kg^{-1} minute^{-1}.***

Similar calculations to those in question **a** will reveal that the volume of CO_2 in 29.27 dm^3 of inspired air was 11.71 cm^3, whereas the volume of CO_2 in 29.27 dm^3 of expired air was 1164.95 cm^3.
Thus the CO_2 output was

$\frac{1164.95 - 11.71}{5 \times 65} = 3.55$ cm^3 kg^{-1} minute^{-1}.

c ***The subject's respiratory quotient (RQ).***

$$\text{The RQ} = \frac{\text{volume of carbon dioxide produced per unit time}}{\text{volume of oxygen consumed per unit time}}$$

$$= \frac{3.55}{4.12}$$

□ Hence the RQ = 0.86.

STUDY ITEM

2.24 (Essay) *(J.M.B.)*

For the following situation describe the physiological responses of the human body and explain how they are brought about:
In an experimental investigation a subject with his nose closed by a clip can only breathe air from and into a gas-tight bag connected to his mouth. The subject breathes and re-breathes air from this bag for a few minutes.

The following points could be made: at first there is no change since the initial p_{CO_2} (0.03 per cent) is very much lower than that in the blood, so removal of carbon dioxide from the blood continues. When the alveolar p_{CO_2} starts to rise, and the carbon dioxide diffusion gradient across the alveoli decreases, the exchange of carbon dioxide will be less efficient and the blood p_{CO_2} will increase. Receptors in the aortic and carotid bodies, and around the medulla oblongata, detect this. Impulses to the ventilation control centre cause the frequency and depth of ventilation to increase. (The sensitivity of the mechanism to increasing p_{CO_2} is enhanced by the concomitant decrease in p_{O_2}, so the change in ventilation is marked.) For a short while the increase in ventilation may compensate for the increased p_{CO_2}, but quickly it becomes unable to reduce blood p_{CO_2} since the p_{CO_2} of the air exceeds that of the blood. Breathing becomes laboured and the subject experiences distress. Unconsciousness may follow. □

STUDY ITEM

2.25 An analysis of human breath

Comparative studies are only of value if sufficient data are to hand to enable a true comparison to be made. The oxygen uptake of individuals of different age, sex, or mass is only comparable if all the data are known.

Questions and answers

a ***Assess the merit of each hypothesis in the light of the data in table [S]8 and the information given earlier in this chapter. Accept or reject each hypothesis on the basis of your assessment.***

Condition or activity	*Respiratory quotient*
Rest, in bed	0.83
Rest, standing	0.80
Walking, 0.89 m s^{-1}	0.85
Walking, 1.34 m s^{-1}	0.93
Walking, 1.79 m s^{-1}	0.88
Walking, 2.24 m s^{-1}	0.94

Table 2
The respiratory quotient for each of the activities shown in table (S)8.

The respiratory quotient for each of the activities shown in table (S)8 is given here in table 2.

Hypothesis 1. This is very unlikely to be the case since at rest the RQ is considerably less than 1.0. Hypothesis rejected.

Hypothesis 2. This may be the case since, as the rate of exercise increases, the RQ approaches unity; however an RQ = 1.0 may also be produced by a 'mixed' metabolism, with some tissues using fat as substrate while others, such as muscle, respire carbohydrate anaerobically. Hypothesis accepted.

Hypothesis 3. The data about oxygen uptake or carbon dioxide output do not tell us anything about the efficiency of the mechanisms for dealing with these gases. It is more likely that the increase in carbon dioxide output and decrease in oxygen uptake are due to a fundamental metabolic adjustment than to a change in efficiency of gas exchange. Occam's razor should be invoked and this hypothesis rejected until further evidence comes to light or until simpler hypotheses are shown to be unsupported.

Hypothesis 4. This is unlikely since the RQ is rising from 0.8 rather than falling further towards 0.7 (which alone would indicate increasing use of fat). The RQ for protein is 0.9 but since the measured RQ exceeds that value it is more likely that the increase is due to the use of carbohydrate.

b ***It is hard to draw certain conclusions from these data because some information is missing. What further data are needed if those in table [S]8 are to be useful?***

Particulars of mass and sex of any subjects used are needed. Information about their age would also be of value. It is only assumed that all these data are comparable. If the walkers were a different age from the resters it would not be acceptable to use the table to draw conclusions about change in RQ with changing activity. □

2.3 The effects of smoking

Assumptions

1 The ability to interpret data presented in the form of line graphs and histograms.

2 An understanding of the terms 'sympathetic' and 'parasympathetic' in the context of the nervous system.

Principles

1 There is very strong evidence that shows smoking to be a major cause of lung damage. Nevertheless it should be pointed out to students that strong correlation does not necessarily imply causality.

2 Intellectual understanding of a fact does not necessarily lead to action; people still smoke.

There are many approaches to this topic. This section attempts to present some of the data and to point out some of the more pertinent features of those data. The topic presents a chance for students to see how increased biological knowledge can be of importance in determining attitudes to social issues. This work can also be a way of testing and reinforcing the knowledge and understanding of how the lung is constructed and how it operates.

Questions and answers

a ***What do you think is the main evidence against this hypothesis?***

People who give up smoking rapidly reduce their chances of dying prematurely. This is a well-known assumption and is supported by the data in *figure 6*.

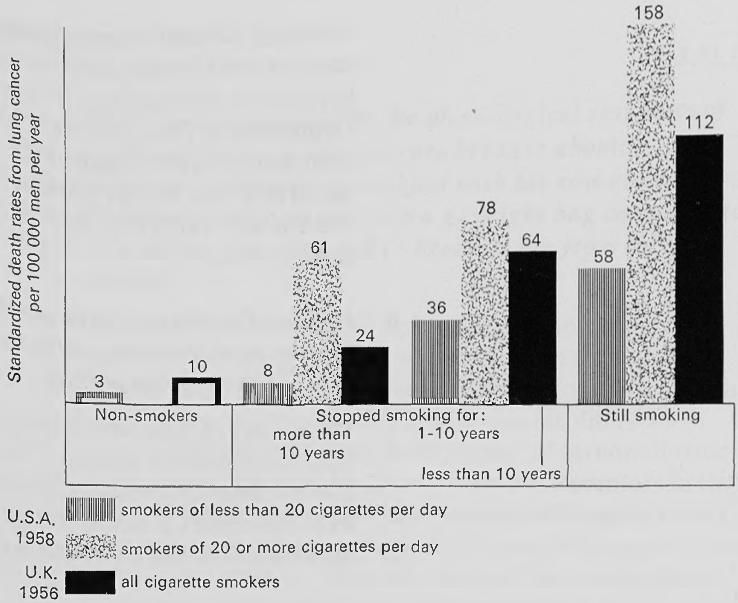


Figure 6
The effect of giving up smoking on the death rate from lung cancer. *Based on Royal College of Physicians of London,* Smoking and health, *Pitman Medical Publishing Co., 1962.*

b ***What do these three graphs suggest about smoking habits and the fall in cigarette smoking?***

There are clear indications from these graphs that there are differences in smoking habits between men and women, and that there may be connections with other social or educational characteristics.

c ***Suggest two different reasons to account for the difference between the two curves in* figure [S]18.**

There may be very different social pressures on the two groups; professional men may be under greater pressure to give up, since fewer of their number smoke. Another reason could be that because of lack of literacy or appropriate reading material or habits, group 5 men are less well informed about the dangers of smoking, and hence less motivated to give up. Many other possibilities may present themselves. It should be noted that, without further data, only speculative answers are possible. Figure (S)18 reinforces one hypothesis, suggesting a correlation between academic attainment and smoking habits.

d ***Express concisely in words the information contained in* figure [S]21 *about the effects of the different levels of cigarette consumption.***

The more cigarettes smoked, the greater the risk of dying from lung cancer, bronchitis, or emphysema.

e ***Account for the differences between the two graphs.***

Women took to cigarette smoking later and more gradually than men (figure (S)16), so the increasing death rate from lung cancer had only produced a slight upturn in the graph for deaths from all cancers by 1978. Since the steep increase in lung cancer for men up to 1963, there has been a steady decline. Reasons for this decline could be discussed.

PART II The *Practical guide*

INVESTIGATION 2A The capacity of the human lungs

ITEMS NEEDED

Portion of lung (optional)

Either
Ink
Kymograph 1/class
Paper for kymograph records 2–4/group
Pen 1/class

Or
Chart recorder 1/class
Cotton thread
Electronic arm 1/class
Eyelet 1/class
Leads, 4 mm
Paper for chart recorder
Pen
Power supply, 9V 1/class
Retort stand 1/class

And
Antiseptic (e.g. Shield, or other antiseptic which does not contain pine oil), in beaker
Soda lime (Carbosorb)

Oxygen cylinder, medical grade, with regulating valve 1/class
Mass, 200 g 1/class
Metre rule 1/group
Mouthpiece 1/group
Nose clip 1/group
Paper towels
Platform scale, kg 1/class
Spirometer 1/class
Stopclock or wristwatch with seconds indicator 1/group

(*Study guide* 2.1 'The control of breathing in humans' and 2.2 'Analysis of human breathing'.)

Principles

1. A spirometer can be used to find the vital capacity of lungs and the various subdivisions of this capacity.
2. The lungs have 'reserve' capacity above that used for quiet breathing; this enables changes in the body's oxygen demands to be easily accommodated.
3. Inhalation only replenishes a relatively small proportion of the air in the lungs with each breath.

In investigations with human subjects, apparatus must be faultless; do not use home-made spirometers. Students should not undertake work with a spirometer unless the teacher is adequately trained in its use. There may also be local regulations specifying qualifications which the teacher must possess. Give careful consideration to the selection of subjects; it may be helpful to know medical histories. The safest course may be for the teacher to be the only subject of such an investigation – always providing that he or she is physically fit. Point out to students the dangers of using spirometers when unsupervised, and explain the biological reasons.

It is necessary to use medical grade oxygen. Although industrial and laboratory cylinders are filled with pure oxygen, harmful dust may accumulate inside. Medical cylinders are checked against this hazard. The instructions provided by the manufacturers of the spirometer should be consulted and followed in detail.

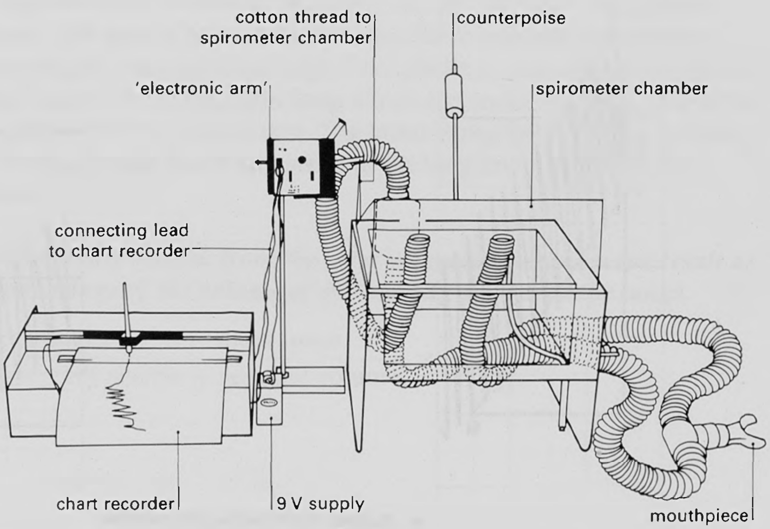
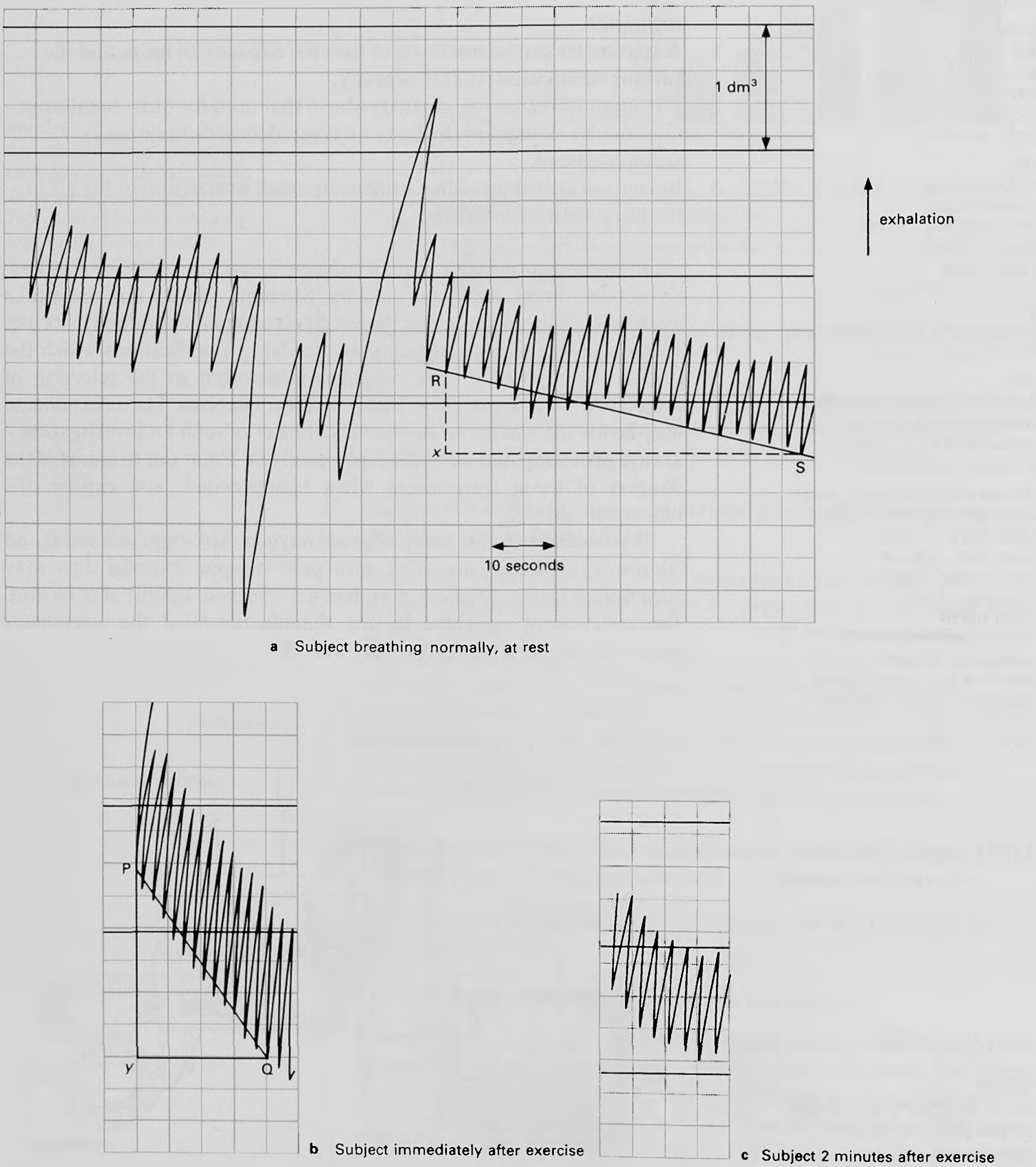


Figure 7
A plan of a spirometer connected to a chart recorder via an electronic arm.

In essence the experimental set-up will be as shown in *figure* 7. (It is possible, however, in this short investigation, to fill the chamber with air instead of oxygen. In this case the carbon dioxide absorber should also be missed out. The student should be instructed to take only a FEW BREATHS before the deep breath required to measure vital capacity.) *Figure 8* shows a spirometer trace produced in circumstances similar to those of this investigation and the next.

Figure 8
A trace from a kymograph, recorded during investigations 2A and 2B.



Questions and answers

a *What are the following volumes for the subject: tidal volume, expiratory reserve volume, inspiratory reserve volume? (See* **figure [P]20.)**

Typical values for adults might be: tidal volume 0.5 dm^3; expiratory reserve volume 0.75 to 1 dm^3; inspiratory reserve volume 2 to 3.2 dm^3.

b *What is the subject's vital capacity?*

This is normally between 3 and 5 dm^3 but may be as high as 6 dm^3 among athletes.

c *Estimate the total lung capacity by multiplying the expiratory reserve volume by six. This has been found to provide a reasonably accurate value.*

In adult humans the total capacity of the lungs is around 5.5 to 6 dm^3.

It should be appreciated that, under the slightly unusual circumstances of this investigation, the subject may be breathing abnormally. If he or she is breathing 'high up' or 'low down' in the lungs, the calculated values of vital and total lung capacity may appear to be unnaturally great or small.

d *From b and c calculate the subject's residual volume.*

This is normally in the order of 1.2 dm^3.

e *Calculate the volume of air that is left in the subject's lungs after a normal expiration. Look at* **figure [P]20.**

This is given by the sum of expiratory reserve and residual volume.

f *Calculate the dead space of your experimental subject in cm^3.*

The dead space consists of two components: the anatomical dead space – the part of the system not vascularized for oxygen uptake (mouth, pharynx, bronchi, larger bronchioles); and the physiological dead space – the areas of the lung which are under-supplied with blood in relation to their ventilation. The latter is negligible during exercise as there are then few, if any, parts of the lung under-supplied with blood.

g *Subtract this volume from the tidal volume and express the result as a percentage of the volume of air already present in the lungs.*

$$\frac{\text{tidal volume} - \text{dead space}}{(\text{expiratory reserve} + \text{residual volume})} \times 100$$

h ***In the light of your calculation comment upon the statement: 'breathing brings fresh air into close proximity with the blood capillaries in the lungs'.***

This question is intended to bring out the idea of partial renewal of air in the lungs at each breath. In fact typical results for the calculation in **g** suggest that the inspired air represents perhaps only 15 per cent of the air still in the lungs.

INVESTIGATION 2B Human consumption of oxygen

ITEMS NEEDED

Exactly as for investigation 2A

(*Study guide* 2.1 'The control of breathing in humans' and 2.2 'Analysis of human breathing'.)

Principles

1 The rate of oxygen consumption of a human is related to the rate of muscular work being performed.

2 Oxygen consumption at rest is a direct measure of the BMR of a subject.

If the exercise is anything more than very light the subject will incur an oxygen debt, and therefore the oxygen uptake either during or immediately after exercise will not give an accurate picture of the energy usage during the exercise. The oxygen debt of the subject may be calculated by subtracting from the amount of oxygen consumed during the recovery time of t minutes the amount consumed during t minutes at rest. For example:

if V = volume consumed in t minutes at rest
and V_r = volume consumed in t minutes during recovery
then $V_{od} = V_r - V \text{ cm}^3$
where V_{od} = the volume of the oxygen debt and t = time of the recovery period.

Small samples of data are included from a typical subject of mass 64 kg and height 177.5 cm (*figure 8a*). Part (*a*) shows the oxygen consumption of the subject at rest; (*b*) shows his consumption immediately after exercise; and (*c*) the consumption two minutes after exercise. The subject ran upstairs and covered a vertical distance of 36.5 metres in 165 seconds.

Questions and answers

a ***Determine how much oxygen the resting subject consumed in five minutes and calculate how much would be consumed, at the same rate, in twenty-four hours.***

Line R to x on *figure 8* represents the subject's oxygen consumption, and is 12.8 mm in length. From the calibration, 19.5 mm are equivalent to 1 dm^3, so his oxygen consumption is

$$\frac{1000}{19.5} \times 12.8 = 656.4 \text{ cm}^3$$

The time taken to consume this oxygen is given by $x - S$ (55 mm). The calibration indicates that 10 mm represent 10 seconds, so $x - S$ represents 55 seconds. If the subject consumed 656.4 cm^3 in 55 seconds he consumed

$$\frac{656.4}{55} \times 300 = 3580.4\,cm^3 = 3.58\,dm^3 \text{ in 5 minutes.}$$

At that rate the subject would consume 1031 dm^3 of oxygen in twenty-four hours.

b ***Calculate the resting metabolic rate of your subject in kJ h^{-1}....***

The amount of oxygen consumed in one hour is

$$\frac{1031}{24} = 42.96\,dm^3$$

This is equivalent to

$$42.96 \times 20.18 = 866.9\,kJ.$$

The metabolic rate of this subject is 866.9 kJ h^{-1}.

c ***Calculate the metabolic rate per square centimetre for the subject***
1 in resting conditions;
2 during or after vigorous exercise.
What significance would you attach to the difference?

For this subject, $W = 64$ kg and $H = 177.5$ cm
Substituting, in the equation given, his surface area

$$\begin{aligned} A &= 64^{0.425} \times 177.5^{0.725} \times 71.84 \\ &= 5.86 \times 42.72 \times 71.84 \\ &= 17974.70\,cm^2 \end{aligned}$$

If the line R to S is taken to be the rate of oxygen consumption at rest, then the metabolic rate per square centimetre of this subject is

$$\frac{866.9}{17974.7}\,kJ\,cm^{-2}\,h^{-1}$$

$$= 48.23\,J\,cm^{-2}\,h^{-1}.$$

If the trace in *figure 8b* is taken to represent oxygen consumption during vigorous exercise, then the line P to y (= 29.2 mm) represents the oxygen consumed and the line y to Q (= 20 mm) represents the time for this consumption. Converting these line lengths gives $\frac{29.2}{19.5}\,dm^3$ of oxygen consumed in 20 seconds.

Hence $\frac{29.2}{19.5} \times \frac{60}{20} \times 60\,dm^3$ of oxygen per hour $= 269.54\,dm^3\,h^{-1}$.
This is equivalent to

$$269.54 \times 20.18 = 5439.29\,kJ\,h^{-1}.$$

Taking the subject's surface area into consideration

$$\frac{5439.29}{17974.7}\,\mathrm{kJ\,cm^{-2}} = 0.30\,\mathrm{kJ\,cm^{-2}\,h^{-1}}$$

$$= 302.61\,\mathrm{J\,cm^{-2}\,h^{-1}}.$$

The amount of oxygen consumed, and the metabolic rate, are greater after exercise than in a subject who has been resting. Exercise is just one of the factors that affect metabolic rate. If BMR is expressed in terms of surface area, very similar values are obtained for animals differing in size as much as a man and a mouse. If BMR is expressed in terms of body mass, then marked differences are apparent between animals of different sizes.

d ***Are the results of measurements before and after exercise strictly comparable? Are the conditions the same for both measurements? If not, state the factors which might vary. What steps could be taken to overcome this variability?***

A subject who has just finished some vigorous exercise usually feels hot. It may be argued that the expired air is at a higher temperature than previously and that this may increase the gas volume in the spirometer. The temperature of the air in the chamber should be measured on both occasions. If it differs considerably, a correction should be made when calculating the volume of oxygen.

e ***Calculate the work done by the subject. Comment upon the work done in relation to the available energy, as revealed by the oxygen consumption.***

In this example,
$m = 64\,\mathrm{kg}$
$g = 9.81\,\mathrm{m\,s^{-2}}$
$h = 36.5\,\mathrm{m}$
$m \times g \times h = 64 \times 9.81 \times 36.5$
$= 22.92\,\mathrm{kJ}$

This work was performed in 165 seconds. The amount of energy available in this time, as indicated by the metabolic rate, was

$$\frac{5439.29}{60 \times 60} \times 165 = 249.30\,\mathrm{kJ}.$$

The calculation from these data reveals that the available energy, as indicated by the oxygen consumption, is considerably in excess of that required for the work performed. The difference between these two figures indicates the overall inefficiency of the human at converting chemical energy into work, especially if relatively unfit!

L 2B.1

STUDY ITEM

~~(P)2.21~~ (Short written answer) *(J.M.B.)*

a ***The amplitude of the pen movement in trace A represents a volume change of about 500 cm³. What is the name given to this volume of human breathing capacity?***

Tidal volume.

b ***In 60 seconds the upper points of trace A have sunk by distance X. What does distance X represent and how can this be useful to an investigator?***

This distance represents the oxygen consumption of the subject. It may be used to determine the subject's metabolic rate.

c ***If the carbon dioxide absorber were removed from the spirometer, what two changes would occur in the continuation of trace A?***

The gradient of the trace would flatten and the frequency and amplitude of the pen movements (tidal volume) would increase.

d ***Explain how the amplitude of trace B can change while that of trace A remains constant.***

B simply records ribcage movements. The diaphragm also moves during breathing and so the tidal volume is a measure of the two components. If diaphragm movements were to increase while those of the ribcage decreased, the tidal volume could remain constant. □

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CHAPTER 3 THE CIRCULATORY SYSTEMS OF ANIMALS AND PLANTS

A review of the chapter's aims and contents

1 The general need for circulatory systems in large organisms is established.
2 The nature of the different types of circulatory system, and the ways in which they match the capabilities of the organism, are investigated.
3 The essential requisites for circulation are stated and then each is examined in turn.
4 The different types of heart are investigated briefly before the human heart is considered in more detail.
5 The complexity of heart function is examined, enabling ideas about co-ordination to be established.
6 The part played by medicine and technology in monitoring and mending the faulty heart is examined.
7 The principle of the changing distribution of blood to meet the requirements of the different tissues is established.
8 The problem of understanding transport in plants is brought out and the point made that such uncertainty is an entirely 'normal' scientific occurrence.
9 Some of the current hypotheses are investigated and students are encouraged to assess the evidence for themselves.

PART I The *Study guide*

3.1 Movement inside plants and animals

Practical investigation. *Practical guide 1*, investigation 3A, 'Movement inside plant cells'.

Assumption

1 Realization that all active cells require a supply of raw materials for metabolism, and metabolic wastes need to be removed.

Principles

1 Mass flow requires energy for the operation of the pump which causes the flow.
2 Larger and more complex organisms require more sophisticated circulatory systems than smaller, simpler ones.

This is the first of two short introductory sections. This section establishes the need in most macroscopic organisms for a system of transport within the organism that is rather more directed and more rapid than molecular diffusion.

Question and answer

a ***What are the essential features of a circulating mass flow system?***

The essential components of such a system are:

1 A transport medium, usually called blood, capable of carrying dissolved materials such as food and oxygen.

2 A pumping device to make the medium circulate.

3 Valves to direct the flow in one direction.

4 Vessels carrying the medium to all parts of the body and back to the heart.

5 An arrangement in the tissues whereby their cells can obtain required substances from the medium and deliver up to it their waste products.

3.2 Circulation in animals

Practical investigation. *Practical guide 1*, investigation 3B, 'Circulation in animals'.

Assumption

1 That the student is familiar with a variety of animals and can understand the examples given in this section.

Principles

1 Different types of animal have evolved different types of mass flow system, which match their mode of life, environment and organization.

2 The demands made on a circulatory system by different parts of the body vary from each other and differ from time to time; the circulatory system must be able to vary accordingly.

This second introductory section makes more reference to circulation, in particular in animals, and the hope is that there will be time to consider how different animals with different levels of organization solve identical problems in many different ways. The question is linked to work on blood vessels, later in the chapter.

Question and answer

a ***What adaptations would you expect to find in the circulatory system that would enable the distribution of the blood to meet the demands of the tissues?***

The distributing vessels (arteries) will require muscle in their walls to enable vaso-constriction to occur. The muscle could be in the form of isolated sphincters or more general distribution of smooth muscle within the tissues of the walls. Both of these features are found. Return vessels (veins) do not need muscle for this purpose (although many veins do possess muscle). Antagonism is provided by the incompressible blood which dilates vessels if their muscle relaxes. Some co-ordination is required, with afferent nerves to the muscle of blood vessels linked via co-ordinating centres (ganglia or the CNS) to receptors which detect the state of activity of different organs.

3.3 Different types of circulation in animals

Assumptions

1 An understanding of the nature of hydrostatic pressure.

2 Familiarity with the idea of diffusion of molecules and with the idea that water moves from a region of high water potential to one where it is lower.

3 Some knowledge of systematics, so as to be able to visualize the examples of animals referred to.

Principle

1 There is a strong correlation between the type of circulatory system possessed by an animal, and its size and activity.

Open and closed circulations

Practical investigation. *Practical guide 1*, investigation 3C, 'The effect of temperature and chemicals on the rate of heart beat of *Daphnia*'.

Principles

1 Open circulatory systems are inherently inefficient at circulating molecules from place to place.

2 It is only possible to develop high blood pressure within closed circulatory systems. The maximum size attainable by an animal possessing an open system is probably limited, at least in part, by the inefficiency of such systems.

Question and answer

a ***Suggest why closed circulatory systems provide a more efficient means of transport than open systems.***

If a molecule at point A in the body has to reach point B in an open system, there will be a time lag before the molecule is moved by chance into the heart. On leaving the heart the molecule may be moving but its direction will not necessarily be that required to reach point B. In a closed system blood vessels will in all likelihood join A to B via the heart, so all molecules originating at A will quickly and inevitably be moved past B.

STUDY ITEM
3.31 Tissue fluid

Assumption

1 A knowledge of the composition of plasma.

Principles

1 Capillary walls are permeable to most solute molecules.

2 The hydrostatic pressure of blood promotes the formation of tissue fluid; the water potential difference between blood and tissue fluid opposes it.

3 There is a net movement of fluid from the blood to the tissues in capillary beds. This fluid is returned to the circulation via the lymphatic system.

4 Diffusion is the main method of getting required molecules from the blood into cells. Circulation merely moves them as near to the tissues as possible and tissue fluid formation simply gives a continuous fluid link between blood and tissue in which diffusion may occur.

One point that is not always clear is that plasma proteins of considerable size diffuse moderately easily out of capillaries. In fact this occurs to a surprising extent; if the mean plasma protein concentration of $7\,\mathrm{g}\,100\,\mathrm{cm}^{-3}$ (most of this being albumin – relative molecular mass 70 000) is taken as producing a plasma water potential of -3.33 kPa, then we may calculate that the values for the water potential of tissue fluid due to plasma proteins are as shown in table 3. Tissue fluid hydrostatic pressure is usually taken as being negligible.

Tissue	*Capillary walls*	*Water potential*	*Plasma proteins (%)*
Liver	discontinuous in sinusoids	−2.83	85
Intestine	fenestrated	−1.67	50
Muscle	continuous	−0.67	20

Table 3
The water potential of tissue fluid in various organs, owing to the effect of plasma proteins.

Questions and answers

a ***In which organs would you expect to find capillary walls that were discontinuous and very permeable?***

In any organs where bulk loading or unloading of capillaries is required. The capillaries of the renal glomeruli and intestinal villi have fenestrated walls with the basement membrane being the only barrier to filtration. In liver sinusoids the capillary endothelium is discontinuous.

b ***Assuming that this is opposed by the water potential difference between the plasma and the tissue fluid, what is the maximum net filtration pressure forming tissue fluid in this capillary?***

The water potential difference between plasma and tissue fluid (-2.66 kPa) will be the opposing force. The maximum net filtration pressure will be

$4.27 - 2.66 = 1.61$ kPa.

c ***How much of the tissue fluid which is formed per unit time will be reabsorbed into the blood at the venous end of the capillary?***

At the venous end the hydrostatic pressure will be insufficient to oppose the water potential difference, so the return of water will be under a pressure of $1.60 - 2.66 = -1.06$ kPa.
This is around two-thirds of the volume of fluid that is leaving the capillary bed.

d ***Can you account for this difference in hydrostatic pressure? How may it be related to the structure or function of these two organs?***

The difference in blood hydrostatic pressure is partly caused by the difference in pressure in the pulmonary and systemic circulations. It may also be the result of different local arrangements, within the tissues, of the constriction or dilatation of blood vessels supplying or draining the organs. It is necessary to have a high hydrostatic pressure in the kidneys to produce the filtration essential for proper renal function. In the lungs, it is important to have only a low pressure, since the volume of tissue fluid there must be kept low in order that the area of surface exposed to air remains as large as possible. Accumulation of fluid in the lung can lead to asphyxia.

e ***Can you suggest what happens to the remaining 2 dm³ of this fluid?***

This is absorbed into the lymphatic system whose vessels permeate every organ and tissue. In the absence of any knowledge about this system, students should be able to suggest that the excess fluid must somehow work its way back to the blood system.

f ***Using the information in this section, explain how this symptom of malnutrition is produced.***

If the protein levels in the blood fall its water potential increases. This means that the difference between the venous hydrostatic pressure and the water potential difference in the capillary beds is reduced. In this case the return of fluid to the bloodstream is impeded and once the capacity of the lymphatic system for reabsorbing fluid is reached, fluid will start to accumulate in the interstitial spaces and will cause the
☐ tissues to swell.

Single and double circulations

Principles

1 A double circulation separates the pulmonary and systemic circulations.

2 A double circulation permits different blood pressures to be established in the two halves of the system, allowing the lungs to function without asphyxia, and still permitting efficient and rapid transport of oxygen to muscles and other tissues.

3 A need for a double circulation is associated with homoiothermy.

The existence of arterioles and venules is ignored at this point since the distinctions between them and arteries and veins are often slightly blurred and they in no way affect the points being made here.

3.4 Different sorts of heart

Practical investigations. *Practical guide 1*, investigation 3D, 'The vertebrate heart in action', and investigation 3E, 'The structure of hearts'.

Assumptions

1 Familiarity with peristalsis as a means of moving material through a tube.

2 Awareness that in all vertebrates the brain has to have a continuous supply of highly oxygenated blood.

Principles

1 The different complexity of the heart in different animals is matched by accompanying modifications of the circulatory system.

2 A pump, vessels, and valves are the only essentials for an effective circulation of blood.

3 The amphibian heart shows an increase in complexity compared with that of a fish but is not completely divided like that of the mammal.

The crayfish is cited as a crustacean example, chiefly because its circulation shows features that are an 'improvement' over that of the locust, while it still retains an open circulation.

It could be pointed out that the heart of *Daphnia* (which is studied in Practical investigation 3C) is very simple, having no attached vessels. It is also relevant that in all these invertebrates the rate of beating is much more susceptible to changes in environmental factors than is the heart beat of homoiothermic vertebrates. All the vertebrates have complex innervation of the heart. The control of invertebrate heart beat is less well documented.

Students may see the close similarity between fish and frog hearts in spite of their apparently different shape. This may help students to realize that, even in mammals, the heart is still essentially a particularly muscular blood vessel.

Question **d** reinforces a point made in the previous section but in a slightly different form. The difference in efficiency between the two types of circulation is of such fundamental importance that the point is worth making again.

Questions and answers

a ***How do body movements in humans contribute to blood flow?***

Veins have muscle in their walls, but it is not used to propel the blood. Instead veins are continually being squashed by nearby skeletal muscles when these contract. Because veins have series of semi-lunar valves in their walls which prevent back-flow, each squashing moves the blood that is in the vein towards the heart.

b ***How else could blood be caused to flow in one direction in a tubular vessel other than by the use of valves?***

Peristaltic contractions could produce flow in a tube without the necessity for valves. Such peristalsis is seen in the dorsal vessel of *Nereis* in which blood is moved forwards. Many other major vessels in this animal (apart from the ventral one) are peristaltic.

c ***What important problem has to be overcome for a single-chambered highly muscular heart to work effectively?***

The muscular chamber cannot be filled. Enough of the energy of systole will be dissipated during the blood's passage round the body for there to be insufficient force left to expand the ventricle once again. With a two-chambered heart the thin-walled atria can be inflated by low pressure venous blood assisted by elastic recoil and negative pressure within the pericardium, and atrial systole has sufficient force to fill the muscular ventricle(s). (Again, this filling is aided by elastic recoil.)

d ***Suggest how the single circulation of the fish might lead to a lack of circulatory efficiency which the divided heart and double circulation overcome.***

The lack of venous pressure in such a circulation leads to a very sluggish, even stagnating, flow in veins, so rapid and efficient circulation of materials is impossible. With a divided heart pressure can be kept low in the pulmonary circulation, which is short and of lower resistance, and then raised very high in the systemic circulation, so that transport throughout the body is efficient and venous return no longer sluggish. In mammals, venous return is further aided by breathing. During each inspiration thoracic pressure falls and this encourages venous blood to flow towards the heart.

e ***What is the importance of the pathway to the skin in a frog?***

The skin is an organ of gas exchange, in addition to the rather poorly developed lungs, which are simple air sacs.

f ***What important problem is presented by the lack of division of a frog ventricle into two separate halves?***

The blood will mix in the ventricle. There still remains contention about the solution. Suffice it to say that there is far less mixing than one might expect and the heart is able to deliver highly oxygenated blood to the head and brain (carotid circulation); well oxygenated blood to the systemic circulation; and deoxygenated blood to the pulmocutaneous circulation.

STUDY ITEM

3.41 (Short answer question) *(J.M.B.)*

Questions and answers

a ***How fast was the kymograph paper moving?***

$0.44\ cm\ s^{-1}$.

b ***1 Describe, very briefly, the action of the heart which corresponds to each of the portions of the trace marked A, B, C, and D.***

A is contraction of the ventricle
B is contraction of the atria
C is contraction of the sinus venosus
D is relaxation of the heart.

2 ***From the appearance of the trace, decide where the thread from the heart was attached to the lever. Copy the unfinished illustration of the kymograph lever and pen and complete it by drawing in the position of the thread that attaches the heart to the lever. Briefly explain your answer.***

The thread must attach to the right of the pivot so that, when the heart contracts, it pulls the lever end downwards and the pen upwards.

c ***From the trace, determine the maximum effect of raising the temperature of the whole heart to 30 °C on (1) the rate and (2) the amplitude of the heart beat.***

1 The rate increases from $0.9\ s^{-1}$ to $1.8\ s^{-1}$.
2 The amplitude increases from 4.5 mm to 7 mm.

d ***What do the traces shown in* figures [S]30e *to* g *suggest about the control of the contraction of the heart?***

Only the treatment given to the sinus venosus had any effect on the heart beat, and that treatment altered the amplitude and frequency of the whole beat. This suggests that the sinus venosus controls the overall rate of contraction. The control treatments show that it is not the conduction of heat from the sinus that influences ventricular systole; this suggests that internal co-ordination is chemical or nervous.

e ***Copy and continue the trace in* figure [S]30h *to show the expected result of stimulating the vagus nerve.***

The trace would show a similar pattern of heart beat, although one would expect both the frequency and the amplitude to be reduced. □

3.5 The mammalian heart in action

Assumptions

1 Awareness that contraction of muscles requires work to be done which makes it necessary to have a supply of metabolites for ATP production.
2 Awareness that the relaxation of contracted muscle is passive.
3 Knowledge that liquids such as blood are incompressible.
4 An elementary knowledge of the structure of the mammalian heart is assumed.

Principle

1 The many refinements and modifications both of structure and function of the heart make it possible to maintain a very complex beat throughout the life of the individual.

Cardiac muscle

move

Practical investigation. *Practical guide 1*, investigation 3D, 'The vertebrate heart in action'.

λ 8 / h, and ~~investigation~~ 3E, 'The structure of hearts'.

Principle

1 The necessity for an antagonist to muscular contraction is fundamental. In the heart, antagonism is provided by elastic recoil.

It may be helpful to ask the students to summarize the properties of cardiac muscle, after reading this section.

STUDY ITEM
3.51 The action of the heart

Principles

1 Heart sounds are the result of valve closure.
2 The major arteries are vital in smoothing the intermittent flow of blood that comes from the heart.

Vibrations are set up by the closure of the valves of the heart and these so-called heart sounds can be heard by a doctor when he applies a stethoscope to a patient. For each heart beat there are two distinct sounds–a 'lub' sound when the atrio-ventricular valves close at the beginning of ventricular systole and a 'dup' sound when the semi-lunar valves close at the end of the systolic phase. It is possible to diagnose diseased heart conditions by the abnormalities heard in the quality and regularity of these sounds.

Questions and answers

a ***Which of the following is most likely to cause the heart sounds heard through a stethoscope?***

A Contraction of the ventricles
B Filling of the ventricles
C Closure of the heart valves
D Contraction of the atria and ventricles

C – closure of the heart valves.

b ***Give a reason for your choice.***

Contraction of the ventricles and/or atria will not make any sound. Filling may make sounds but they will not be audible as two distinct, sharp 'noises'.

c ***Explain briefly how the intermittent action of the mammalian heart is converted into a steady flow of blood.***

Arteries are flexible to some extent. They expand when blood is pumped into them and accommodate the high volume of blood discharged into them at ventricular systole. During diastole the return of the arteries to their original diameter helps to force the blood onwards at a steady rate. □

The origin of heart beat

Principle

1 No extrinsic stimulus is necessary for a heart beat to occur; the contraction of the cardiac musculature arises spontaneously 'within' the muscle of the sinus venosus vestige.

Conduction of the excitation wave

Principle

1 The propagation of a wave of excitation via specialized muscle tissue throughout the heart is responsible for the co-ordination of heart beat.

STUDY ITEM
3.52 The cardiac cycle

The details are omitted from the *Study guide* because students should try to understand the graph of pressure changes, using only their understanding of what has preceded this section. The details are as follows. **Phase 1 The period of isometric relaxation.** The ventricles are closed chambers at this stage; the atrio-ventricular valves have been shut by the pressure built up during systole and remain shut until opened by the influx of blood in phase 2. The semi-lunar valves shut as soon as the pressure in the great arteries exceeds that of the ventricles; this occurs at the end of ventricular systole. The contracted muscle fibres of the ventricles now relax but they cannot lengthen in readiness for the next contraction without the help of some outside agency. This phase takes its name from the tendency of the fibres to stay the same length (isometric) even though they are relaxing. As we have already seen there is some lengthening, however, due to the elastic recoil of heart tissue. During the period of isometric relaxation, which lasts for 0.08 second when the heart is beating at a rate of 75 beats per minute, both the atria and the ventricles are in the diastolic condition. Blood, impelled by venous pressure, flows into the relaxed atria throughout this phase.

Phase 2 The period of rapid filling. The pressure in the atria cannot exceed that of the venae cavae which is low, being no more than 0.7 kPa. The difference between atrial and ventricular pressure, however, becomes greater because elastic recoil will tend to increase ventricular volume, causing an automatic decrease in pressure. The ventricles therefore exert a sucking effect. As soon as the difference in pressures is such that atrial pressure exceeds ventricular, the atrio-ventricular valves are forced open and blood is able to flow rapidly into the ventricles. This phase of rapid filling lasts for 0.19 second.

Phase 3 The period of diastasis. When elastic recoil ends there is no aid to filling and it slows down or comes to an end. At 75 heart beats per minute there is a period of diastasis of 0.19 second during which a little blood enters the heart. This period becomes progressively shorter as the rate of heart beat increases during exercise.

Apart from the suction effect of the dilating ventricles, the filling of the heart in phases 1, 2, and 3 is dependent on the pressure generated by ventricular systole; the heart fills itself by the way in which its own contractions serve to force blood round the body and back to the heart again.

Phase 4 The period of atrial systole. Triggered off by the excitation wave received from the sino-atrial node, the muscle fibres of the two atria contract and exert a compression force on the blood contained within the atria. As a result of this, blood is forced into the ventricles. Atrial systole, which is of 0.1 second duration, does not appear to play a very important part in heart action. It may serve to stretch the muscle fibres of the ventricles but the main role of the atrium seems to be little more than that of a reservoir. By contracting, the atria expel their contained blood into the ventricles, thus ensuring that they are fully primed with blood. The atrial walls are very thin in comparison with those of the ventricles and they contribute nothing to blood pressure; there is no serious decline in the efficiency of circulation even when the atria are diseased and incapable of contraction. In hearts with diseased atrio-ventricular valves the atria make a positive contribution by forcing blood through the constricted apertures.

Phase 5 The period of ventricular systole. The atria conduct the excitation wave from the sino-atrial node to the atrio-ventricular node; the time delay at the latter prevents overlap of atrial and ventricular systoles. The excitation wave spreads down the Purkinje fibres adjacent to the septum and up those on the lateral walls, so that the last part of the ventricles to contract is the region nearest to the apertures of the great arteries. This arrangement allows the maximum amount of blood to be squeezed out of the ventricles as the final wave of contraction spreads upwards from the base. At the beginning of the ventricular systole the atrio-ventricular valves are open and the semi-lunar valves closed. The first increase in ventricular pressure presses the flaps of the atrio-ventricular valves upwards so that they close the openings between atria and ventricles; the valve flaps are restricted in their range of movement by the tendons attaching them to the papillary muscles. The ventricles are therefore once more completely enclosed chambers and their initial muscular effort is exerted on blood for which there is no immediate outlet. Blood, like all fluids, is relatively incompressible and so, although the muscle fibres develop tension, they cannot shorten. Blood pressure rises rapidly during isometric contraction. The moment it exceeds the pressure in the aorta and the pulmonary artery the semi-lunar valves are forced open and blood is ejected into these vessels. Once ejection has started the muscle fibres are free to contract and as they do so the ventricles pump out their contained blood.

Isometric contraction lasts for 0.04 second and ejection for 0.3 second. At the end of the ejection the muscle fibres of the ventricles relax and this event completes the cardiac cycle. Each cycle takes 0.8 second. From figure (S)36 it will be seen that ventricular systole lasts for less than half this time.

Pressure in the ventricles drops as the muscle fibres relax and quickly falls below the pressure in the aorta and pulmonary artery. When this happens blood will be forced back towards the heart but cannot enter it because it fills the pockets of the semi-lunar valves and so closes them.

Questions and answers

a ***During which of the numbered phases of the beat, 1 to 5, would you expect each of the following events to occur?***

1 Rapid filling of the ventricles.

2 Increase in tension in the ventricle muscle fibres without any change in their length.

1 Phase 2 is expected to be the period of most rapid filling of the ventricles. The atrial pressure exceeds that of the ventricles and the ventricles appear still to be undergoing a decrease in pressure, perhaps as a result of elastic recoil. The combined result of these two components will be to fill the chamber rapidly.

2 The very beginning of phase 5 would show this phenomenon. There is a dramatic rise in pressure within the ventricles and yet the aortic valves are closed. There will be no passage of blood out of the heart, so this increase in pressure is not accompanied by shortening of muscle fibres.

b ***What might account for the rise in pressure in the aorta during phase 1?***

The elasticity, not just of the aorta immediately next to the heart, but also of the major arteries round about, will result in there being considerable back pressure as a consequence of elastic recoil. This will force blood not only onwards in the system but also back against the semi-lunar valves.

c ***If the rate of heart beat is to increase during exercise, one or more parts of the cardiac cycle must take less time. Which of the five phases could be shortened with least influence on the effectiveness of the heart beat?***

The most likely phase is 3, where little appears to be occurring of any great importance. This is indeed the case and, during violent exercise when the heart may be beating at, say, 180 beats per minute, the phase of diastasis may be absent altogether. □

Electrocardiograms (ECGs) and Catheterization of the heart

These short passages are included as examples of the ways in which the activity of the heart may be monitored in a human patient. Other methods include:

taking the pulse;
determination of blood pressure by using a sphygmomanometer;
listening to heart sounds;
X-ray examination, having first injected into the heart or coronary arteries a solution of radio-opaque material.

3.6 Heart surgery and the heart-lung machine

Principles

1 Cooling the body reduces the rate of metabolic activity.
2 Most heart disease is self-induced and avoidable.
3 The finest materials and the best design will not produce a machine that can in all respects satisfactorily replace the 'natural machine' for circulating blood. Nevertheless, by understanding how the body performs such tasks, the job of the medical engineer is clarified and he is able to produce better and better substitutes.

This section does not concentrate simply upon the technology behind the use of heart–lung machines but instead tries to point out the drawbacks and problems that are inevitable when you consider the biological functions that have to be performed. There is little to be gained by the student simply knowing how open-heart surgery is performed or what the operating procedures involved are. There is much to be gained from a consideration of the uneasy marriage between engineering and physiology that tries to produce substitutes for fundamental body processes and structures. The extent of such technology needs neither to be belittled nor exalted.

3.7 The nature of blood vessels

Practical investigation. *Practical guide 1,* investigation 3F, 'Arteries, veins, and capillaries'.

Assumptions

1 An ability to interpret micrographs at an elementary level.
2 Familiarity with collagen as a strong but inelastic protein.

Principles

1 Arteries are thick-walled to withstand high blood pressure, and have plenty of muscle in their walls to assist with the control of blood distribution.
2 Muscular arteries and arterioles are the vessels that control distribution most completely, aided by pre-capillary sphincters.
3 Capillaries are the exchange points of the circulation and are well adapted to that end, having very thin, sometimes fenestrated or discontinuous walls.
4 Capillary dilatation and collapse are not caused by any active change effected by the capillaries themselves, but are the result of the flow of blood to them, as controlled by the arterioles and sphincters.

No discussion of the role of nerves in the walls of the large vessels is embarked upon. Students should be aware that any control of circulation will probably require both interoception and also the passage of motor nerve impulses to the muscles effecting the control.

Students will probably be unfamiliar with the different muscle types that are met in the body and so the term 'smooth' muscle will have no special significance. It would be useful, perhaps, to point out that the muscle found in the vessel walls is of a type that is adapted to slow, sustained contractions and does not easily fatigue.

Questions and answers

a ***What function do you think is served by the vasa vasorum?***

These vessels supply the tissues of the thick walls with oxygen and metabolites.

b ***What signs might be looked for in an elderly patient as a result of this change?***

Loss of elasticity will increase the peripheral resistance, which will in turn lead to an increased blood pressure (hypertension).

c ***In what major way does the wall of this vessel differ from that of an elastic artery such as the aorta?***

The muscular artery has the same basic structure but the elastic tissue is reduced to a rather well-defined but thin elastic sheet – the internal elastic lamina (IEL) – in the tunica intima, and more diffuse elastic tissue in the tunica adventitia. The tunica media is mainly composed of smooth muscle.

d ***How do you think that***
1 the muscles in the arteriole walls and
2 the pre-capillary sphincters
assist in controlling blood flow?

The muscles in arterioles reduce the blood flow through the vessel when they contract: this gives a general control of blood distribution and controls peripheral resistance and systemic blood pressure. Capillary sphincters control the entry of blood into the capillaries. There is far too little blood in the body to fill all the blood vessels at once.

Exchange between the lumen of the capillary and the surrounding tissues in the continuous capillary is believed to occur in three ways:

1 Passive diffusion through the cytoplasm – gases, ions, and metabolites of small molecular mass.
2 Transport by pinocytosis – proteins and some lipids.
3 Passage through the intercellular space – certain white blood cells are thought to migrate in this way by a process called diapedesis.

In fenestrated capillaries the permeability is much greater and molecular labelling techniques have demonstrated that the fenestrations permit the rapid passage of macromolecules smaller than the plasma proteins.

e ***Why do you think that valves are unnecessary in the veins of the thorax?***

The reduction in thoracic pressure that occurs with every inspiration, together with recoil of the heart, are sufficient to keep blood flowing into the atria without any back-flow.

The flow of blood through vessels

Principles

1 Resistance to flow within a blood vessel is related to the radius of the vessel and hence to its cross-sectional area. The smaller the vessel, the greater the resistance offered.
2 Blood pressure falls as work is done on overcoming peripheral resistance.
3 The volume of blood flowing through any type of vessel in the system at any one time is the same.
4 The velocity of blood flow decreases as the resistance to flow increases.

Students often find the problems of pressure, resistance, and velocity very confusing. It may help to point out that the capillaries lack any real tensile strength and hence cannot withstand any great pressure. Pre-capillary resistance to blood flow is an essential protective device.

STUDY ITEM

3.71 (Short answer question) *(J.M.B.)*

Questions and answers

a ***State how the rate of flow of the liquid in a particular section of the system could be measured.***

If some sort of marker (perhaps a small air bubble or a tiny polyphenylethene bead) were introduced, its passage could be timed, in any of the transparent tubes, against a centimetre scale.

b ***Account for the different rates of flow***
1 from A to B compared with C to D;
2 from C to D compared with E to F.

1 A to B is a tube of larger diameter than C to D. For the same volume of liquid to pass in both tubes, the flow will have to be faster in the latter tube.

2 The very narrow tubes (E to F) offer much greater wall area in contact with the liquid and hence greater resistance to flow. This will reduce the flow rate in these tubes.

c ***Suggest how the apparatus might be simply modified to determine the pressure at different positions in the circuit.***

An inverted T-shaped tube can be used as a simple manometer.

d ***Whereabouts on* figure [S]49 *would you expect the rates of flow to be equal but the pressure difference to be at its greatest?***

At the inlet and outlet of the one-way pump.

e ***Copy and complete the graph* (figure [S]50) *to show changes in pressure of blood as it passes through the vessels of a mammal.***

The graph continues successively downwards in the three remaining columns. □

3.8 The control of circulation

Assumptions

1 An elementary understanding of reflex arcs and of the existence of the autonomic nervous system. (See Chapter 11.)
2 Knowledge that motor nerves release transmitter substances onto effector organs. (See Chapter 11.)
3 Familiarity with the principle of homeostasis.

Principles

1 The flow of blood through the circulation is variable and meets the demands made by the different tissues.
2 Maintenance of correct blood pressure is vital to the survival of the individual.

3 Control of the circulation is on two levels – the control of heart beat and adjustments to the peripheral circulation.
4 The circulation is under the control of the autonomic nervous system.
5 Cardiac frequency is normally kept as low as possible by vagal tone.

The amount of detail about the nervous system is limited although it may cause problems with those who have not done O-level biology. It is, however, vital to make the point about autonomic control.

The points of principle are that all the time the cardiac frequency is kept as low as is compatible with life, rather than unnecessarily high; and that this is achieved quite involuntarily and adjusted by reflex to meet the demands of tissues. Note that the adjustments cannot be anticipatory – increase in cardiac frequency does not occur in order to permit more muscular work to be undertaken sometime in the future.

Questions and answers

a ***Use the foregoing information to explain these symptoms.***

The paleness is a result of the constriction of the arterioles supplying the cutaneous circulation and the cold skin reflects this reduced blood flow. The rapid pulse is the manifestation of the effect of adrenalin on cardiac frequency and the clamminess of the skin results from sympathetic stimulation of the sweat glands.

b ***Why do you suppose that it is dangerous to give a patient who is suffering from a haemorrhage a 'nice hot cup of tea'?***

The increased blood temperature caused by the hot tea causes dilatation of vessels constricted as a result of sympathetic stimulation; this reduces still further the blood pressure in more vital parts of the circulation and exacerbates the shock.

c ***Why is it likely that someone who is about to faint will become very pale?***

A fall in the blood pressure in the vessels supplying the brain produces the onset of fainting. Reflex compensatory constriction of cutaneous arterioles, by sympathetic stimulation, may provide extra blood for the circulation to the head and prevent fainting. Simple treatment for fainting (lowering the head and raising the feet) has a logical physiological basis.

A brief consideration of the influence of one or two drugs administered to control circulation is included in order to establish the idea that circulation is not simply under nervous control.

3.9 Transport inside plants

Practical investigation. *Practical guide 1*, investigation 3G, 'Transport inside plants'.

Assumptions

1 Sufficient knowledge of plant stem anatomy to understand the terms xylem, phloem, cambium, cortex, and parenchyma.
2 An understanding of the basic principles behind tracer work, using ^{14}C.

Principles

1 The transport of metabolites occurs in the sieve tube elements of the phloem and may be in both upward and downward direction in the plant.
2 Phloem translocation is too rapid to be accounted for by diffusion.
3 Sieve tube elements are living.
4 Metabolic processes are associated with translocation in the phloem.
5 The examination of sieve tube element structure does not solve the problem of the mechanism of translocation since it is not clear how reliable a representation of living tissue is given by micrographs of dead material.

This topic provides a fitting contrast with the apparent certainty with which circulation in mammals is approached. Students should be encouraged to realize that the uncertainty does not devalue the work of research in this field nor mean that the topic should not be included in our studies here. The questions at the end are not intended to support any particular view on translocation but rather to enable students to see for themselves that the available data can support more than one hypothesis.

Although reference is made in the *Study guide* to the analysis of phloem exudate from severed aphid stylets, the data in table (S)9 are from exudate obtained from cuts made into the bark of *Ricinus*. Such a detailed analysis has not yet been performed on the exudate from aphid stylets.

Questions and answers

a ***Explain how the concentrations of these sugars support a mass flow hypothesis of translocation in the phloem.***

During the summer there will be synthesis in the leaves and transport to, and perhaps storage in, the roots. After leaf fall and the cessation of synthesis, a higher concentration of sugars is expected in the roots than in the shoots. So these data agree with the prediction that, if mass flow mechanisms operate, they require a source–sink concentration gradient. Here is one.

b ***Show how these data could be used to support both the simple mass flow hypothesis and one which suggested complex metabolic intervention, to account for the translocation of the 2,4-D.***

Photosynthesis produces sugars and this will cause a concentration gradient such that mass flow away from the leaf follows. The 2,4-D is carried passively by the mass flow. Alternatively, the synthesized sugars are used as a respiratory substrate for an active process that transports 2,4-D out of the leaf independently of any mass flow which may or may not be occurring.

c ***What was the point of the treatment in which sucrose was added with the 2,4-D and then the plants were left in the dark?***

It adds weight to the idea that it is somehow the sugars, manufactured during photosynthesis, that are responsible for the movement, although it does not discriminate between the two hypotheses.

PART II The *Practical guide*

The investigations related to this chapter appear in *Practical guide 1, Gas exchange and transport in plants and animals.*

ITEMS NEEDED

Elodea leaves, young, maintained in warm (20 °C), bright conditions
Tradescantia stamens

Pond water
Water

Forceps, fine 1/1
Microscope 1/1
Microscope slides and coverslips
Pipettes 1/1
Stopclock or wristwatch with seconds indicator 1/1

INVESTIGATION
3A Movement inside plant cells

(*Study guide* 3.1 'Movement inside plants and animals'.)

Principle

1 Leaf cells of *Elodea*, or staminal hair cells of *Tradescantia* show protoplasmic streaming.

This investigation provides a useful starting point for a discussion of the need for movement of materials inside plants.

Cyclosis is often slow, especially in the winter, and it may be necessary to observe the specimen under illumination for as long as twenty minutes. It must be ensured that the leaf used is young and has been kept warm (around 20 °C) and illuminated before use. It must also be realized that not all the cells in a leaf will readily show the phenomenon. Students should not be allowed to expect immediate and rapid movements! It is tempting to ascribe too great an importance to cyclosis; it should be pointed out that in a leaf such as that of *Elodea*, diffusion alone will account for a considerable amount of all required movement of materials.

Questions and answers

a **_Explain how chloroplasts in a cell of_ Elodea _can appear to move from one cell to another._**

Although the leaf is thin there are usually two layers of cells in the mid-line, and all the cells have a depth which is greater than the focal depth of the microscope. The chloroplasts move in all three planes and may pass into or out of focus while progressing round a cell. Often a chloroplast at the edge of one cell moves out of focus, as one at the edge of the adjacent cell moves into focus, thus giving the impression of movement from cell to cell.

b **_What do your measurements of the rate of chloroplast movement indicate about the cause of that movement?_**

The rate increases with time and this is attributable to the heating effect of the microscope lamp. (A change in light intensity alone can also cause movements although these are very slow.)

Rates may reach 5 mm per minute or more.

Time taken to move set distance (s)
23.2
20.5
17.4
14.2
12.1
13.1
14.0

Table 4
The rate of movement of chloroplast in a leaf cell of *Elodea*. (The leaf cell was illuminated and the time taken for a chloroplast to move between two fixed points adjacent to the cell was noted. Readings were repeated at intervals of one minute; the light was left on.)

c **_From your observations can you suggest a likely mechanism that could be the cause of chloroplast movement?_**

The observations alone suggest little about the mechanism, save that it is temperature-dependent and so may be chemical, involving ATP, or purely physical, resulting from random molecular movement. Since the effect is to produce a clear *flow* of cytoplasm and to move comparatively large structures round the cell, it is unlikely that the cause is thermal agitation alone. It has been suggested that the continual breakage and reformation of weak cross-links between the protein molecules in the cytoplasm are the underlying cause.

d **_What functions could be performed by the movements of chloroplasts?_**

The movements could distribute the products of photosynthesis all round the cell or aid the uptake by chloroplasts of substrates whose supply is only local. It is suggested that the mobility of chloroplasts promotes more efficient use of incident light.

e **_In what ways is movement within staminal hairs of_ Tradescantia _the same as or different from the movement of chloroplasts in_ Elodea?**

In *Elodea* the cytoplasm is not easily seen. This, together with the fact that adjacent chloroplasts move at different rates, gives the impression that chloroplast movement is independent of the cytoplasm. In *Tradescantia* the movement of the cytoplasm can be easily seen around the cell wall, round the nucleus, and in fine strands between the two. In this cytoplasm small particles may be seen moving in all directions. This movement is not Brownian movement, since definite flow occurs, but stationary particles can be seen to vibrate and that may be random molecular agitation.

INVESTIGATION
3B Circulation in animals

ITEMS NEEDED

Either
Asellus 3/1

Plasticine
Water

Hand lens 1/1
Microscope 1/1
Microscope slides and coverslips
Pipettes
Test-tube or specimen tube 1/1

Or
Xenopus tadpole, or guppy 1/1

MS-222 in pond water

Cottonwool
Microscope 1/1
Watch-glass 1/1

(*Study guide* 3.2 'Circulation in animals'.)

MS-222 in pond water
Ethyl 3-amino benzoate (Tricaine; methane sulphonate), 0.05 g
Pond water, 100 cm^3
Dissolve the MS-222 in the water and use this solution to immobilize the animal. (*TAKE CARE* in preparing and storing the solution.)

Principles

1 Circulation of a fluid such as blood permits the distribution of materials dissolved within it.

2 Counter-current flow may increase the exchange occurring between two flowing media.

3 Flow of a fluid may be achieved with a pump, one-way valves, an incompressible medium, and vessels within which the medium travels.

The investigation is divided into two parts. The first is extremely easy and good observations should be reported within a short time of capturing and mounting either the small crustacean or the fish or tadpole. The second part, involving the displacement of the abdominal plates (pleopods) is a little more difficult but is well within the capabilities of most students. The blood (haemolymph) of Crustacea is a colourless fluid consisting of plasma and amoeboid leucocytes. The plasma contains haemocyanin, a copper-containing compound which is bluish when oxygenated and colourless when deoxygenated.

Students may find it difficult to displace one of the pleopods without crushing the animal. It is better to try other specimens than to persevere to the point of damage with one animal. In fact it may not be necessary to displace the pleopod at all in some specimens and it may be visible as a separate, fluttering structure, dorsal to the last walking leg and ventral to the uropods. If the moving blood stops, this is a sure sign that too much pressure has been applied and the animal is unlikely to recover.

Questions and answers

a ***Were particles moving in both directions along the limb?***

Yes; there are usually at least two streams moving in opposite directions.

b ***If so, do they follow the same path in both directions?***

Sometimes there are two streams flowing into a limb and one back into the body or *vice versa* (*figure 9*).

c ***Do particles move with a constant velocity?***

No; the movement is irregular or pulsatile.

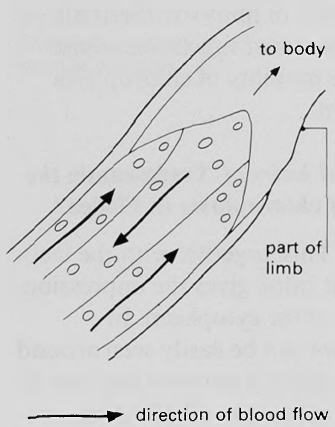


Figure 9
Blood flow in a limb of *Asellus*.

d ***Can you see blood vessels in any of the limbs?***

No; in places the appendages seem to be full of moving fluid (blood) and there must be partitions of some sort, allowing a two-way flow. The partitions themselves are invisible. The animal contains blood spaces known collectively as the haemocoel in which most of the blood (haemolymph) is found. There are also short, distributing arteries from the heart to major organs, which contain valves. These vessels cannot be seen.

e ***Suggest a possible cause of the colourless appearance of the structure in contrast to the other parts of the body.***

The cuticle covering the pleopod might be much thinner or less heavily sclerotized than the rest of the body covering.

f ***Can any moving particles be seen inside this structure? If so, describe their paths and velocity.***

Particles (cells) are visible, moving in the pleopods. The direction of movement is generally from the posterior to the anterior end of the animal.

g ***Which way are particles moving near the edge of the structure and what may be the significance of this?***

Blood moves in one direction while water is continually propelled past the pleopods in the opposite direction. This suggests that a counter-flow system is present, which would maintain a steep diffusion gradient for solutes between the water and the haemolymph in the pleopods.

h ***Suggest a possible function for the whole structure.***

From observing the *Asellus* in a test-tube it may be noticed that water is moved backwards (in a posterior direction) over the posterior parts of the body. The pleopods are seen to 'flutter'. They have a large surface area and, probably, thin walls. This, together with the counter-flow of water and blood mentioned in question **g** would indicate that the pleopods could be responsible for the uptake of oxygen.
Caution must be exercised. A 'thick' cuticle will not necessarily reduce gas exchange significantly and it is more likely that these organisms use their entire body surface for gas exchange, with the extra abdominal appendages both increasing the surface area of the animal and helping to maintain water flow over it.

INVESTIGATION
3C The effect of temperature and chemicals on the rate of heart beat of *Daphnia*

ITEMS NEEDED

Daphnia, healthy culture 1/class

Acetylcholine solution (1 part in 1000 parts pond water)
Adrenaline solution (1 part in 1000 parts pond water)
Aspirin solution in pond water
Ethanol, 1 % solution and 10 % solution, in pond water
Ice
Pond water or suitable substitute, preferably filtered

Beaker, 10 cm^3 or 25 cm^3 1/group
Cottonwool
Microscope 1/group
Mounted needles 2/group
Petri dish, small 1/group
Pipettes 2/group
Stopwatch 1/group
Syringe, 10 cm^3 1/group
Thermometer 1/group
Water baths or means of maintaining pond water at constant temperature 3 or 4/class

(*Study guide* 3.3 'Different types of circulation in animals'.)

Assumption

1 Awareness that many factors affect the rate of metabolism; they include external temperature, hormones, age, surface area to volume ratio, and muscular activity.

Principles

1 By changing external variables in a controlled way it is possible to illustrate their effect on the metabolic rate and hence on the cardiac frequency of a poikilotherm such as *Daphnia*.

2 This investigation may also be used to introduce the idea of temperature coefficient (Q_{10}).

3 Individual organisms of one species may vary markedly in their response to identical treatments, necessitating statistical comparison of results.

Ideally students should see a heart within a living creature pumping blood round the body. This may be done to a limited extent with crustaceans and other semi-transparent creatures. There is little doubt that experiments on a living frog heart have considerable impact on students, but there is a growing reaction against the use of frogs for this purpose. This investigation, using *Daphnia*, may be preferred by some teachers and students.

If it is wished, instead of heating water in a water bath, the animal can be surrounded by a circular heating coil in the dissecting dish, connected to a 6-V battery. This will gradually heat the water in the dish and the cardiac frequency may be estimated at 5° or 10 °C intervals. An additional, larger Petri dish outside the small one could also be filled with water at the appropriate temperature to help to reduce heat loss from the experimental chamber. A 'dummy run' without *Daphnia*, using a thermometer with its bulb immersed in the cottonwool could be included to determine the extent of any temperature fluctuations. Time is saved if, before the lesson begins, water baths containing filtered pond water at the required temperatures are set up, so that students can obtain water from them as and when it is needed.

There will be considerable variation in the data gathered (see table 5). Records of the rate of heart beat at any temperature should be collected and the mean and standard deviation of the class results recorded (see table 6).

	Temperature (°C)			
Daphnia	0	10	20	30
A	75	82	92	178
B	71	85	96	180
C	65	93	93	190
D	60	128	155	260
E	54	151	150	278
F	56	142	168	272
G	47	150	140	328
H	48	—	153	308
I	39	—	234	318

Table 5
The rate of heart beat of individual *Daphnia* in pond water at different temperatures. (The heart rate is given as beats per minute.)

Treatment	*Heart beat mean rate* (minute^{-1})	*Standard deviation*	*Sample size*
Surrounding water 0 °C	181.57	52.89	7
Surrounding water 10 °C	194.75	45.94	9
Surrounding water 20 °C	249.00	18.81	4
Surrounding water 30 °C	296.85	35.45	7
Surrounding water 40 °C	350.40	18.32	5
Adrenaline in pond water at 20 °C (step 11)	311.67	69.81	3
Acetylcholine in pond water at 20 °C (step 11)	243.67	37.75	3
1 % ethanol in pond water at 20 °C (step 9)	218.00	68.40	4
10 % ethanol in pond water at 20 °C (step 10)	116.60	78.99	5

Table 6
Class results of investigations into the effect of different treatments on the heart beat of *Daphnia.*

The heart must be observed with transmitted light if it is to be properly visible.

Students may be interested to try the effects of other chemicals such as thyroxine on *Daphnia*, although this raises the problem of how we might expect hormones found in vertebrate animals to act in invertebrates.

Questions and answers

a ***What information does the graph give you about the effect of temperature on the rate of heart beat of* Daphnia?**

The graph should show an increase in cardiac frequency from around 5 °C, and the rate of increase may steepen. At around 40° to 50 °C there will be a sudden fall in the rate. The graph illustrates the lack of an adequate homeostatic mechanism of temperature control in this animal, a feature to be expected in any poikilotherm.

b ***Determine the Q_{10} from your own results. Is the Q_{10} consistent for the complete range of temperatures used? If not, why not?***

Within a range of about 10 °C above and below its 'normal' environmental temperatures, the rate of a metabolic process is expected to double for every 10 °C rise in temperature. *Daphnia* heart rate has a more complex relation to temperature than does a single enzyme-controlled reaction; thus it may not be expected to show a $Q_{10} = 2$. Many other factors apart from temperature may be influencing the heart's action. At higher temperatures (40° to 50 °C) the relation between the two rates will not hold because of the deleterious effect of the extreme temperature.

c *If these same investigations were carried out on a human subject, in what respects would you expect the results to differ from those obtained with* Daphnia?

Humans are homoiothermic and their cardiac frequency should not reflect the environmental temperature changes, since they possess an adequate homeostatic mechanism.

d *Describe the effect on the cardiac frequency of* Daphnia *of any of the chemicals you used in step 11. Can you put forward hypotheses to explain any of these effects?*

Some sample data are given in table 6. Hypotheses may relate to what the students already know about the action of such drugs and hormones on vertebrates, or they may relate to toxicity. Care should be taken to point out the difficulties of using vertebrate hormones on invertebrates.

INVESTIGATION
3D The vertebrate heart in action

ITEM NEEDED

Ruler, mm 1/1

(*Study guide.* Study item 3.41 (short answer question); section 3.5 'The mammalian heart in action'.)

Assumptions

1 The surgical procedures referred to are assumed to have no effect upon the normal rate, rhythm, or contracting sequence of the frog heart.

2 The various treatments that are employed here are assumed to have the same effect on the heart beat of a pithed frog as they would have in a live animal.

Principles

1 The heart muscle is myogenic and does not require external stimuli in order to contract.

2 The beating of the heart chambers is sequential and is co-ordinated by the heart itself.

3 Nervous and hormonal stimuli are able to alter the rate and rhythm of the heart beat, but have little or no effect on its co-ordination.

At the time at which this *Practical guide* was written there was considerable discussion about the desirability of carrying out in schools experiments which involved pithed animals. It was felt that, whatever the outcome of these discussions, it was unnecessary and undesirable for this investigation to be performed in school. Experiments using pithed frogs have, however, been a mainstay of experimental physiology for many years, and many features of the control of heart beat can very readily be demonstrated in this way. This investigation provides actual traces produced from such investigations in a school and gives students an opportunity to analyse the resulting figures and to draw appropriate conclusions. The experimental details of the preparation of the frog are not given in the *Practical guide.* They are reproduced here so that teachers may the more easily answer questions about the techniques, should they arise, and so, perhaps, make the work a little more immediate to interested students.

Pithing a frog

Before pithing a frog, the animal is stunned by holding it by the legs, ventral side up, and bringing the head down sharply against the edge of a bench. The stunned frog is held firmly in a cloth, dorsal side up, and the head is bent downwards. The point of a blunt seeker is slid back along the mid-line of the head until it slips into the depression of the foramen magnum. The point of the seeker is pressed firmly into this depression and forwards into the brain. It is pulled back with upward pressure to destroy the brain but is not removed completely. It is turned round through 180° and is pressed back into the neural canal with an upward pressure as far as it will go. The frog is tested for reflexes by pinching each foot. If this fails to evoke a response, pithing has been successful. It could be pointed out to students who may be concerned about the frog suffering that an animal without a central nervous system cannot feel pain. The appearance of a pithed frog is perfectly acceptable to students.

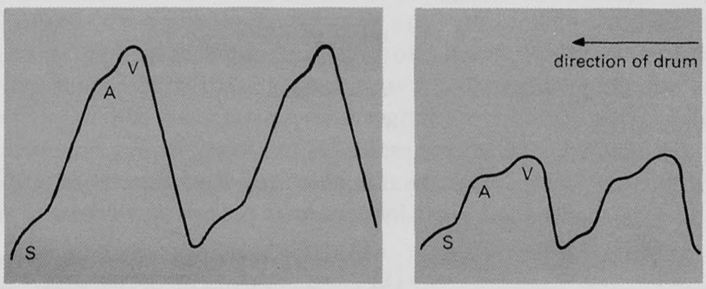
Questions and answers

The effect of temperature on heart beat (1)

a ***Examine traces 1 and 3. Explain the shape of the traces, taking into account your knowledge of the structure of the frog's heart.***

The traces show quite clearly that there are three contractions:
the sinus venosus
the atria
the ventricle (see *figure 10*).

Figure 10
Examples of recordings of a frog's heart beat.



Much depends on the method of attachment of the heart to the lever. Here, contraction causes an *upward* movement of the heart lever. Also, the speed of the kymograph drum obviously affects the shape of the trace.

S = portion related to the contraction of sinus venosus
A = portion related to the contraction of atria
V = portion related to the contraction of ventricle

The ventricular contraction produces the greatest movement of the pen, which is to be expected since the ventricle is the largest (most muscular) chamber. (The absence of these features from many of the traces in the *Practical guide* is a result of poor adjustment of the mechanical systems used in recording the beat.)

b ***Calculate the cardiac frequency: that is, the number of beats every minute for the heart, at each of the three temperatures. What is the effect of changing temperature on the cardiac frequency?***

The cardiac frequencies are:

Temperature (°C)	Frequency (beats s^{-1})
10	0.32
20	0.68
34	1.05

The effect of increasing the temperature of the heart is to increase the cardiac frequency. The effect of decreasing the temperature is to decrease the cardiac frequency.

c ***Are there any other differences or similarities between the traces at these three temperatures?***

Assuming that the mechanical system was unchanged between recordings it would appear that the reduced temperature resulted in a loss of definition between the phases of contraction and that an increase in temperature accentuated them. There is no significant change in amplitude with changing temperature alone.

The effect of temperature on heart beat (2)

d ***Calculate the cardiac frequency for each of the four treatments by measuring the mean distance between the major troughs on the trace, in mm. What is the cardiac frequency in traces 1 to 4?***

The frequency of heart beat in the four treatments is:

	Rate of heart beat (s^{-1})
before treatment	0.55
ventricle warmed	0.55
atria warmed	0.54
sinus venosus warmed	0.69

e ***In what way does this investigation explain how the change in heart beat rate with increasing temperature is brought about?***

The warming of the atria and ventricle has no significant effect on the rate of beating of the heart. Warming the sinus venosus produces an increase in rate. If the mean rate of beating in traces 1 to 3 is calculated it is found to be $0.55\,s^{-1}$. The increase in rate produced by warming the sinus venosus (an increase of $0.14\,s^{-1}$) is equivalent to an increase of slightly more than 25 %. This suggests that the increased temperature, however it is caused, affects heart rate by acting primarily on the sinus venosus.

The co-ordination of the heart beat

Figure 11 shows the position of the Stannius ligatures. The disruption of the co-ordination of the heart beat is a result of physical crushing of the conducting bundles within the heart muscle, so that the waves of depolarization that emanate from the nodes cannot act upon the next 'pacemaker'.

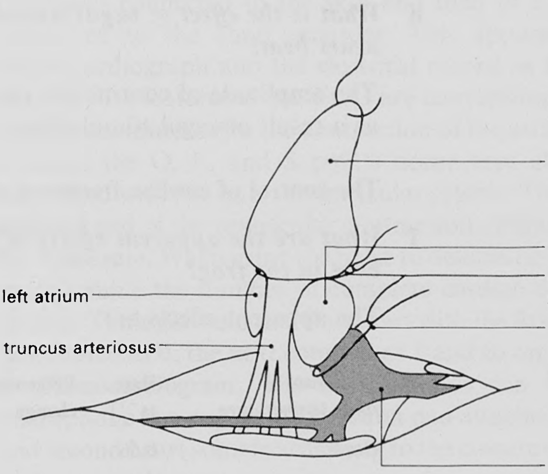


Figure 11
A diagram to show the position of the first Stannius ligature.

f ***Suggest a model for the control of heart beat that is consistent with the results of Stannius ligature experiments as reported here.***

The results of these experiments suggest that the intrinsic rhythm of the whole heart originates in the sinus venosus, which acts as a pacemaker for the entire heart. They suggest that the impulse spreads from the sinus venosus to the atria and ventricle in turn. They also suggest that in the absence of controlling impulses from the sinus venosus, the atria themselves initiate a co-ordinating impulse which can spread to the ventricle. Although the experiments do not allow the myogenic properties of individual muscle 'cells' to be deduced, they certainly permit the conclusion that extrinsic initiation of contraction is not necessary for any of the chambers of the heart.

The control of cardiac frequency (1)

The uneven nature of the traces in figure (P)32 occurs because the Ringer's solution in which the whole preparation is bathed conducts the stimulus to all parts of the preparation. The voltage at which these records were taken was sufficient to cause general muscular stimulation in the thorax of the frog.

g *What is the effect of vagal stimulation on cardiac frequency?*

Vagal stimulation reduces cardiac frequency.
Pre-stimulus frequency: $0.64\,s^{-1}$
Post-stimulus frequency: $0.45\,s^{-1}$
This represents a 30 % decrease in the rate of contraction.

h *What is the effect of vagal stimulation on the amplitude of the heart beat?*

The amplitude of contraction appears to show no significant change as a result of vagal stimulation.

The control of cardiac frequency (2)

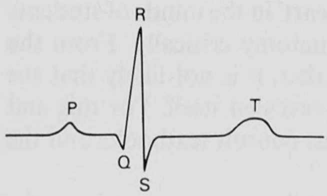
i *What are the apparent effects of these two chemicals on the heart beat in the frog?*

The apparent effects are:

Treatment at room temperature	**Rate** (s^{-1})	**Percentage change**
Control	0.66	–
$+\frac{1}{1000}$ adrenaline	0.96	+45
$+\frac{1}{1000}$ acetylcholine	0.53	−20

j ***It is thought that the vagus nerve releases acetylcholine when it is stimulated. Another nerve, the so-called 'accelerator' nerve, releases noradrenaline (a chemical very similar to adrenaline). Both of these nerves enter the heart at a spot known as the pacemaker. In the frog, this pacemaker is within the muscular wall of the sinus venosus. The vagus nerve originates in the brain of the animal. Can you produce a model of the control of heart beat in a vertebrate such as the frog, using all the information now at your disposal?***

It should be possible to suggest that control is intrinsic and that alteration in rate is a result of stimulation of one or other nerve that acts on the pacemaker in the sinus venosus, via a chemical transmitter, to speed or slow the heart. It is quite likely that students will suggest a role for adrenaline related to their own experience of the effect of adrenaline on their heart rate. The problems of relating such a model to what is occurring in the frog's heart could be pointed out.



The P wave corresponds to atrial systole.
The QRST complex corresponds to ventricular systole.

Figure 12
The normal electrocardiogram.

Additional or alternative procedures

The following procedures may be used to investigate something of the action of the human heart.

1 **Electrocardiogram.** The electrical currents set up when the heart muscle contracts can be measured easily on a human subject, using electrodes connected to the skin and then to a battery-run amplifier connected to the chart recorder. This apparatus is known as an electrocardiograph and the electrical record as the electrocardiogram (ECG). Five features of the ECG are conventionally described – the P wave is associated with the contraction of the atrial muscle fibres (atrial systole); the Q, R, and S points occur very close together and are associated with the start of ventricular systole. The T wave is associated with the end of the ventricular contraction. (*Figure 12.*)

2 **Pulse rate.** When using the pulse to determine heart rate, the object is to determine the number of complete cardiac cycles there are in one minute. Timing should therefore start with the first pulse and this should be counted as 0; the next counted as 1 and so on.

3 **Phonocardiogram.** The heart sounds can be recorded, using a microphone in a cup-shaped suction pad attached to the chest. The first and second heart sounds are owing to the closure of the atrio-ventricular and aortic valves respectively.

INVESTIGATION
3E The structure of hearts

(*Study guide* 3.4 'Different sorts of heart' ~~.)~~ and 3.5 'The mammalian heart in action'.)

ITEMS NEEDED

Cardiac muscle, prepared slide, L.S. 1/2
Heart, fresh, sheep 1/group
Heart, preserved, of locust and rat or mouse (as preserved from work of investigations 1B and 1E) 1/class
Dissecting board 1/group
Microscope 1/2
Microscope, stereo 1/2
Petri dishes 3/1
Scalpel 1/group
Scissors 1/group
Specimen tube 2/class
Vernier calipers 1/group

Assumptions

1 Elementary knowledge of the appearance of tissues such as muscle, connective tissue, and blood vessels under the microscope, or access to atlases of histology.
2 Familiarity with the location and function of the four chambers of the mammalian heart.

Principles

1 The structure of the different hearts is closely allied to the way in which they function and the type of circulation which they support.
2 Common features of all the hearts are the possession of musculature and valves which enable them to produce a directional flow by a simple contraction.
3 The separation of the two 'halves' of the mammal heart reflects the 'double' nature of the mammal circulation.
4 The copious blood supply to the mammal heart indicates the massive work load sustained and the need for an adequate supply of metabolites and oxygen.

From biology lessons, first aid classes, or general reading, all the members of the class will probably think that they know how a heart is constructed. A real mammal heart, as opposed to a textbook diagram of one, is a most complicated, three-dimensional structure. The aim of this investigation is to widen the definition of 'heart' in the minds of students and encourage them to approach heart anatomy critically. From the brief examination of the mice dissected earlier, it is not likely that the concept of a double circulation will have revealed itself. For this and other details of blood systems students must consult textbooks and the *Study guide.*

The class should organize itself so that while some are examining the preserved hearts, others are studying the histology of cardiac muscle.

When purchasing hearts from slaughterhouse or butcher, try to obtain at least one heart that has a reasonable length of aorta and vena cava attached. 'Cleaned' hearts are often little more than ventricular stumps.

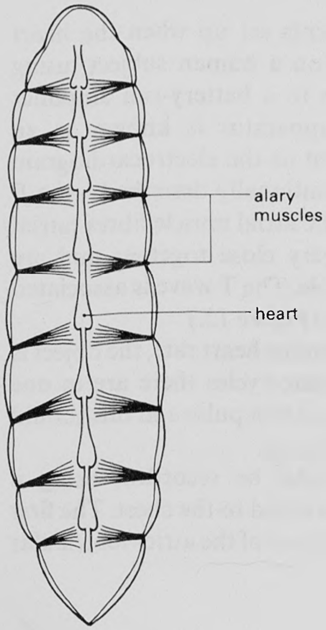


Figure 13
The inner surface of a dorsal strip of exoskeleton of *Locusta.*

Questions and answers

a ***Describe the heart of a locust as far as possible, from the preserved tissue at your disposal.***

The 'heart' is a long tube lying dorsally along the whole length of the thorax and abdomen. It is closely attached to the ventral surface of the dorsal cuticle. It is pale in colour. Slight bulges corresponding with the segments of the body may be seen – these are the chambers of the heart, and there are 13 of them. The entrance to each chamber is controlled by valves. When the heart muscle relaxes blood is sucked in through these openings. When the heart contracts the valves close and the blood is forced to move forward. (See *figures 13* to *15*.)

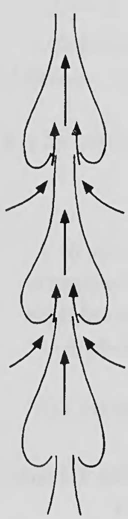


Figure 14
A diagram of part of the heart of *Locusta*, showing valves.

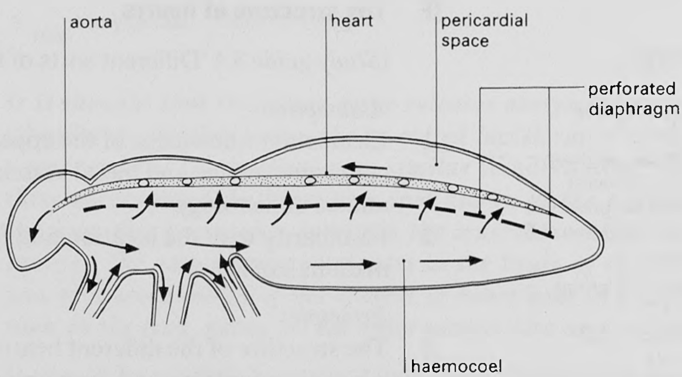


Figure 15
The circulation of blood in *Locusta.*
Figures 13–15 are based on Clarke, W. M. and Richards, M. M. The locust as a typical insect, *John Murray, 1976.*

b ***Which hearts exhibit signs of possessing their own circulatory system?***

Hearts of mammals have a network of vessels visible over the outside surface. These are the coronary vessels and are conspicuous in large hearts.

c ***How does the total volume of the atria compare with the total volume of the ventricular space in any one heart?***

The total volume of the atria should equal that of the ventricles; in a living heart this could hardly be otherwise, blood being an incompressible fluid. In the dead sheep's heart the atria may appear to be of much smaller volume; this is entirely due to the fact that these thinner-walled chambers collapse so much when not stretched.

d ***Is there any anatomical evidence that the ventricles could produce a higher blood pressure than the atria?***

Differences in the thickness of ventricular and atrial walls. If a heart wall is largely made of muscle, thick tissue can presumably exert more force when it contracts than thin tissue. If this is so then more force will be exerted by the ventricles than by the atria, causing a higher blood pressure.

e ***How are the valves prevented from blowing back into the atria when the ventricles contract?***

The valves between the atria and ventricles are anchored by tendons – the chordae tendinae – to papillary muscles on the inner surface of the ventricles. These valves prevent blood from flowing back into the atria during ventricular systole. This should be apparent in step 5. The muscles associated with the bicuspid valve are larger than those holding the tricuspid valve and this may be related to the greater force exerted by the thicker muscle wall of the left ventricle. The fact that the tendons are attached to muscles at all is related to the fact that the space in the centre of the valve changes in area as the heart contracts and a changing length of tendon is needed during this time to maintain effective closure of the valve.

f ***Does your examination of the interior of the heart suggest that the pressure produced by the two ventricles is equal or unequal? Give your reasons.***

See answer f. The wall of the right ventricle is only one-third as thick as that of the left ventricle. Pressure in the pulmonary circulation of mammals is much less than in the systemic circulation.

g ***What is the function of the semilunar valves?***

These valves prevent the return of blood to the ventricles on completion of systole.

h *As far as you are able, relate the structure of cardiac muscle to the functions which it has to perform.*

The interconnected 'cells' suggest that a degree of co-ordination of contraction should be possible. This is clearly essential if the heart is to function at all. The prolific vascular supply within the muscle underlines the need of this tissue for rapid and copious supplies of metabolites and of oxygen, and for the removal of wastes. The individual muscle 'cells' are very small and the intercalated discs at the junctions between adjacent 'cells' permit a rapid spread of excitation.

INVESTIGATION
3F Arteries, veins, and capillaries

ITEMS NEEDED

Artery and vein, prepared slide, T.S.; mounted on the same slide 1/2
Either
Cardiac muscle, prepared slide, T.S.* 1/2
Or
Lung, prepared slide, T.S.* 1/2
Or
Thyroid gland, prepared slide, T.S.* 1/2
And
Heart vessel sections, fresh or frozen from previous investigation

Cotton
Eyepiece graticule 1/1
Hand lens 1/1
Hooks 2/1
Masses, 10 g
Micrometer, stage 1/class
Microscope 1/1
Retort stand 1/1
Rule, metre 1/1

* To show capillaries in T.S.

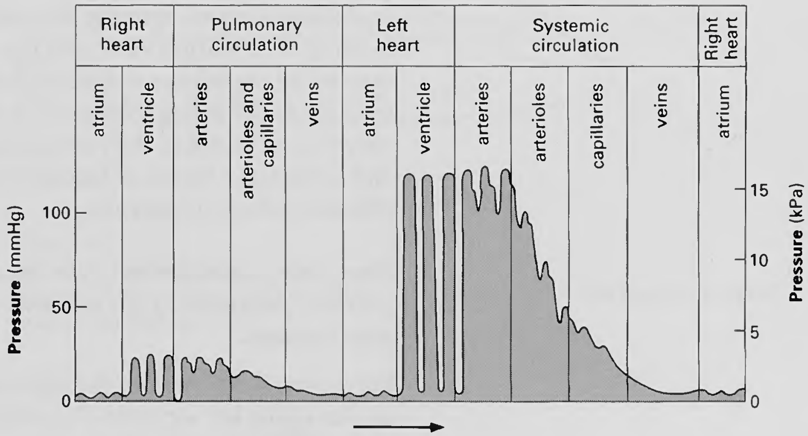
(*Study guide* 3.7 'The nature of blood vessels'.)

Assumptions

1 Ability to identify tissues within the microscopical sections (perhaps with the assistance of an atlas of histology).
2 Ability to use an eyepiece graticule and stage micrometer.
3 Knowledge that the flow of blood in blood vessels is continuous and not pulsating; this implies a 'smoothing' of the pulsed cardiac output.

Principles

1 The structure of blood vessel walls is closely related to the functions which they perform.
2 Capillaries are the sites of exchange between blood and other tissues, and structural features of capillary networks contribute to this function.
3 Blood circulating round the body travels at different velocities in different places, and under a gradient of pressure (see *figure 16*).



Pressure fluctuations produced by three heart beats are shown in each section. Note the fall in pressure as blood traverses the arterioles of the systemic circulation.

Figure 16
The pressure in different parts of the mammalian circulation.
Based on Marshall, P. T. and Hughes, G. M., Physiology of mammals and other vertebrates, *2nd edn, Cambridge University Press, 1980.*

This investigation is limited to one simple experiment and to examination of only three types of blood vessel and is therefore not comprehensive. There is scope for increasing the detail and types of vessel studied, but histological work can demand considerable experience which students will be unlikely to possess and distinctions between other types of blood vessel are largely artificial. No reference is made to valves in veins or to the presence and possible function of so-called 'pericytes' in the capillary systems.

Students should be encouraged to use atlases of histology only to enable them to identify tissues and not as a substitute for their own observations.

Questions and answers

a ***How do the artery and vein compare, with regard to***

1 the percentage increase in length on loading

2 the ability to return to the original length on unloading?

The artery is expected to show a greater percentage increase in length on loading and a greater ability to return to the original length on unloading.

b ***How might the differences noted in your answer to question a, and the distribution of the different tissues noted in step 4, relate to the function of these two types of vessel in the body?***

The extensibility of the artery correlates well with the greater proportion of elastic fibres in its walls, while the inextensibility of the vena cava correlates with its high proportion of collagen. Both of these properties must be of use in the body. Students may suggest that the artery may be stretched by the outflow of blood at ventricular systole and that its walls recoil during diastole. It is hoped that they may realize that this will smooth the blood flow. A simple model (*figure 17*) may be useful to help to convey this concept.

It may be realized also that the 'pulse wave' is not going to travel into the venous system and thus there is potential stagnation within veins; hence it is vital, especially if the valves are to operate, that these vessels do not simply expand as blood moves into them or there would be no effective return of blood to the heart. It is important to stress that the fact of circulation does not demand the presence of muscle in the vessel walls. Its function relates chiefly to alterations in the distribution of blood within the system and local adjustments to blood pressure necessitated by the body's changing activities.

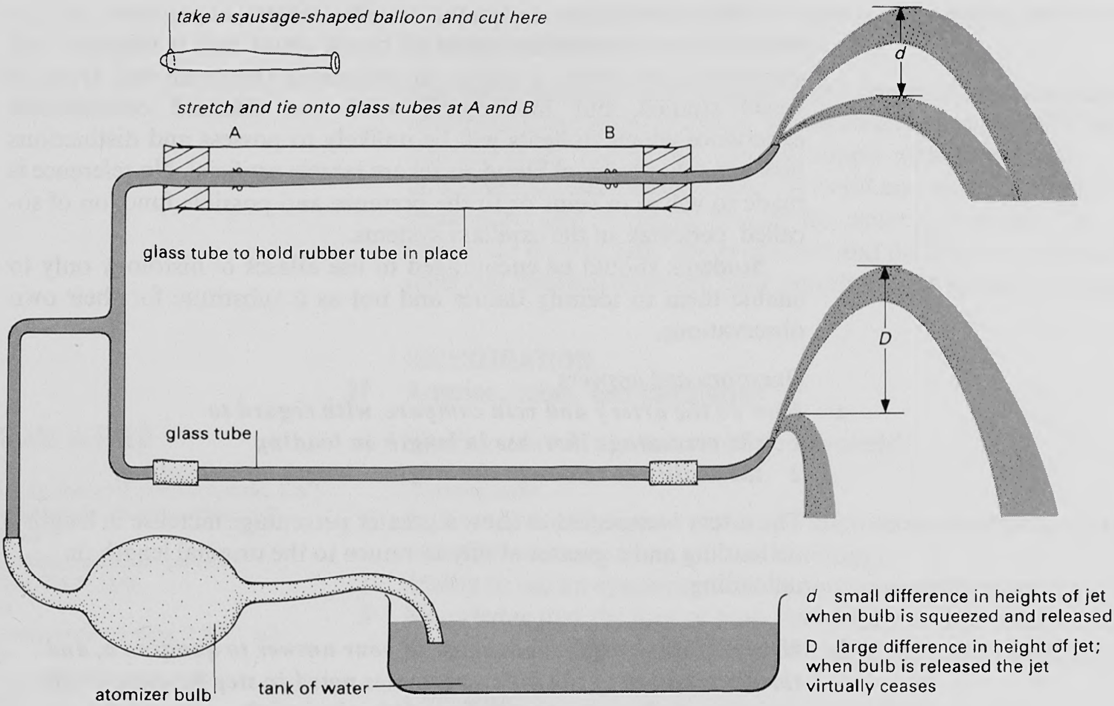


Figure 17
A model to show the different effects of elastic and rigid tubes.

c ***How do the living cells in the walls of an artery or vein obtain oxygen?***

There are small blood vessels (the vasa vasorum) in the walls of arteries and veins.

d ***What is the significance of the differences noted between the artery and vein and the capillary?***

The wall of the capillary consists of a single layer of cells, by contrast with the three layers of cells of both the artery and vein walls. This single layer of endothelial cells may be less than 0.1 μm thick and it thus facilitates the rapid exchange of materials between blood and other tissues. Capillary networks present a large surface area for this exchange.

e ***How does the diameter of a capillary compare with the diameter of a red blood cell? What is the significance of this comparison?***

The diameter of a capillary is very similar to that of one red blood cell (8–10 μm). By virtue of this fact, each capillary has a large wall area in relation to the volume of blood it contains, increasing the potential for exchange.

INVESTIGATION
3G Transport inside plants

ITEMS NEEDED

Impatiens sp., healthy, vigorously growing plants, to provide 2 shoots per student

Methylene blue, aqueous, about 0.005 %
Water

Beaker, 100 or 250 cm^3 1/1
Razor blade 1/1
Scalpel 1/1
Test-tubes 2/1
Test-tube rack 1/1

(*Study guide* 3.9 'Transport inside plants'.)

Principles

1 Growth in any region of a plant requires substantial supplies of metabolites. These are transported chiefly in the phloem.

2 Excess sugars are exported from organs of supply (such as mature leaves) and will be moved to regions of demand, either upwards or downwards in the plant.

The ringing of the *Impatiens* cutting could be performed three weeks in advance of this practical session.

Questions and answers

a ***Describe the distribution of ^{14}C in the control shoot after 24 hours as shown in the autoradiograph. How does the distribution of ^{14}C differ in the two shoots?***

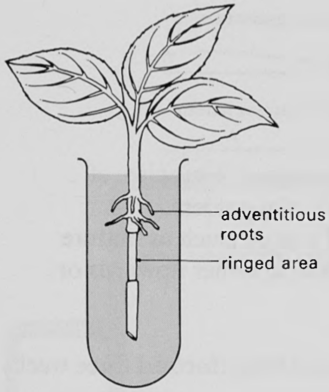
In the control shoot (A), ^{14}C is present in the fed leaf, the entire stem, and a few leaves near the apex (more being seen in leaves on the same side of the stem as the fed leaf). The fed leaf must have been an exporting leaf under these circumstances. There is a marked difference between the two shoots. In the experimental shoot (B), there is abundant ^{14}C as far as the ringed portion of the stem; beyond this there is only a faint trace in the stem and none at the apex.

b ***Examine the micrograph of a transverse section through privet stem*** **(figure [P]37).** ***What can you deduce about the probable pathway of ^{14}C in the shoots shown in*** **figure [P]36?**

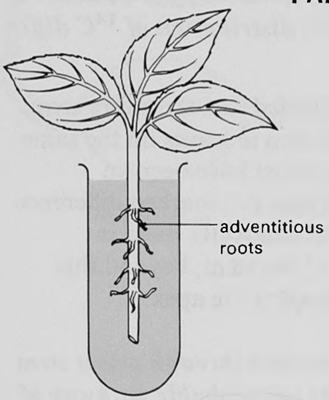
The path taken by ^{14}C would appear to lie in the outer part of the stem (cortex and phloem), not the xylem.

c ***What criticism can you put forward about the design of this experiment?***

The surface of the fed leaf would have had to be damaged in order for sucrose to be absorbed there (in fact, the surface is abraded). This treatment and the absorption of the sucrose are both highly unnatural and it would not be surprising if the 'export pattern' from that leaf was somewhat abnormal. The shoot was not attached to the parent plant so there were no roots acting as a sink for the sucrose. It must be questioned whether a true picture of sugar transport in the intact plant is obtained by this technique.



Experiment



Control

Adventitious roots develop over whole of submerged area and are slower to develop than those of the ringed stem.

Figure 18
The results of ringing a shoot of *Impatiens.*

d ***Draw a diagram of the two* Impatiens *shoots to indicate when and where the adventitious roots formed. How can you explain the difference?***

Adventitious roots were first formed on the ringed shoot where they developed only above the ringed area. In the control, adventitious roots developed over the whole of the submerged area. The ringing removed the epidermis, cortex, and phloem and food materials were therefore unable to reach below the ring. They accumulate above the ringed area, hence the earlier formation and better growth of adventitious roots.

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CHAPTER 4 BLOOD AND THE TRANSPORT OF OXYGEN

A review of the chapter's aims and contents

1 The role of blood as a transporter of oxygen is discussed. This role is common to a large variety of animals and their pigments are studied.
2 The biochemistry of haemoglobin shows that on a molecular level, too, structure is closely allied to function.
3 The dissociation curve is explained and its operation investigated. Adaptability to a changing environment is a theme that occurs here and this is continued into an examination of humans under oxygen stress.
4 A variety of situations is examined in detail, in order that the students may take examples that are quite familiar to them and see how they illustrate the principles of homeostasis and adaptation that we have been placing before them all along.
5 The final two sections that deal with the neonatal problems and blood technology illustrate the important overlap between biology, medicine, and engineering.

PART I The *Study guide*

4.1 Respiratory pigments

Practical investigation. *Practical guide 1*, investigation 4A, 'Examination of blood'.

Assumptions

1 Familiarity with enough systematics to enable the students to visualize the animal groups given as examples.
2 Sufficient knowledge of protein structure to realize that a protein may be composed of joined polypeptides.

Principles

1 The efficiency of blood in the transport of oxygen is determined by the presence of a respiratory pigment, the ability of the pigment to combine with oxygen, and the concentration of the pigment in the blood.
2 There are several respiratory pigments and all seem to consist of a protein and a non-protein prosthetic portion. They are conjugated proteins.
3 Different pigments are restricted to particular groups of animals.
4 The form of the pigment found in a species reflects an adaptation to the environment in which the species lives.

5 Pigments may not only be used to transport oxygen but also to store it.

STUDY ITEM

4.11 Respiratory pigments

This item surveys the variation among respiratory pigments that is found in the animal kingdom. Students should be aware of this variety to balance the fact that haemoglobin is usually the only one seen and examined in practical work. The four types of haemoprotein mentioned are probably the main but not the only types of respiratory pigment.

Questions and answers

a ***Which are the most efficient of these pigments in terms of the molecules of oxygen carried by each atom of metal?***

Haemoglobin and chlorocruorin.

b ***Which pigment is the most efficient in terms of the concentration of oxygen per unit volume of blood?***

Haemoglobin.

c ***How can your answers to a and b be related?***

Although both haemoglobin and chlorocruorin carry one molecule of oxygen per atom of metal, haemoglobin is usually found in very high concentrations closely packed in cells. Hence there is a greater concentration of the pigment per unit volume of blood.

Students could be reminded that the red blood cell possesses no nucleus and no cytoplasmic organelles. Hence it has almost the entire internal volume in which to pack haemoglobin.

d ***How would you account for these facts?***

The limited rate of activity of these animals means that their oxygen requirements will also be limited. The small amount that is dissolved in sea water is sufficient to fulfil their requirements.

e ***What additional function does haemoglobin appear to serve in diving animals and* Arenicola *compared with terrestrial animals like humans?***

The haemoglobin acts as a device for storing oxygen against the time when it would otherwise not be available. In the invertebrates, haemoglobin seems to have arisen independently and to have been retained in many different species as an adaptation which enables them to withstand longer or shorter periods of oxygen deficiency. In terrestrial animals, oxygen is usually readily available and reserves stored in the blood are not required. (Students could read about the function of the *rete mirabile* in Cetaceans.) □

The structure of haemoglobin

Practical investigation. *Practical guide 1*, investigation 4B, 'The carriage of oxygen'.

Assumptions

1 That the preceding section has been read.
2 Knowledge that polypeptides are chains of amino acid residues.
3 An understanding of chemical oxidation.
4 Familiarity with the concept of partial pressures.

Principles

1 Oxygen is attached to the haemoglobin at particular binding sites.
2 The binding of oxygen is cooperative in haemoglobin, with each successive oxygen addition facilitating the uptake of the next.
3 The iron(II) of the haem groups is not oxidized during the oxygenation of haemoglobin.
4 The cooperative binding and the Bohr effect are explained by the breaking or stabilizing of salt-links within the molecule, increasing or decreasing its affinity for oxygen.
5 The Bohr effect enables the loading and unloading of oxygen to match the demands of the tissues through which the blood is passing.

Students whose experience of organic chemistry or biochemistry is limited may find this short section somewhat daunting. It is not absolutely essential as a prelude to the work that follows but it does explain the cause of some of the effects that are referred to later. It could be returned to when protein structure is dealt with, together with enzyme action, in Chapter 5.

STUDY ITEM
4.12 Haemoglobin and the carriage of oxygen

Assumption

1 An understanding of the concept of partial pressures and how to calculate them from data about the percentage composition of air.

Principles

1 Blood is saturated with oxygen at the lungs. This oxygen is released to tissues as blood passes; the extent of the release is related to the p_{O_2} of the tissues.
2 The relation between p_{O_2} and percentage saturation describes a sigmoid curve. This gives a steep working range and rapid unloading of oxygen in the tissues.

Students are required to plot a graph. They will see the dissociation curve again. This exercise is to try to prolong their contact with the curve and to give them working knowledge of its construction, since the ability to understand it is so fundamental to the work in this chapter.

Questions and answers

a ***Using the figures from the first column of table [S]13 as the abscissa, plot a graph of p_{O_2} against the percentage saturation of Hb.***

—

b ***In the lungs of a man the p_{O_2} is 13.3 kPa. What is the percentage saturation of Hb at this level?***

97.5 per cent.

c ***In the venous blood the p_{O_2} is 5.3 kPa. What is the percentage saturation at this level?***

74.0 per cent.

d ***When the p_{O_2} of blood is low, is the percentage saturation of the Hb high or low?***

Low.

e ***When the p_{O_2} of blood is high, is the percentage saturation of the Hb high or low?***

High.

f ***Is the relation between p_{O_2} and percentage saturation a linear one?***

No, there is a steep, almost linear increase at first and then a flattening of the curve. The curve is sigmoid.

g ***Consider the top of the curve, in the p_{O_2} range 6 to 14 kPa. Would a large change in p_{O_2} (say 2 kPa) have a small or large effect on the amount of oxygen carried by the Hb?***

A small effect; maximum change would be around 9 per cent.

h ***Calculate the partial pressure of oxygen***
1 in the atmosphere; and
2 in the expired air.

1 in the atmosphere $\frac{21.0}{100} \times 101.3 = 21.27\,\text{kPa}$

2 in expired air $\frac{16.5}{100} \times 101.3 = 16.71\,\text{kPa}$

i ***Is there likely to be much variation in the amount of oxygen loaded into Hb at the lungs because of variations in the oxygen content of the atmosphere?***

At both of the p_{O_2} values calculated in **h**, Hb would be nearly 100 per cent saturated. From the curve it would look as though the atmospheric p_{O_2} needs to fall to around 10 kPa before there is much drop in saturation. A p_{O_2} of 10 kPa would exist if there were 9.9 per cent oxygen in the atmosphere. Question **b** also suggests that the 'normal' alveolar p_{O_2} is well above this low value.

j ***What is the effect, on the amount of oxygen carried by the Hb, of small changes in p_{O_2} within the range 1 to 5 kPa?***

Large changes in the percentage saturation of Hb.

k ***The p_{O_2} in tissue fluids ranges from 0.7 to 4.0 kPa. What will be the influence of this low partial pressure on oxyhaemoglobin?***

Oxygen will be given up rapidly to the tissues.

l ***If more oxygen were used in the tissues because of exercise, what would be the effect on the dissociation of oxyhaemoglobin?***

Exercise would decrease the p_{O_2} in the tissues. The effect of this would be to increase the dissociation of Hb, making considerably more oxygen available to the tissues. □

STUDY ITEM
4.13 The oxygen haemoglobin dissociation curve

Assumptions

1. Knowledge that active muscular contraction produces a rise in local temperature and in p_{CO_2}.
2. Awareness that the acquisition of blood containing a respiratory pigment in red blood cells need not have been a sudden evolutionary occurrence but may be viewed as the result of a series of small developments from a primitive condition.

Principles

1. At a given p_{O_2} an increase in temperature or in p_{CO_2} reduces the percentage saturation of haemoglobin.
2. The decreased affinity for oxygen by haemoglobin in conditions of increased p_{CO_2} or increased temperature are examples of physiological adaptation.
3. Myoglobin has a greater affinity for oxygen than does haemoglobin at p_{O_2} of less than 8 kPa.
4. Myoglobin can be used as an oxygen reservoir, releasing its oxygen when other sources are unavailable.
5. The nature of the pigment used in particular organisms or tissues may reflect the local environmental or physiological circumstances.

This item should develop the students' understanding of the dissociation curve. Most of the questions are straightforward and should cause little difficulty.

Questions and answers

a ***Describe, in general terms, the effects that these factors have on the form of the curve.***

The curve moves to the right and flattens as p_{CO_2} and temperature increase, losing its sigmoid shape.

b ***Consider blood in an environment at a p_{O_2} of 2 kPa. What is the effect of***

1 increasing the temperature from 20 °C to 38 °C; and

2 increasing the p_{CO_2} from 2.7 kPa to 10.7 kPa?

Both of these treatments will reduce the percentage saturation of the haemoglobin in the blood by causing it to release some of its oxygen. The temperature change, **1**, would produce a fall in percentage saturation from around 70 per cent to around 20 per cent. The p_{CO_2} change, **2**, would cause a smaller drop from around 40 per cent to around 15 per cent.

c ***What influence would you expect increasing body temperature to have upon the Bohr effect?***

It would increase the Bohr effect.

d ***Of what practical value are these effects for a tissue such as exercising muscle?***

As tissue does more metabolic work its demand for oxygen increases. Its production of heat and carbon dioxide help in meeting that demand, by promoting the dissociation of oxyhaemoglobin.

e ***If fully saturated blood passes through the 'tonic' muscle when its p_{O_2} is 5 kPa, what will be the effect on the saturation of both the haemoglobin and the myoglobin?***

The haemoglobin saturation will fall by around 40 per cent. If the myoglobin is already fully saturated, its saturation will not change. If it is not fully saturated, it will become so, taking its oxygen from the haemoglobin.

f ***Describe how this muscle tissue may obtain oxygen if its p_{O_2} falls from 5 to 3 kPa and then from 3 to 1 kPa during particularly prolonged activity.***

As the p_{O_2} of the muscle falls, it obtains more oxygen from the haemoglobin to begin with. At 3 kPa or less the haemoglobin is carrying relatively little oxygen. Oxygen is then obtained from the myoglobin – at 3 kPa it is still nearly 80 per cent saturated and represents a considerable reserve of oxygen.

g ***Suggest how the presence of myoglobin in these muscles may contribute to the fact that they seldom show any signs of fatigue.***

What other feature of these 'tonic' muscles would you expect to find as part of their protection from fatigue?

The myoglobin acts as a reserve 'store' of oxygen within the muscle. At a p_{O_2} in the tissue of less than 8 kPa, myoglobin will absorb oxygen from the haemoglobin in the blood. Hence the muscles are unlikely to run out of oxygen. They will however require a supply of metabolizable substrate. The 'tonic' fibres are often particularly rich in glycogen.

h ***Describe the differences in the relation between percentage saturation and p_{O_2} for the two animals.***

In *Arenicola* the tissue p_{O_2} has to fall to around 1 kPa before oxygen is released, whereas in Man release of oxygen starts at 9 or 10 kPa. Full saturation of the haemoglobin only occurs at p_{O_2} greater than around 10 kPa in Man, whereas the haemoglobin in *Arenicola* is fully saturated at around 2 kPa.

i ***To what extent do these differences reflect differences in the environment and way of life of the species?***

The lugworm leads a sluggish life in sand, pumping water through its burrow which has a low oxygen concentration (see section 4.2). It is essential, therefore, for the animal to have blood that can be fully saturated with oxygen from a low environmental concentration. However, being sluggish, it does not require a large amount of oxygen. Hence the low oxygen concentration in its tissues. In Man, with his high oxygen requirement, oxygen is released at p_{O_2} between 2 and 6 kPa. This could never happen in *Arenicola*. Man obtains his supply of oxygen from the atmosphere where p_{O_2} is high (13.3 kPa in the alveoli).

j ***Arrange these stages in the order in which you consider they might have occurred. Explain the reasons for your choice.***

The order should be:
Composition similar to sea water (2)
The production and incorporation of a respiratory pigment (3)
A dissociation curve which has a marked S-shape (5)
Increased concentration of pigment (1)
☐ The production of blood cells (4)

STUDY ITEM
4.14 (Short answer question) (*J.M.B.*)

Questions and answers

a ***Express these results graphically to show the relationship between partial pressure of oxygen in kPa and the percentage saturation of haemocyanin with oxygen.***

b ***What two pieces of information does the shape of both curves give about the possible usefulness of haemocyanin as an oxygen carrier in the living animal?***

The pigment is relatively highly saturated at low environmental p_{O_2}. It shows a steep working range, meaning a good release of oxygen as the p_{O_2} falls.

c ***Using the graph, determine the partial pressure of oxygen when the haemocyanin is 50 % saturated with oxygen at a low concentration of carbon dioxide.***

Approximately 2.9 kPa.

d ***If this partial pressure of oxygen in c is kept constant, but the concentration of carbon dioxide is increased from the low to the high, what will be***
1 the effect and
2 the resultant percentage saturation of the haemocyanin with oxygen?

1 The effect will be the release of oxygen from the haemocyanin.
2 The resultant percentage saturation will be 18.5 per cent.

e ***How is the effect given in d of use to the living animal?***

If the animal exercises, its demand for oxygen will increase as will its output of carbon dioxide. This latter effect causes oxygen release which satisfies the demand. □

4.2 When oxygen is scarce

Principle

1 A change in the demand by an organism for oxygen will be met by changes in the operation of the systems that supply the tissues with oxygen.

Exercise and training

Assumption

1 Knowledge that ATP is the universal 'energy currency' and is used to provide energy to working systems such as muscles.

Principles

1 Respiration is generally aerobic in Man; anaerobic respiration may be used but it leads to the accumulation of the toxic waste substance, lactic acid.
2 The oxygen debt is the amount of oxygen needed by an organism after exercise, mainly to remove the lactate produced by anaerobiosis during exercise, but also to recharge myoglobin and haemoglobin.
3 The amount of work that an athlete can perform is determined by the

total equivalent oxygen available to him or her (the maximum oxygen consumption over the period of the exercise plus the maximum oxygen debt sustainable).

4 Training can increase the rate of oxygen absorption, the maximum oxygen debt, and the cardiac output of an individual.

5 The type of training undergone by an individual should match the type of demands to be made by the event in which he or she participates. Aerobic events demand aerobic training.

It is necessary here to bring in some reference to aerobic and anaerobic respiration (see *Study guide I*, Chapter 5). The chemical details are kept to a minimum and the biochemical mechanisms behind the switch to and from anaerobiosis need not at this stage be mentioned. The questions are largely quantitative but should present few problems.

STUDY ITEM

4.21 The effects of training

Questions and answers

a ***From the data in the table calculate the cardiac output in dm³ minute⁻¹ of the three athletes at rest.***

In each case calculate

$$\frac{\text{heart rate} \times \text{stroke volume}}{1000} = \text{dm}^3\ \text{minute}^{-1}\ \text{cardiac output.}$$

Hence:

Pre-training	*Post training*	*International*
4.61	4.58	4.61

b ***Which of the rows of data [table (S)15] indicates most directly the athletes' ability to sustain oxygen debt?***

'Lactate, maximum'. This indicates the maximum concentration of lactic acid that the individual can tolerate in the blood.

c ***Describe the changes that have occurred to the heart as a result of the training in the international athlete compared with the pre-training athlete.***

The resting heart rate has decreased by 50 per cent. However, the resting stroke volume has doubled and the maximum has increased by 70 per cent. Thus, although the resting cardiac output shows no change, the maximum output is up by 60 per cent. This has been accompanied by an increase in heart volume of 60 per cent, achieved by the growth of new heart muscle, as well as by increased extension of existing muscles.

d ***Why do you think that there are no data given for ventilation apart from its rate?***

The ventilation is intimately concerned with getting oxygen into the blood and the data for cardiac output and maximum oxygen uptake give the information about that function. Tidal volume is irrelevant, since so many other factors have a more fundamental influence on the efficiency and extent of oxygen uptake from the atmosphere.

e ***There is a very big difference between the data for the normal individual after training and those for the international athlete. Apart from the duration and extent of the training, what is the main factor determining the features such as maximum cardiac output and maximum oxygen uptake?***

The other important factor is the individual's physique and his or her capacity for response to training.

f ***Explain briefly the method by which you would obtain a value for maximum oxygen uptake ($\dot{V}_{O_2max}$).***

Use a Douglas bag and collect expired air for a known length of time. Determine its volume and percentage oxygen content. The change in oxygen content in comparison with atmospheric air tells you how much oxygen has been absorbed during the time of collection. Provided that this measurement was made during maximum intensity of work, this is $\dot{V}_{O_2max}$. □

STUDY ITEM

4.22 Oxygen debt

Principles

1 During short periods of high rate of activity, oxygen supply cannot meet demand and so the tissues accumulate an oxygen debt.
2 This oxygen debt must be repaid after the period of activity.
3 Individuals vary in the extent of the oxygen debt they can carry. This affects athletic performances.

Questions and answers

a ***What short-term beneficial effect will the athlete gain from the presence of lactic acid in the blood?***

It will cause a change in the stability of oxyhaemoglobin and promote oxygen release to the tissues requiring the most oxygen. (See section 4.1.)

b ***How far can he run at this speed before becoming completely exhausted?***

His maximum oxygen demand is $0.2\,dm^3$ per second $= 0.2 \times 60 = 12\,dm^3\ minute^{-1}$. Aerobic respiration can only satisfy $4\,dm^3\ minute^{-1}$, however, and he is therefore accumulating an oxygen debt at the rate of $8\,dm^3\,minute^{-1}$. The maximum debt that he can sustain is $15\,dm^3$ which will be reached after $\frac{15}{8} = 1.875$ minutes. In this time he will have run $1.875 \times 60 \times 8 = 900$ metres.

c ***What proportion of the energy that he has then used is associated with the oxygen debt?***

The total oxygen used, at $0.2\,dm^3\,second^{-1}$ is $1.875 \times 60 \times 0.2 = 22.5\,dm^3$. Of this, $15\,dm^3$ is oxygen debt. $\frac{15}{22.5} = 0.67$. Thus 67 per cent of the total oxygen needed (energy used) is associated with the oxygen debt.

d ***From the graph, what is the approximate value of the maximum oxygen debt sustainable by this athlete...?***

Answer B: approximately $21\,dm^3$.

e ***What is represented by the three areas, L, M, and N on the graph?***

L represents the oxygen debt; M represents the amount of oxygen used during the exercise period; and N represents the repayment of the oxygen debt. It is worth noting that the debt takes far longer to repay than it does to acquire.

f ***What is the maximum rate of oxygen consumption by this athlete?***

$5\,dm^3\,minute^{-1}$. The athlete was completely exhausted and it may be assumed that his rate of uptake had reached a maximum, as had his oxygen debt. Additionally, the oxygen consumption appears on the graph to have reached a plateau.

g ***Which runner will win each of these two races?***

Race 1
In 50 seconds A can take in $2.5\,dm^3$ O_2 and can incur an oxygen debt of $15\,dm^3$.
∴ Total energy available will be proportional to $15 + 2.5 =$ *17.5*.
In 50 seconds B can take in $3.33\,dm^3$ of oxygen and incur an oxygen debt of $10\,dm^3$.
∴ Total energy available to B will be proportional to $3.33 + 10 =$ *13.33*.
∴ *We should expect A to win over 400 metres.*

Race 2
In 14 minutes A can take in $3 \times 14 = 42\,dm^3$ of oxygen.
∴ Total energy available to A will be proportional to $42 + 15 =$ *57*.
In 14 minutes B can take in $4 \times 14 = 56\,dm^3$ of oxygen.
∴ Total energy available to B will be proportional to $56 + 10 =$ *66*.
□ ∴ *We should expect B to win over 5 km.*

Diving birds and mammals

Assumption

1 A familiarity with the anatomy of the mammal lung and associated structures.

Principles

1 Forced or prolonged submersion in birds and mammals leads to a classic diving response of selective anaerobiosis and vasoconstriction, together with bradycardia.
2 Most spontaneous dives by many birds and mammals are performed aerobically.
3 Increased pressures at depth can cause many physiological difficulties, partially overcome by a degree of collapse of the chest and elimination of gas from air-filled spaces.

This work is a useful recapitulation of some of the principles and facts that emerged from earlier work on gas exchange. Although passing reference is made to Man, the examples are intentionally chosen from other vertebrate groups in order to show that the (perhaps familiar) pattern of response is not exclusively Man's preserve and to give a little respite from the emphasis on human physiology.

STUDY ITEM
4.23 Diving and the use of oxygen

Questions and answers

a ***State concisely what these facts suggest about the use of oxygen by these birds during diving.***

They suggest that the dives are short enough to utilize reserves of oxygen without having to call significantly upon anaerobiosis.

b ***Describe the main differences between the heart rate***
1 immediately before
2 during
3 immediately after
the two dives shown in* figure [S]66, *and the heart rate at rest (the part of the trace marked A).

1 Immediately before a dive the rate increases markedly (approximately doubling).
2 During a dive the rate is slightly less than that at rest, increasing slowly during the dive.
3 Immediately after a dive the rate increases rapidly to the level it attained immediately before the dive, and remains there.

c ***From the evidence of* figure [S]66, *would you say that these ducks employ oxygen-conserving adjustments during spontaneous dives?***

Probably not; there does not seem to be any significant bradycardia and there is increased ventilation before the dives; it seems more likely that a maximum oxygen supply is made available and used throughout the dive.

d ***Feeding periods for the Weddell seal may last for as much as 10 hours. What advantage does this seal gain by diving aerobically?***

Over a long period of time it can spend relatively more time below the surface than it could if it were diving anaerobically. This means that it has more time to feed because it needs to spend less time recovering from anaerobiosis.

e ***If aerobic diving is used for feeding, under what circumstances would you expect the seal to use its anaerobic diving capacity?***

It would need to use longer, anaerobic diving for underwater swimming when searching for new air holes through the ice.

f ***Explain briefly the ways in which each of the adaptations mentioned in the preceding paragraph may be of advantage to the diving mammal.***

Elimination of gas from spaces lined with soft tissues and from the exchange regions of the lung will prevent the residual gases, such as nitrogen, from dissolving in the blood under pressure. Collapse of the chest wall and alveoli is one way to achieve this elimination. The oblique diaphragm and compliant thorax offer little structural resistance to collapse, unlike the reinforced air passages. These remain open in spite of the pressure, so the air collects in the dead space. Partial expiration before submersion reduces the volume of gas in the lungs that could cause problems. Moving quickly to a depth where collapse of alveoli occurs reduces the time during which the gas is under pressure whilst still present in the alveoli; thus it also reduces the time during which nitrogen can be absorbed. □

Breathing at high altitude

Assumption

1 Knowledge that every dm^3 of oxygen absorbed by a human is associated with the release of approximately 20 kJ, at s.t.p.

Principles

1 Within limits, the body's physiology automatically adjusts to the change in pressure resulting from living at or visiting high altitudes – long-term homeostasis.

2 The low p_{O_2} in the atmosphere at very high altitudes is the ultimate reason for the inability of Man to exist under those conditions.

The newborn baby

Assumption

1 Knowledge that carotid arteries originate from the apex of the aortic arch.

Principles

1 Relatively sudden modification of circulatory and respiratory physiology is essential at birth because of the drastic environmental change.
2 Foetal circulation is single; adult circulation is double.
3 Medical assistance is normally necessary in the case of premature babies, who have not yet matured sufficiently for independent survival.
4 Many neonatal problems are self-correcting in time; medical help often merely mimics natural modifications that the body would make were it mature enough.
5 Lung surfactant reduces alveolar surface tension and makes inflation possible.

Some additional ideas may be of help in answering questions that may arise during a study of this section and may add to the sometimes limited explanations contained in the *Study guide*.

Because of the precise relation of the vessels entering and leaving the heart, and because of laminar flow and preferential streaming, most of the blood crossing the foramen ovale actually enters the right atrium from the inferior vena cava. This blood is highly oxygenated and it is this blood, therefore, that enters the left ventricle and aorta and goes to supply the arteries to the head.

The interval between the delivery of the infant and the cessation of umbilical cord pulsations, as the umbilical vessels constrict, allows the blood to be redistributed between the placenta and the infant. Occasionally the infant is over-transfused with blood from the placenta and this can lead to problems as the circulation becomes sluggish, because of the elevated blood viscosity resulting from the excess of red blood cells. On the other hand early clamping of, or a tear in, the cord may lead to the infant having a deficiency of red cells and hence anaemia.

During foetal life the high pulmonary vascular resistance is a result of the constricted, thick muscular walls of the pulmonary arterioles. The cellular mediators of the dilatation of these vessels which follows birth are poorly understood but may include kinins and, as suggested, prostaglandins. Over the months following birth the arteriolar walls thin out, with a loss of muscle cells and a more limited ability to constrict.

Why the ductus arteriosus should respond in the opposite way from the pulmonary artery to a rise in oxygen is not known. The cellular mediators of the change are poorly understood. It is believed that certain prostaglandins are synthesized in the ductus wall and help maintain patency during foetal life.

The exact composition of the lung surfactant has yet to be fully worked out, but it is known that the phospholipid dipalmitoyl lecithin is the predominant molecule in it, making a significant contribution to the action of the fluid. The surfactant is secreted by pneumocytes or type II cells of the epithelium.

Foetal blood cells have low diphosphoglycerate levels and it is this, in part, that accounts for the high oxygen affinity of foetal Hb.

The explanation for the continued patency of the ductus arteriosus is not always straightforward. If the arterial oxygen concentration is low this will cause the ductal muscle wall to dilate. The low arterial p_{O_2} may be an indirect effect of high pulmonary artery pressure, which causes right to left shunting of blood through the foramen ovale. Circulatory fluid overload does not usually lead to raised blood pressure in the newborn, and we are not exactly clear how overload acts to promote ductal patency.

Questions and answers

a ***Examine* figure [S]69. *Which vessel carries the most highly oxygenated blood?***

The umbilical vein.

b ***In the adult the carotid arteries carry highly oxygenated blood towards the brain. Why would this appear to be impossible in the foetus?***

Because the blood from the umbilical vein appears to mix with the deoxygenated blood from the superior vena cava in the right atrium. However, blood returning to the heart via the inferior vena cava streams preferentially through the foramen ovale to the left side of the heart; poorly oxygenated blood returning from the superior vena cava preferentially streams into the right ventricle and, ultimately, much will perfuse the placenta and become oxygenated again.

c ***Why is it essential that blood flow to the lungs is not stopped altogether during the development of the foetus?***

As the foetus grows in size, the raw materials for lung growth are needed in increasing quantities and these must be supplied by blood in the pulmonary artery.

STUDY ITEM
4.24 Foetal haemoglobin

Questions and answers

a ***Show how the possession of foetal haemoglobin is vital to the survival of the foetus.***

The p_{O_2} of foetal tissues is very low and corresponds to the steep working range of the foetal Hb curve. If this p_{O_2} were as high as it is in the adult, foetal Hb would be unable to unload.

b ***Explain why it is necessary to replace foetal haemoglobin with adult haemoglobin after birth.***

After birth the tissue p_{O_2} rises so that it is above the full saturation level for foetal Hb. For this reason a change of molecules is necessary so that the blood may unload its oxygen in adult tissues. □

4.3 Blood technology

Assumptions

1 Awareness of the significance of maintaining a constant water potential of blood.

2 Familiarity with the respective roles of antibody and antigen.

3 Knowledge of the ABO system of blood grouping.

Principles

1 Blood contains many components, each with a specific role. Whole blood or its individual components may be given by one individual to another to restore full function in the recipient.

2 Blood group matching is vital for donations of cells but not for most non-cellular components.

3 Lack of blood may occur through injury or because of disease or damage to haemopoietic tissue (such as bone marrow).

4 Blood is a living tissue and has limited storage time outside the body; it is metabolically active.

The *Study guide* goes into considerable detail both about the manipulative techniques and the use of the various factors. They illustrate the application of an increasingly sophisticated technology to a vital area of medical practice and serve also to show clearly the many functions that are performed by blood; this will redress the balance after the preceding emphasis on blood's oxygen-transporting function.

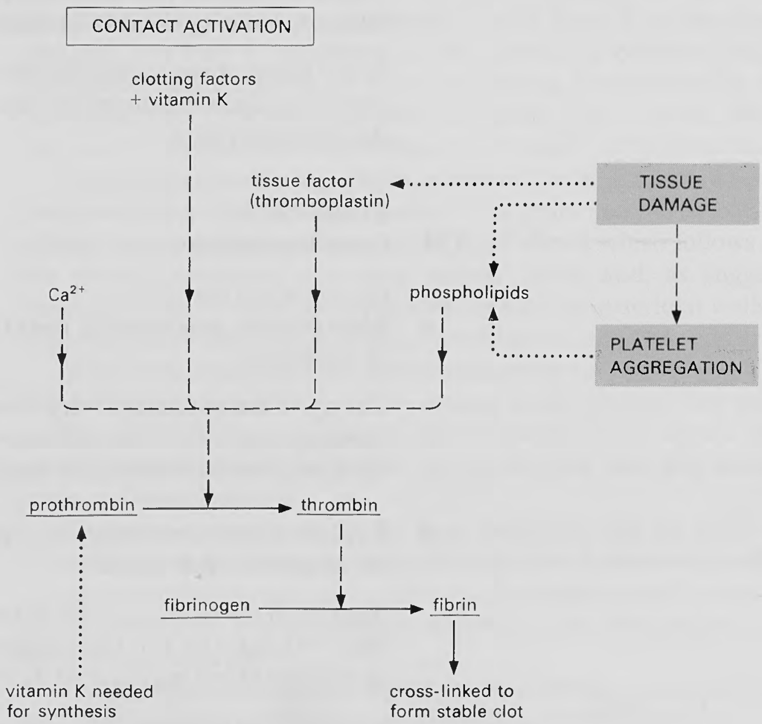

Figure 19
The coagulation of blood.

Very little detail about clotting is included. A slightly more complete diagram is included in this *Guide.* The phospholipids come from the damaged membranes of local tissues and from platelet breakdown. The role of vitamin K is interesting. It can be obtained in the diet and/or by biosynthesis during the normal activities of the gut flora. It is not only essential for the synthesis of prothrombin but it is also implicated in the synthesis of factors VII, IX, and X. If it is absent, or if an antagonist (such as dicoumarin or Warfarin, which are structural analogues of vitamin K) is present, an abnormal prothrombin is synthesized which does not bind calcium. Prothrombin, along with perhaps eight of the other factors, is manufactured in the liver.

PART II The *Practical guide*

The investigations related to this chapter appear in *Practical guide 1, Gas exchange and transport in plants and animals.*

INVESTIGATION 4A Examination of blood

ITEMS NEEDED

Blood, mammalian; citrated

Carbon dioxide supply
Carbon monoxide supply
Detergent, household, liquid
Dextrostix strips 2/1
Ethanol, or ethanol and ethoxyethane (diethyl ether)
Giemsa stain
or
Leishman's stain
Oxygen supply
Saline, buffered
Sodium chloride solution, saturated
Sodium chloride solution, weak (0.89 %)
Water, distilled

Centrifuge 2/class
Centrifuge tubes
Cottonwool
Dale and Laidlaw tube 1/1
Eyepiece graticule 1/1
Flowmeter, if possible 1/class
Lancets, sterile
Microscope 1/1
Microscope slides and coverslips
Petri dish 1/1
Pipettes, teat 2/1
Plastic tubing *(continued)*

(*Study guide* 4.1 'Respiratory pigments'.)

Anticoagulant for mammalian blood. Mix saturated solutions of the following (*TAKE CARE:* they are toxic and harmful):
Ammonium ethanedioate (ammonium oxalate), 3 parts
Potassium ethanedioate (potassium oxalate), 2 parts
4–5 cm^3 of this mixture in the empty container for every 100 cm^3 of blood are more than enough to prevent clotting.

Alternative 10 per cent sodium citrate solution, aqueous. Add 10 cm^3 of this solution to the empty container for every 100 cm^3 of blood to be collected.

Giemsa stain
Giemsa stain, 1 g
Methanol, 66 cm^3 (*TAKE CARE:* toxic and highly flammable)
Propane-1,2,3-triol (glycerol), 66 cm^3
Put the powdered stain into a conical flask. Add the glycerol and heat at 56 °C for 90 to 120 minutes. Remove the heat, add the methanol, mix thoroughly, and allow to stand for 7 days at room temperature before filtering.

Phosphate buffered saline – pH 7.2
Disodium hydrogen phosphate, 1.15 g
Potassium chloride, 0.20 g
Potassium dihydrogen phosphate(V), 0.20 g
Sodium chloride, 8.00 g
Dissolve the salts in 1 dm^3 of distilled water. It may be necessary to

ITEMS NEEDED *(continued)*

Rubber caps to fit syringe nozzles 4/1
Stage micrometer 1/class
Stopclock or wristwatch with seconds indicator 1/1
Syringe, plastic, 10 cm^3 2/1
Syringe, plastic, 20 cm^3 4/1
Water bath at 37 °C 1/class

adjust the pH. (Giemsa stain and the buffer are mixed immediately before use, as instructed in the *Practical guide.*)

Leishman's stain

Leishman's stain, 0.15 g
Methanol, 100 cm^3 (*TAKE CARE:* toxic and highly flammable.)

Assumptions

1 Some understanding of osmosis.
2 Awareness that the cell membrane has limited tensile strength.
3 An understanding of the fact that blood normally clots outside the body unless a chemical anticoagulant is added to it.

Principles

1 Blood consists of large numbers of different types of cell suspended in a watery medium (plasma).
2 There are many varieties of white cell distinguishable, after staining, by their structural features.
3 Blood cells are fairly easily damaged by mechanical disturbance and this leads to the assumption that the water potential of blood must be carefully maintained.
4 Detergent will disrupt cell membranes (because of their lipid content) and hence release haemoglobin from the red blood cells.
5 Haemoglobin reacts reversibly with some gases and irreversibly with others. This difference is of profound significance both for effective normal gas exchange and for cases of poisoning by noxious gases.
6 Blood transports numerous substances apart from respiratory gases and this role of distribution is as fundamental as the role of blood in gas transport.

Because blood changes rapidly once it is released from the body, it is essential to use fresh samples wherever possible. Students should be able to obtain drops of reasonable size from thumb or forefinger but this should never be demanded of them against their wishes. The teacher should always be willing to provide some in such cases. It is most important that all students should follow a strict procedure in obtaining blood samples and never deviate from it. It is recommended that they follow the procedure detailed by the Association for Science Education in *Education in Science*, April 1979, and in *Topics in safety*, 1982 (see Appendix). In particular, it is essential that sterile lancets are used and no lancet should be used more than once. After use, the swabs and lancets should be sealed and autoclaved and then thrown away. The reasons for care and discipline should be pointed out to students as part of their biological training. *Note*: there may also be local regulations relating to blood sampling.

The practical investigations are grouped into the four procedures according to the ideas they generate rather than the techniques they employ. It is therefore important for students to read through the whole of the investigation and plan the use of their time before the practical period. For example, both the centrifuging steps take time and should be performed simultaneously.

The main difficulty is in obtaining a sufficient supply of blood. Larger supplies may either be bought from a biological supplier or obtained through a local abattoir.

Procedure 1b. If the smear is overstained (too blue) immerse the slide in buffered saline for a while. In making a good smear it is essential to see that the end of the second slide is perfectly straight and smooth.

Procedure III. Sufficient carbon monoxide for the class can be collected in a strong plastic bag from a car exhaust.

Procedure IVb. It is important to prevent temperature fluctuations, since the speed of clotting is twice as fast at 37 °C as it is at average room temperature of around 20 °C.

Questions and answers

a ***What volume of the blood sample is made up of cells?***

Cells which settle at the bottom of the tube occupy 45 per cent of the total volume and the clear plasma above them occupies 55 per cent. The figure '45' is known as the packed cell volume (PCV) or haematocrit. With sufficient centrifuging it should be possible to distinguish a greyish-red layer of leucocytes above the red cells and above this, just below the plasma, a thin, creamy layer of platelets. It should be remembered that storage of blood leads to changes in PCV.

b ***What is the size (diameter and thickness in micrometres) of the red blood cells?***

Normally a population of red cells can be seen to vary appreciably in diameter. It should also be remembered that the measurements of diameters in a dried film is artificial since the cells shrink on drying – diameters are 8 to 10 per cent less than when suspended in plasma. Typical diameters of red cells in a dry film might be between 6 and 8.5 μm, with an average thickness near the periphery of 2.2 μm.

c ***Why is the central region of the red blood cell less densely stained than the outer rim?***

This is a result of the biconcave disc shape of the cell.

d ***How many types of white cell can you find? (Identify them with reference to*** **figure [P]40*****). Describe them, using diagrams and a table, paying particular attention to the size and shape of the nucleus and the colour and amount of cytoplasm.***

There are at least five types of white blood cell. They are subdivided into two main classes, depending upon the shape of the nucleus and the nature of the granules which may occur in the cytoplasm. Figure (P)40 gives a convenient summary and guide for this identification.

e ***What are the proportions of red cells, white cells, and platelets?***

The number of each per mm^3 of blood is:
red cells – about 5 000 000
white cells – about 4000 to 11 000
platelets – about 300 000.

f ***In what ways are the blood cells affected by the various liquids used in this procedure? Can you account for your observations?***

Red cells placed in distilled water or very weak solutions swell and burst (haemolyse) because water moves through the membrane by osmosis. The ability of the normal red cell to withstand a high water potential results from its biconcave shape; this allows the cell to increase its volume by about 70 per cent before the surface membrane is stretched – once this limit is reached lysis occurs. Detergents cause the membranes of red cells to rupture. In concentrated solutions water passes out of the cells by osmosis, causing them to shrink, often into fantastic shapes.

g ***How does the centrifuged sample of whole blood compare with that of the blood–detergent mixture (procedure IIa)?***

When liquid detergent is added to fresh whole blood, the appearance changes from cloudy to clear. During centrifugation ruptured membranes are driven to the base of the tube, forming a pellet of 'ghosts'.

h ***How do the blood samples exposed to the various gases compare in colour with the sample exposed to atmospheric air?***

sample plus oxygen – bright red
sample plus carbon monoxide – cherry red
sample plus carbon dioxide – no colour difference between this blood and the deoxygenated blood is likely to be visible to the eye. Carbon dioxide combines with haemoglobin to form 'carbaminohaemoglobin'. This compound has a different absorption spectrum from haemoglobin.

i ***Were any of the observed colour changes reversible? Can you think of any medical implications of your answer?***

The reactions with oxygen and carbon dioxide are both completely reversible; the reaction with carbon monoxide is generally irreversible. Carbon monoxide has a higher affinity for haemoglobin (200 to 300 times greater) than oxygen has. In individuals exposed to atmospheres containing carbon monoxide, 'carboxyhaemoglobin' gradually replaces oxyhaemoglobin in the blood and death occurs as a result of anoxia (lack of oxygen).

j ***What is the concentration of glucose in the plasma compared with whole blood? What conclusion can you reach from the answer?***

The figure should be the same, indicating that plasma provides the medium for the transport of glucose. In the blood of a normal, healthy adult there is approximately 0.1 g of glucose in every 100 cm^3 of blood.

k ***Record the clotting time of your blood in seconds. What is the mean and standard deviation of clotting time at this temperature within the class group?***

Quoted values for this method of determination, at a constant 37 °C, are between 6 and 12 minutes.

INVESTIGATION
4B The carriage of oxygen

ITEMS NEEDED

Blood, citrated (see investigation 4A).

Aqueous ammonia (ammonium hydroxide solution), 5 mol dm^{-3}
Detergent, dilute solution (0.5 cm^3 in 10 cm^3 water)
Oxygen supply
Potassium hexacyanoferrate (III) solution

Beakers, 25 cm^3 3/1
J-tube 1/1
Rubber tubing, about 4 cm in length, to fit J-tube and syringe, 1 cm^3 1/1
or
Screw adjustment to fit J-tube 1/1
Ruler, mm-ruled 1/1
Specimen tube with stopper 1/1
Syringes, 5 cm^3 or 10 cm^3 2/1
Water bath at 35 °C 1/1

(*Study guide* 4.1 'Respiratory pigments'.)

Aqueous ammonia (ammonium hydroxide solution), 5 mol dm^{-3}
Aqueous ammonia (ammonium hydroxide), concentrated, 33.5 cm^3
(*TAKE CARE:* this is toxic and corrosive.)
Water, distilled, 66.5 cm^3
Potassium hexacyanoferrate(III) solution.
Potassium hexacyanoferrate(III), 20 g
Water, distilled, 100 cm^3

Assumptions
1 The knowledge that blood consists of a liquid plasma in which red blood cells are suspended.
2 An understanding of the concept of 'saturation of blood with oxygen'.

Principles
1 Bubbling oxygen through blood will saturate blood so that it is carrying a maximum volume of oxygen per unit volume of blood.
2 Potassium hexacyanoferrate(III) solution causes haemoglobin to release all the oxygen that it is carrying.

The addition of the ammonia to the blood after oxygenation makes it run very freely in the capillary tube, absorbs any carbon dioxide present, and has the property of causing rupture of the cell membranes. Therefore the detergent is used merely to reduce surface tension in the capillary tube and this seems to assist the movement of the liquids, prevent too much breaking up of the bubbles, and help the tiny bubbles of oxygen forming in the blood to rise to its surface and burst. The hexacyanoferrate and the detergent may cause the formation of a precipitate when mixed, but, with the method used here, this should not prove to be a problem and any such slight precipitate may be ignored.

Obtaining blood may be a problem. Unfortunately a large volume is necessary for the whole class. Citrated blood may be bought from suppliers, or may be available from local abattoirs.

Oxygenation of the blood in step 1 must be done slowly or the bubbles will pour ever the edge of the tube. At step 6 it is vital to move the sample well away from the open end of the tube since the increase in temperature will cause the air to expand and may cause the loss of fluids from the J-tube.

The appearance of air bubbles within the blood is quite instructive and students will quickly find ways to get them to move up the column of blood in step 9.

Each tube should be rinsed thoroughly, immediately after each estimation, to prevent the accumulation of material which will block the bore.

Additional procedures

1 Repeat the method but do not oxygenate the blood in step 1.

2 Centrifuge whole blood and then repeat the procedure, using
a plasma
b red cells suspended in small volumes of plasma.

Some students may find it is as much as they can do to make one or two determinations; others will find that they take less time to work through the procedure and will welcome the addition of those given above.

The theoretical bases for the technique are not easy to fathom. It would seem that the cyanoferrate complex is responsible for forcing a change in the shape of the haemoglobin molecule such that oxygen can no longer be bound. It is probably incorrect to refer to an oxidation state change in the Fe(II) atom in the haem as being in any way involved.

Questions and answers

a ***What assumptions are inherent in this method of estimating the oxygen content of blood?***

It is assumed that all the red cells are ruptured and that all the oxygen they are carrying is released by the treatment. Also it is assumed that, by having oxygen bubbled through it, the blood will become fully saturated.

b ***Collect as many results as you can for identical blood samples and calculate the mean and standard deviation of the volume of oxygen carried. The mean value will probably be somewhat below the quoted value for fully saturated mammalian blood. Why would you expect this to be the case?***

Fresh whole blood, when fully saturated, will carry up to about 20 cm^3 of oxygen per 100 cm^3 of blood at 35 °C. The method used here will probably show about 15 to 17 cm^3 oxygen carried per 100 cm^3 blood (see table 7). The fresher the blood the fuller the saturation. If the blood is not very fresh it will have suffered some cell death and cell disruption. Experimental error may also account for a displaced mean.

Volume of oxygen released from 100 cm^3 blood (cm^3)
19.5
16.0
10.6
35.0
12.0
15.0
8.0
5.7
8.5
14.6
11.6
7.8

Table 7
Class results for the volume of oxygen carried by sheep's blood at 35 °C.

c ***You have little real evidence from this investigation that it is the haemoglobin that transports the oxygen in the blood. How would you extend the investigation to test this hypothesis?***

Centrifuge the blood and separate the plasma. Test the plasma and cell fractions separately, using this technique. You would expect no oxygen to be detectable in the plasma and a much increased oxygen release from the cells (see table 8).

Sample	*Percentage oxygen*
Human whole blood	19.78
Sheep whole blood	13.36
Sheep plasma	2.23
Sheep resuspended cell concentrate	26.45

Table 8
The mean percentage of oxygen released from different blood samples.

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(Both of these guides are helpful when dissecting or describing these two animals.)

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CHAPTER 5 CELLS AND CHEMICAL REACTIONS

A review of the chapter's aims and contents

1 Our knowledge of cell structure has been dependent on developments in microscopy.
2 The internal structure of eukaryotic cells involves elaborate membrane systems.
3 Biological systems conform to the Laws of Thermodynamics despite superficial evidence to the contrary.
4 The role of enzymes in biological reactions is to lower the activation energy of the reactants.
5 Owing to their structure, enzymes are very specific in their action and have optima of pH and of temperature for their activity.
6 The energy for anabolic reactions is derived from step-by-step catabolic reactions.
7 The initial stages of the breakdown of glucose are anaerobic and result in some release of energy. If oxygen is available, the later aerobic stages result in the release of far more energy.
8 The complex activities of living cells are compartmentalized by the membrane systems within the cells, which thus function as integrated and co-ordinated units.

PART I The *Study guide*

5.1 Cells and the cell theory

Assumptions

1 Some knowledge of the cellular basis of living organisms which may be recalled by students who have studied the subject at a lower level.
2 That an understanding of the specialized structure and function of some cells (for example, red blood cells) will have been derived from earlier chapters.
3 An appreciation of magnification and scale, which is important for a proper understanding of this section.

Principles

1 Our understanding of the cellular basis of living organisms has evolved over the last three centuries.
2 Technological developments in microscopy have played a central part in our understanding of cells.
3 There are fundamental differences between plant and animal cells.
4 The relation between surface area and volume has important consequences for cell size.

Though Robert Hooke first described and drew plant cells over three centuries ago, the cellular basis of animal tissue was not finally established until the early nineteenth century. This gave rise to the cell theory of Schleiden and Schwann. Throughout this time our understanding of cell structure depended on developments in light microscopy. However, by the late nineteenth century the manufacture of microscopes, or more particularly that of lenses, had reached such standards that technology no longer placed serious constraints on further advances. Nevertheless the power of resolution of the light microscope was now limited more fundamentally. The resolving power of a light microscope depends upon the numerical aperture of the objective and the wavelength of the incident light. The numerical aperture of the best lenses cannot be improved upon, while the wavelength of visible light is about 0.5 μm. Thus the best resolving power that can be achieved with a light microscope is about 0.2 μm. Though minor further advances were made in our interpretation of cell structure, attention became focused on the biochemical aspects of cellular function. It was not until the development of the electron microscope that our understanding of cellular structure took a fundamental step forward.

STUDY ITEM

5.11 Cells and microscopy

Principles

1 The quality of drawings made from the microscope is determined partly by the quality of the instrument itself and partly by the accuracy of the draughtsman's observation.

2 Observation does not simply depend upon an image on the retina, but is equally dependent on the conceptual framework within which the observation is made.

3 Most of the significant developments in light microscopy had taken place by the end of the nineteenth century.

4 There are similarities as well as differences between plant and animal cells.

5 Different staining techniques and different forms of light microscopy all contribute to our interpretation of cellular structure.

Questions and answers

a ***In what ways do the three drawings differ from each other?***

There are clearly many features which are visible in the most recent drawing but are absent, misplaced, or inaccurately recorded in the earlier examples. These features include differential wall thickness, intercellular spaces, cytoplasm in some cells, nuclei in some cells, and the absence of acute or re-entrant angles.

b ***Which of these differences do you think can be attributed to improved microscopy over the 265 years separating the three drawings?***

The first four of the features listed above may now be visible owing to improved microscopy, though the thickened walls of the xylem vessels and the cell nuclei can be seen in the second drawing.

c ***Which of these differences do you think can be attributed to poor observation on the part of the person making the drawing?***

Acute angles in the corners of some cells and re-entrant angles in others are features which are almost certainly due to poor observation. The same may be said of the presence of nuclei in the centre of so many of the cells shown in the second drawing. The recording of all of these features may well have been affected by the draughtsman's prior knowledge of cell structure. Thus by the time the second drawing was made, the presence of nuclei in plant cells was well established and the draughtsman may thus have felt compelled to 'observe' nuclei in most of the cells drawn. An understanding of the three-dimensional shape of the cell was not as well developed at that time as it is today. This in turn may have affected the apparently poor observation of acute and re-entrant angles in some of the cells.

d ***Which of the periods covered by drawings a to b or b to c showed the greatest development of microscopical techniques?***

Most of the features depicted in **c** (the most recent drawing) are also shown in **b**. The presence of cytoplasm in some of the cells is the conspicuous exception. As we have seen above, many of the other poor features of **b** are almost certainly due to poor observation and recording, and not to inadequate microscopes. Most of the significant developments in light microscopy had already taken place *before* drawing **b** was made.

e ***Construct a table to highlight the similarities and differences between the two types of cell.***

The similarities and differences between the plant and animal cells shown in figure (S)83 are summarized in table 9.

<table>
<tr><th></th><th></th><th>Plant cell</th><th></th><th>Animal cell</th></tr>
<tr><td>Similarities</td><td>1</td><td colspan="3">Both cells are bounded by a plasma membrane.</td></tr>
<tr><td></td><td>2</td><td colspan="3">The cytoplasm of the two cells appears similar.</td></tr>
<tr><td></td><td>3</td><td colspan="3">Mitochondria are distributed in the cytoplasm of both cells.</td></tr>
<tr><td></td><td>4</td><td colspan="3">Both cells contain a nucleus with a nucleolus.</td></tr>
<tr><td>Differences</td><td>1</td><td>Has a large central vacuole bounded by the tonoplast.</td><td>1</td><td>No large vacuole.</td></tr>
<tr><td></td><td>2</td><td>Is surrounded by a cell wall.</td><td>2</td><td>No cell wall.</td></tr>
<tr><td></td><td>3</td><td>The cytoplasm of this cell contains chloroplasts.</td><td>3</td><td>No chloroplasts.</td></tr>
<tr><td></td><td>4</td><td>No centrosome.</td><td>4</td><td>A centrosome is present.</td></tr>
<tr><td></td><td>5</td><td>No secretory granules.</td><td>5</td><td>Secretory granules are present in this cell.</td></tr>
</table>

Table 9
The similarities and differences between typical animal and plant cells.

f ***Suggest why it was more than a century after the description of plant cells, before animal cells were identified as individual entities.***

The plant's cell wall forms a very clear line of demarcation between one cell and another. In animal tissues, however, the plasma membrane is much less easily differentiated. It is worth noting that as early as the beginning of the eighteenth century Leeuwenhoek observed both unicellular animals and spermatozoa; but his examination of animal *tissues*, such as the cardiac muscle shown in figure (S)81b, did not reveal to him the boundaries between one cell and the next.

g ***Construct a table to show those features which are highlighted by the different forms of microscopy.***

Table 10 summarizes these features.

Feature	*Bright field*	*Dark ground*	*Positive phase contrast*	*Negative phase contrast*
Nucleus: 1 Membrane	Faintly defined.	Clearly defined.	Clearly defined.	Not very clearly defined.
2 Content	Some internal detail visible.	Some differentiation of detail.	Some differentiation of detail.	Little differentiation of detail.
Cell membrane	Faint, but clear.	Conspicuous and sharply defined.	Rather blurred.	Conspicuous.
Other cell contents	Not clearly defined or differentiated.	Very clearly defined and differentiated.	Quite clearly defined with some differentiation.	Clearly defined with some differentiation.

Table 10
The features of a human cheek cell highlighted by different forms of microscopy.

h ***What important factors are absent from these photographs? How might these factors contribute still more to our understanding of cell structure?***

Two-dimensional photographs lack any real depth of focus. Thus the three-dimensional inter-relationship of the various cell components cannot be truly appreciated. In the examination of real material under the microscope, some understanding of this third dimension may be obtained by the use of the fine focus. Colour is also missing from these particular photographs. Though unstained living material may not be coloured, the differential refraction of light of different wavelengths may give rise to colours which reveal additional information on cell components.

i ***What problems of interpretation are there in the microscopic examination of stained cells and of those which are viewed with different forms of microscopy?***

Staining techniques and different forms of microscopy give rise to artefacts. Part of the problem of interpretation involves distinguishing

these from genuine cell components. Students may like to make their own preparations of cheek cells similar to those shown in figure (S)84.
☐ They could also investigate the effects of stains such as methylene blue.

Cell size and cell shape

Principles

1 Most cells fall within a relatively narrow size range.
2 The relationship between surface area and volume changes as the linear dimension increases.
3 This changing relationship has implications for cell size and cell shape.

The section is introduced by a brief discussion of the two principal units of length used in the descriptions of cells.

STUDY ITEM
5.12 Surfaces and volumes

Questions and answers

a ***What are the surface areas of the two cells at the extremes of the size range?***

A spherical cell of diameter 0.5 µm has a surface area of 0.785 μm^2. A cell of similar shape and diameter 20 µm has a surface area of approximately 1257 μm^2. This and the following calculations can easily be carried out with the aid of an electronic calculator.

b ***By what factor has the surface area increased with this same increase in diameter?***

By 1600 times (that is, by 40^2).

c ***What are the volumes of each of the two cells at the extremes of the range?***

Application of the formula $\frac{4}{3}\pi r^3$ gives volumes of approximately 0.06545 μm^3 and 4189 μm^3 for the two hypothetically spherical cells.

d ***By what factor has the volume increased?***

The volume has increased by 64 000 times (that is, by 40^3).

e ***What is the implication of your answers to b and d for the upper limits of cell size?***

The fact that the volume increases at a faster rate than the surface area and that materials must enter and leave the cell through the surface means that there will come a point when this surface is no longer sufficient to serve the needs of the cell. This can be overcome to a certain extent by specialization of shape, but communication within the cell then becomes difficult. This is discussed briefly in the concluding part of this section.

f *Suggest reasons for the lower limits of cell size.*

Cells have a minimum size which is determined by the range of molecules which they must contain. In addition to the genetic material, each of the whole range of enzymes and other molecules must be present in sufficient numbers to permit collisions to take place between reacting molecules. The concentration of particular molecules within cellular organelles facilitates this. □

5.2 The ultrastructure of the cell

Assumptions

1 That students have some knowledge of cell components, derived from the previous section.
2 An understanding of scale and of the relationship between the units micrometre (μm) and nanometre (nm) (this is particularly important for the interpretation of electronmicrographs).

Principles

1 The resolving power of the electron microscope is much greater than that of the light microscope owing to the much shorter wavelength of the electron beam.
2 This greater resolution makes it possible to examine and interpret the details of structures which are too small even to be seen with the light microscope.
3 The electron microscope has important limitations.
4 There are fundamental differences between prokaryotic and eukaryotic cells.

STUDY ITEM
5.21 Electronmicrographs of cell structure

Questions and answers

a ***What additional information is available from the transmission electronmicrograph which cannot be derived from the photomicrograph?***

Figure (S)86b provides much additional information about the cell components. Some internal structure of the chloroplasts, mitochondria, and nucleus can also be seen. Portions of the bases of the two flagella, together with the flagellar swelling, are clearly visible. The cytoplasm can be seen to be traversed by numerous membranes. A number of other smaller bodies can be discerned, though their detailed structure cannot be determined from this electronmicrograph.

b ***Examine the mitochondrion labelled in* figure [S]86b. *Use the magnification given to determine its dimensions.***

The labelled mitochondrion, and indeed the others clearly visible in this cell, are about 0.5 μm in diameter.

c *Remember that this electronmicrograph is taken from a thin section through a cell. From the evidence of this thin section describe the three-dimensional structure of a mitochondrion.*

The appearance of the mitochondrion in section would suggest that the organelle is bounded by two membranes, the inner one of which has a number of flat folds or finger-like projections. All the mitochondria visible in this cell would appear to be spherical or slightly ovoid in overall shape.

d *Assuming that the mitochondria are evenly distributed through the cytoplasm, how many mitochondria are there in this cell? What else must you assume in making this calculation?*

About 60 mitochondria are visible in this section, which is approximately 50 nm thick. Reference to the three parts of the figure would suggest that a *Euglena* cell is about 10 μm across. The section shown in figure (S)86b thus represents only $\frac{1}{200}\left(\frac{50\,\text{nm}}{10\,\mu\text{m}}\right)$ of a *Euglena* cell. However, each mitochondrion is about 0.5 μm thick and would thus appear in 10 $\left(\frac{0.5\,\mu\text{m}}{50\,\text{nm}}\right)$ consecutive sections. The total number of mitochondria in the cell is therefore *very* approximately

$$60 \times \frac{200}{10} = 1200 \text{ mitochondria.}$$

It must be assumed that the mitochondria are indeed approximately spherical in shape.

e *What additional information is provided by the scanning electronmicrograph shown in* figure [S]86c?

It suggests a furrowed surface to the pellicle of the *Euglena.*

f *Bearing in mind what we have said about the techniques of preparing specimens for electronmicroscopy, what inherent problems are there in the interpretation of electronmicrographs?*

It has already been explained that living material for electronmicroscopy must first be killed, fixed, and dehydrated before embedding in a hard supporting resin. Only then can it be sectioned. Each of these stages may introduce artefacts which are open to misinterpretation when the electronmicrograph is examined.

g *From the evidence of* figures [S]86 *and* [S]87 *what are the principal differences between prokaryotic and eukaryotic cells?*

The most conspicuous difference is the apparent lack of internal organization in the prokaryotic cell. Membrane-bound cell organelles such as mitochondria, chloroplasts, or nuclei are not present, nor is the cytoplasm traversed by the endoplasmic reticulum. □

5.3 Cells and molecules

Assumption

1 A basic knowledge of elementary chemistry.

Principles

1 There is a basic similarity in the composition of living material, though the proportions of the different elements may vary between organisms.

2 Four-valent carbon plays a central role in the compounds which constitute living material.

STUDY ITEM

5.31 The composition of living material

Questions and answers

a ***What are the four elements mentioned above which make up about 85 to 95 per cent of the body mass of a human being or a corn plant?***

Carbon, oxygen, hydrogen, and nitrogen form 87.4 per cent of the total dry mass of a human being and 95.7 per cent of the total dry mass of a corn plant. In the human being calcium and phosphorus, as important constituents of bone, also form a significant proportion of the body mass.

b ***What other feature do these six elements have in common?***

They are the smallest elements that readily form strong stable covalent bonds with each other. This is the first step in the formation of molecules and macromolecules.

c ***In each case suggest a reason for the abundance of these elements in the human body.***

The first four are the major constituents of carbohydrates, fats, and proteins. The proportion of protein in mammalian tissue is very much higher than it is in most plants. Nitrogen is an important constituent of protein. The reason for the abundance of calcium and phosphorus has been mentioned already in answer to question **a**. □

The concluding paragraphs of this short section are concerned with the central role of carbon in the chemical constitution of living material.

5.4 The form and function of cellular organelles

Assumption

1 Some acquaintance with cellular organelles gained from the section on the ultrastructure of cells (section 5.2).

Principles

1 The cytoplasm is not a uniform 'soup' of compounds in an aqueous medium, but a complex, highly organized, membrane-bound collection of organelles, each with a specific function.

2 Fundamental differences exist between animal and plant cells.

During the last few decades work with the electron microscope has taken our understanding of cellular structure to a level that was undreamed of with the light microscope. Some of the major organelles are bound by a double membrane envelope. These include the nucleus, the chloroplasts and other plastids, and the mitochondria. Other organelles, such as the microbodies and lysosomes, are bound by a single membrane. Most of these organelles, and others described in the *Study guide*, are illustrated by electronmicrographs. The chloroplast will be considered in Chapter 7. There are some fundamental differences between plant and animal cells and these two cells are highlighted in the text and in the following Study item.

STUDY ITEM

5.41 Plant and animal cells

Questions and answers

a ***Which, of A and B, is a plant cell and which is an animal cell?***

The presence of a large central vacuole, chloroplasts, and a cellulose cell wall clearly identify figure (S)98 as a plant cell (in fact it is a section through a cell from a leaf of timothy grass, *Phleum pratense*). The animal cell in figure (S)99 is an exocrine cell from a rat pancreas.

b ***Make a large sketch of each of the cells and include more detail of representative areas to show the following structures: plant cell wall, plasma membrane, chloroplast, vacuole, nucleus, nucleolus, mitochondria, and endoplasmic reticulum. Label these and any other visible structures on your drawings, and add a scale.***

This is intended as an exercise in observation and recording. It will be necessary for students to return to the electronmicrographs earlier in this section to identify the various organelles specified.

c ***What is the approximate thickness of the cell wall you have drawn?***

Application of the scale suggests that the wall is approximately 0.1 μm thick.

d ***What problems might be created by the presence of a relatively rigid cellulose cell wall around each plant cell?***

Clearly communication between cells may be hindered by the presence of an inert cellulose barrier between one cell and the next.

e ***What features might the cellulose cell wall possess which could ease the problems mentioned in your answer to question d?***

If there were pores in the wall to make communication possible. Such channels do exist, but are not visible in this particular figure but electronmicrographs of bordered pits are shown in figure (S)182.

f *What features would such organelles have to possess in order to exist as free-living prokaryotic cells?*

As well as being able to derive energy from some external source, such organelles would have to have some means of reproduction in order to exist as free-living organisms. □

5.5 The importance of cell membranes

Assumptions

1 A knowledge of the widespread existence of membrane-bound organelles and membrane systems within cells gained from previous sections of this chapter.

2 A background in basic chemistry for the understanding of parts of this section.

Principles

1 Membranes and membrane systems are important, not only in controlling the passage of materials in and out of the cell, but also in minimizing diffusion within the cell.

2 The differential permeability of biological membranes is related to the phospholipid bilayers of which they are composed.

The concept of biological membrane structure based on the fluid mosaic model is introduced as an example of the importance of relating function to structure at the molecular level. Though the detailed relationship of membrane proteins and phospholipids may not be fully understood, their overall functions are outlined.

STUDY ITEM

5.51 The uptake of mineral ions by plant tissue

The data on which this Study item is based are taken from Smithers and Wilson (1968) who also outline the practical details of their experiment. (See also page 209.)

Principles

1 The absorption of mineral ions into living cells is not merely a passive process, but is actively controlled by the cells. This control involves the expenditure of energy.

2 Cells are able to carry out preferential absorption of ions from mixed solutions.

Questions and answers

a ***Suggest a reason why the carrot or red beet tissue is cut into thin discs for such an investigation.***

Discs of the size and shape described have a high ratio of surface area to volume, which permits more rapid absorption from the surrounding solution.

b ***Why is it unnecessary to cut the maize roots in a similar way?***

The roots are thin and already have a high ratio of surface area to volume.

c ***What practical steps would you take, after removing the discs from the solution, before determining the amount of ion remaining in the solution?***

The discs should be thoroughly washed with distilled water and the 'washings' added to the remaining solution. This can then be made up to a convenient volume, say twice that of the original solution, before the determination of the remaining ion concentration is made.

d ***How might the amount of ion remaining in the solution be measured?***

The most obvious method, and probably the one known to most students, is titration. Flame photometric, colorimetric, and microdiffusion methods can also be used.

e ***What do the differences in the rates of absorption under these two forms of aeration suggest about the mechanism of absorption of*** Br^- ***and*** PO_4^{3-}***?***

The greatly increased rate of absorption of ions when air, as opposed to nitrogen, is bubbled through the solution, suggests that the absorption is an active process involving the expenditure of energy by the absorbing tissue.

f ***Suggest a mechanism for the absorption of these ions when nitrogen only is bubbled through the solution.***

The slow absorption of ions under these conditions is presumably due to passive diffusion.

g ***What effect does increasing the concentration of cations (***Na^+ ***and/or*** K^+***) have on the rate of uptake of cations?***

It is clear from the results shown in table (S)20 that the rate of absorption of both K^+ and Na^+ is increased if the initial external concentration is increased.

h ***What is the effect on the rate of absorption of each of these ions when they are in mixed solution?***

The rate of absorption of both ions appears to be depressed, though that for K^+ is affected very much more than that for Na^+. It may be that both ions compete for absorption.

i ***Suggest a mechanism that will explain your answer to question h.***

The most obvious explanation is that a mechanism is employed by the cell which transports both Na^+ and K^+ across the plasma membrane.

The two ions compete for this mechanism which shows a preference
☐ for Na^+.

5.6 Metabolism, the Laws of Thermodynamics, and the role of enzymes

Assumptions

1 Some knowledge of enzymes, and the conditions which limit their action, gained from previous courses (these are likely to have focused chiefly on digestive enzymes).

2 Some appreciation of the Laws of Thermodynamics.

Principles

1 Metabolism consists of catabolic, or energy-releasing, reactions which drive anabolic, or energy-consuming, reactions.

2 These processes of energy transfer are governed by the First and Second Laws of Thermodynamics.

3 The energy release from catabolic processes takes place over a number of small-stage reactions which permit the controlled transfer of energy.

4 Many metabolic reactions are speeded up by enzymes, which lower the activation energy required for the reaction.

5 Enzyme activity is normally specific to one reaction.

Some of the concepts involved in this section, especially those of entropy and activation energy and the role of enzymes in lowering the latter, are not easily grasped. The analogies given in the *Study guide* should prove helpful, but those who are also studying other science subjects may find the ideas more easily understandable than those who are not.

The properties of enzymes

Practical investigation. *Practical guide* 2, investigation 5A, 'A metabolic pathway in yeast'.

STUDY ITEM

5.61 Enzymes and pH

Principle

1 Enzymes have an optimum pH, which may vary from one enzyme to another.

Questions and answers

a ***Plot a graph of the data.***

The independent variable (pH) should be placed on the x axis and the dependent variable (rate of activity) on the y axis.

b ***What is the pH value at which urease appears to have its maximum rate of activity (known as the optimum pH)?***

This is difficult to establish precisely from the graph, but would appear to be at about pH 6.9 to 7.0, at which point the rate of activity is about 0.9 to 1.0 arbitrary units.

c ***Within what range of pH values does the enzyme act at over 50 per cent of its maximum rate of activity?***

Between approximately pH 5.9 and 8.2 the rate of activity of the urease is at or above 50 per cent of its maximum rate.

d ***What is the optimum pH of the enzyme pepsin?***

This can be seen to be about pH 2.0.

e ***Within what range does pepsin act at over 50 per cent of its maximum rate of activity?***

Data for pH values less than 1.0 are not shown on this graph. At about pH 3.5 the rate of activity falls to 50 per cent of its maximum value.

f ***What would you expect to be the pH of the stomach contents?***

If pepsin is to act at anywhere near its maximum rate, the pH of the stomach contents must be about 2.0. This is in fact close to its actual value in most cases. □

STUDY ITEM
5.62 Enzymes and temperature

Principles

1 Enzymes have an optimum temperature for activity.
2 Caution must be exercised when extrapolating results. Early trends are not necessarily continued.
3 Control experiments eliminate variables other than the one under investigation.

Questions and answers

a ***On the same axes, plot graphs of the theoretical and experimental results.***

The independent variable (temperature) should be plotted on the x axis and the dependent variable (yield) on the y axis.

b ***Calculate, in arbitrary units, the inhibitory effect which comes into operation at higher temperatures.***

The inhibitory effect can be calculated by subtracting the actual reading from the theoretical value at each temperature.

c ***Draw another graph to show this effect.***

Temperature is again plotted on the x axis, with the inhibition values on the y axis.

d ***What assumption was made when the theoretical yields were calculated?***

It was assumed that the early trend would continue indefinitely with an increase in temperature; that is, the increase is exponential.

e ***Does the inhibitory effect follow the same or a different pattern of change as the theoretical yield?***

It can be seen from the graph plotted in answer to question **c** that the inhibition also increases exponentially.

f ***Without reference to the experimental results, calculate the theoretical yield of the product at 60 °C.***

The theoretical yield at 60 °C would be twice that at 50 °C, that is, 96 mg per 10 minutes.

g ***Estimate, from the experimental results, what the actual yield would be at 60 °C.***

It can be seen from the inhibition figures, that the inhibitory effect is approximately doubled for each 5 °C rise in temperature. This would give an inhibitory effect of about 96 mg per 10 minutes at 60 °C, which is equal to the theoretical yield at that temperature. The projected actual yield would therefore be zero at 60 °C.

h ***The experimenters who carried out this work drew the conclusion that the tissue extract contained an enzyme, or enzymes, responsible for breaking down the substrate. What evidence is there to support this conclusion?***

The system of substrate and extract is thermolabile. This might indicate the presence of an enzyme or enzymes in the tissue extract.

i ***Can we rule out the possibility that the substrate itself is being broken down by heat?***

From the evidence available here the possibility that the substrate itself is thermolabile cannot be ruled out. However it is clearly not being broken down to form the same end-products as those brought about by the action of the enzyme.

j ***What further experiments would you recommend, in order to discover if the tissue extract does in fact contain an enzyme, or enzymes?***

A control experiment to discover the effect of heat on the substrate alone is clearly necessary. Useful additional evidence could be provided by heating and then cooling the tissue extract before using it. Using different dilutions of the tissue extract or running the experiment at different pH values would provide still more evidence. □

The mechanism of enzyme action

Principles

1 The mechanism of enzyme-catalysed reactions involves the formation of an enzyme–substrate complex.

2 Many of the properties of enzymes reflect the fact that they are proteins.

3 Models of enzyme action involve the existence of an active site on the enzyme molecule which binds with the substrate molecule.

Since the early work on protein structure in the 1960s, molecular biologists and biophysicists have elucidated a great deal of the mechanism of enzyme action. Some of the ideas arising from this and from earlier research are outlined in this section. The 'lock and key' and the 'induced fit' models are discussed as alternative, but not necessarily mutually exclusive, hypotheses. Finally the role of inhibitors is considered.

STUDY ITEM

5.63 The 'lock and key' model of enzyme action

Questions and answers

a ***How can each of the observations 1 to 4 be explained in terms of the 'lock and key' model of the activity of enzymes?***

1 A key can serve equally well to lock or unlock a padlock. Hence the analogy can be used to interpret the reversible aspect of enzyme activity.

2 This would be expected if enzyme molecules acted as keys. Up to a point a given number of keys can open an increasing number of padlocks, but there is a limit to this when each key is operating at its fastest rate. From then on additional padlocks (that is, substrate molecules) will have no effect on the rate of opening (that is, the rate of reaction).

3 Presumably the same key (succinic dehydrogenase) will fit into two padlocks (the substrates succinic acid and malonic acid). But it will only open the former; hence only with succinic acid is hydrogen lost. Thus the active site of the enzyme only fully fits the pattern of succinic acid. However, because it can loosely fit into malonic acid, it will prevent combinations with succinic acid and thus the reaction is inhibited. The lock and key analogy can therefore be used to interpret competitive inhibition.

4 A key can only enter a padlock if it is in direct contact with it. We know that molecules move more at higher temperatures and thus come into more frequent contact. Hence you would expect the initial increase of enzyme activity with temperature.

b ***To what extent do any of these observations indicate that the model may be inadequate?***

At about 45 °C something apparently happens to the lock and key mechanism, and it may be argued that this model is no longer tenable. However, it could be assumed that the molecular pattern of the enzyme becomes disrupted so that the reaction becomes impossible. Enzymes are proteins and the influence of high temperature on proteins (such as egg albumen) is well known. Thus the idea of the active site of the enzyme being disrupted would appear credible. Indeed it is suspected that the hydrogen bonds between the polypeptides of the protein are broken and the alpha-helical structure of the protein unwinds. That is, the protein is said to be denatured. □

Practical investigations. ***Practical guide 2*****, investigation 5B, 'Enzyme extraction', investigation 5C, 'The course of an enzyme-catalysed reaction', and investigation 5D, 'An enzyme-catalysed synthesis'.**

5.7 Cellular respiration and the mitochondrion

Assumptions

1 A knowledge of basic chemistry, particularly of oxidation–reduction reactions.
2 A knowledge of the earlier sections of this chapter, in order to place the reactions described in their appropriate sites in the cell.

Principles

1 Cellular respiration consists of a large number of enzyme-catalysed steps which permit the controlled transfer of energy.
2 Such steps are essentially oxidative, involving the removal of hydrogen atoms by a hydrogen acceptor.
3 The first stages in the oxidation of glucose take place in the cytoplasm and result in the production of pyruvic acid coupled with the production of relatively small amounts of ATP and $NADH_2$.
4 Under aerobic conditions the pyruvic acid is oxidized further to carbon dioxide. This takes place in the mitochondria and results in the production of large amounts of $NADH_2$ and hence of ATP.
5 The oxidative breakdown of pyruvic acid is via a cyclical pathway.

It is important that students understand the overall principles which have been outlined above and do not become preoccupied with the biochemical details of the breakdown pathway. Thus glycolysis can be considered as the splitting of a six-carbon sugar which, after some rearrangement, gives rise to two molecules of the three-carbon pyruvic acid. A relatively small amount of energy is available for transfer from this breakdown. The oxidation of pyruvic acid can be thought of as an initial removal of carbon dioxide to form a two-carbon compound, which combines with a four-carbon compound to form the six-carbon citric acid. Two further decarboxylations and some rearrangement once again produce the four-carbon compound, which can recombine with a further molecule of the two-carbon product of the decarboxylation of pyruvic acid. This cyclical process enables large amounts of energy to be transferred to anabolic reactions in the cell. Students able to cope with more detailed biochemistry can obtain such information from a number of sources mentioned in 'Suggestions for further reading'. However, they should not be tempted to do so at the expense of the overall picture outlined in the *Study guide.*

Oxidation

Practical investigation. *Practical guide* 2, investigation 5E, 'The uptake of oxygen as a measure of metabolism'.

The citric acid cycle – oxidation in the mitochondrion

Practical investigation. *Practical guide* 2, investigation 5F, 'Respiratory quotient'.

5.8 The cell as an integrated unit

Principle

1 The numerous metabolic reactions occurring in any living cell are co-ordinated and integrated by a variety of mechanisms.

This section provides the student with the opportunity to consider the cell as a whole. In spite of the fact that cellular metabolism is compartmentalized by the cell's membrane systems, it is nonetheless highly co-ordinated and integrated into a single functional unit.

PART II The *Practical guide*

The investigations related to this chapter appear in *Practical guide 2, Chemical reactions in organisms.*

INVESTIGATION 5A A metabolic pathway in yeast

ITEMS NEEDED

Yeast, dried

Fructose, 0.2 mol dm^{-3}
Galactose, 0.2 mol dm^{-3}
Glucose, powdered (*continued*)

(*Study guide* 5.6 'Metabolism, the Laws of Thermodynamics, and the role of enzymes'.)

Sugar solutions. Allow 20 cm^3 of each solution for 10 to 12 experiments. For the fructose, glucose, and galactose solutions, dissolve 36 g in 1 dm^3 distilled water. For the sucrose and lactose solutions, dissolve 68 g in 1 dm^3 distilled water.

ITEMS NEEDED *(continued)*

Glucose, 0.2 mol dm^{-3}
Lactose, 0.2 mol dm^{-3}
Sucrose, 0.2 mol dm^{-3}
Water
Yeast extract

Balance 1/class
Beaker, 250 cm^3 1/class
Conical flasks or beakers, 100 cm^3 5/class
Durham tubes 10/group
Graph paper
Measuring cylinders, 50 or 100 cm^3 2/group
Pipettes 2/group
Ruler 1/group
Spatula 1/group
Stopclock or stopwatch 1/group
Test-tubes 10/group
Water baths, thermostatically controlled at temperatures of 20 °C, 35 °C, and 50 °C 3/class

Assumptions

A knowledge of the following:

1 The specificity of enzymes.
2 The other properties of enzymes and enzyme-catalysed reactions.
3 The role of fermentation and, more generally, respiration.
4 The concept of Q_{10} and the range of values expected in physiological processes.

Principles

1 Many physiological processes have features which resemble the properties of enzymes, for example, specificity, temperature dependence, and pH optimum. This reflects the fact that metabolic pathways are sequences of enzyme-catalysed reactions.
2 Organisms determine which compounds are absorbed and respired through the specificity of the proteins, which transport and metabolize these compounds. These proteins can possess a high degree of discrimination between very similar compounds (for example, glucose and galactose).
3 Without the appropriate enzymes the conversion of glucose to ethanol and carbon dioxide is an extremely unlikely event. A large number of enzyme-catalysed reactions ensures that the conversion proceeds rapidly. Other enzymes couple this conversion to the synthesis of ATP.
4 Enzymes perform a great variety of functions within cells.

In order to complete this investigation in just over one hour it is essential that stages 1 to 3 and 9 of the procedure are carried out well in advance.

This investigation uses a semi-quantitative method of measuring carbon dioxide production because it is reliable and fairly rapid. A class can investigate several aspects of fermentation and pool the results at the end. (It is not expected that one student would be able to cover all of the suggested experiments within one hour.)

A number of other experiments can be substituted for, or added to, those suggested in the procedure. For instance, the yeast suspension could be boiled before use. As well as investigating potential substrates that possess very similar molecular structures (for example, glucose and galactose), you might find it helpful to include other classes of compounds that are potential substrates for fermentation (for example, amino acids or fatty acids). It is essential to provide a textbook or diagram and, ideally, models which will show the molecular structure of each substrate being used.

Because this investigation examines only the initial substrates and final products of fermentation it does not show that the metabolic pathway is a series of chemical reactions. A question is included to discuss this point in the light of evidence obtained with extracts from yeast cells. Though such an extract contains no living cells, it may still catalyse the following chemical conversion:

$$\text{glucose} + \text{ADP} + \text{phosphate} \xrightarrow{Mg^{2+}} \text{ethanol} + \text{carbon dioxide} + \text{ATP}$$

At the end of the last century the Buchner brothers in Germany regarded this extract as a single substance and gave it the name 'zymase'. Subsequent chromatographic and chemical analysis showed that 'zymase' is a mixture of several enzymes, each catalysing a distinct chemical reaction. Together these form a chain of reactions which bring about the overall conversion.

Questions and answers

a ***Do your results suggest that the metabolism of yeast cells shows a preference for one sugar rather than another? If so, which sugar is preferred, and what features of its molecular structure distinguish it from the other sugars which you have tested?***

It is important that students make the connection between the effectiveness of sugars as substrates and their molecular structure. They will then appreciate how enzymes might distinguish between these molecules through the complementary structure of their binding sites. (Rather than record the *complete* structures of various sugars it is more important to note the *differences* between their structures. For instance, though galactose is not fermented, it differs from glucose, which is fermented, only in the distribution of —OH and —H groups around a single carbon atom; they are stereoisomers.)

b ***If a yeast cell were presented with several sugars in its environment what type of mechanism might ensure that it would absorb most rapidly the sugar that it could metabolize most efficiently?***

Discrimination in the absorption of different compounds could be achieved in two ways:

1 Two compounds, A and B, diffuse freely into a cell along their concentration gradients. Because of the specificity of the cell's enzymes one compound, A, is metabolized, but the other is not. As long as A is removed as a result of metabolism it will continue to move into the cell long after the concentration of B has reached equilibrium across the cell membrane.

2 The molecules of certain compounds cross cell membranes at specific sites where they bind to 'carrier proteins'. The specificity of these proteins allows discrimination in the absorption of these compounds.

c ***In what way does the rate of fermentation vary with temperature? Though the data obtained in this investigation are semi-quantitative it may be possible to estimate the Q_{10} value for fermentation. Does your estimate agree with what you expect for physiological processes?***

The rate of fermentation increases with temperature up to about 40 °C. At temperatures above this the rate falls. In the temperature range of 20° to 40 °C the value of Q_{10} is very high, ranging from 3 to 4. This is significantly higher than the value of 2 often quoted as 'typical' of the Q_{10} value for physiological processes. Above 40 °C the Q_{10} value drops below 1.

d ***Summarize the evidence that you have obtained to suggest that an enzyme (or enzymes) could be involved in fermentation.***

Fermentation shows a specificity towards its substrate and a temperature optimum. Both of these characteristics are typical of enzyme-catalysed reactions.

e ***Cell-free extracts of yeast can carry out alcoholic fermentation. That is, they will catalyse the conversion of sugars into ethanol and carbon dioxide in the absence of living cells. Suggest how you might find out whether such an extract contains a single enzyme or several enzymes responsible for fermentation.***

Chromatography is the key technique and students should be familiar with it from previous courses in biology and chemistry. A number of experiments are possible. The two described here are typical of the biochemist's approach to analysing physiological systems.

1 Assume that the extract is a mixture of enzymes and attempt to separate them into groups by chromatography. If successful, determine whether the groups are separately capable of fermentation. If they are not, add them back together to reconstitute the original extract and check that this is still capable of fermentation. In this way it can often be shown that more than one protein is required to catalyse a particular metabolic conversion.

2 Use chromatography to analyse what compounds, other than ethanol and carbon dioxide, are produced during the fermentation of glucose by the yeast extract. If a compound (for example, fructose-6-phosphate) is identified in this way, it is then possible to determine whether it is an intermediate in the metabolic pathway proceeding to carbon dioxide and ethanol. This is done by adding the particular compound alone to the extract and then looking for the production of ethanol and carbon dioxide (see below).

If

$$\text{glucose} \xrightarrow{\text{extract}} \text{fructose-6-phosphate} \xrightarrow{\text{extract}} \text{ethanol} + \text{carbon dioxide}$$

then

$$\text{fructose-6-phosphate} \xrightarrow{\text{extract}} \text{ethanol} + \text{carbon dioxide}$$

After demonstrating that more than one reaction is involved the next step is to identify and purify the enzymes that catalyse these reactions.

INVESTIGATION
5B Enzyme extraction

ITEMS NEEDED

Germinating seeds (barley), untreated 24 g/group

Iodine in aqueous potassium iodide solution (*continued*)

(*Study guide* 5.6 'Metabolism, the Laws of Thermodynamics, and the role of enzymes'.)

Iodine in aqueous potassium iodide solution. Dissolve 6 g potassium iodide in about 200 cm^3 distilled water; add 3 g iodine crystals and make up to 1 dm^3 with distilled water; stand for 24 hours to allow iodine to dissolve.

ITEMS NEEDED *(continued)*

Sodium chlorate(I) (sodium hypochlorite) solution, diluted to 1 %
Starch suspension, 0.1 %
Water, distilled
Water, sterile

Beaker, 250 cm^3 1/group
Centrifuge 1/class
Centrifuge tubes
Conical flask with bung, 100 cm^3 1/group
Dish (to germinate the barley grains) 1/group
Filter paper
Incubator, set at 25 °C 1/class
Measuring cylinder, 100 cm^3 1/group
Mechanical homogenizer 1/class or pestle and mortar 1/group
Muslin or nylon stocking (3 or 4 pieces to fit over beaker) 1/group
Pipette, dropping 1/group
Test-tubes 2/group

Starch suspension, 0.1 %. Mix 1 g starch with a little cold distilled water; stir into about 800 cm^3 of boiling, distilled water; when cool, make up to 1 dm^3.

If a mechanical homogenizer is available it may be more practical for several groups to pool their barley in order to prepare the extract. The initial surface sterilization of the grains is important. Germinating grain can produce unpleasant fungal growths and it could be argued that the ability to degrade starch was derived in part, or wholly, from the micro-organisms rather than the grain itself.

Principles

1 It is possible to extract the non-living components from living cells and study them in isolation. Ideally students should experience this for themselves. If they only use purified enzymes obtained from suppliers, they may fail to associate these compounds with living systems.

2 Many enzymes that are dissolved in the cytoplasm can be extracted by homogenizing or grinding the tissue in order to break open its cells. The cell debris can then be removed by filtration and centrifugation.

3 Amylase from barley grains is an unusually stable protein and it will not require any special conditions for extraction for its activity to be retained. This is exceptional. (See question **d** below.)

Questions and answers

a ***Which parts of the germinating barley, do you think, have been discarded in the extraction process?***

The husks or pericarp with insoluble endosperm.

b ***What tests could be performed on the discarded material to reveal why the seeds should contain starch-digesting enzymes?***

Iodine solution could be used to test for the presence of starch, and Benedict's test for the presence of reducing sugars. The presence of these compounds within the barley grain makes sense if the starch-digesting enzyme is also present.

c ***How would you obtain evidence that the starch-digesting activity of the extract is indeed due to the presence of an enzyme (that is, an amylase)?***

Boil the extract and then test for its ability to degrade starch. The loss of this activity would be consistent with heat denaturation of an enzyme.

d ***Many enzymes are likely to denature (lose their specific protein structure and enzyme activity) when they are removed from the special conditions of the cytoplasm. Were any special precautions taken to ensure that the barley extract retained its ability to degrade starch?***

No. Barley amylase is unusual in being very robust in the range of conditions it can tolerate. The extraction of most intracellular enzymes must be carried out at low temperatures (0 ° to 4 °C), and in solutions which are well buffered at a particular pH and have suitable concentrations of specific ions. The most appropriate conditions must be determined separately for each enzyme.

INVESTIGATION
5C The course of an enzyme-catalysed reaction

ITEMS NEEDED

Enzyme extract prepared in investigation 5B

Iodine in aqueous potassium iodide solution (see page 122)
Starch suspension, 0.2 %
Water, distilled

Colorimeter with red filter 1/group
Colorimeter sample tube 1/group
Conical flask or beaker, 50 cm^3 1/group
Graph paper 1/group
Measuring cylinder, 25 cm^3 1/group
Stopclock or stopwatch 1/group
Syringes, disposable plastic, 1 cm^3, or pipettes, dropping 2/group
Syringe, disposable plastic, 5 cm^3 1/group
Test-tube 1/group
Water bath, set at 25 °C 1/class

(*Study guide* 5.6 'Metabolism, the Laws of Thermodynamics, and the role of enzymes'.)

Starch suspension, 0.2 %. 2 g dm^{-3} distilled water made up as described on page 123.

Several students could share a single colorimeter. Stable colorimeters are essential for this work and it is better to share a reliable machine rather than use a number of erratic ones. The students should be given the opportunity to find out by preliminary trial how to obtain consistent readings. For example, the preparation of the conversion graph for starch concentrations (see stages 10 and 11 in the procedure) provides good practice. The readings obtained should produce a smooth curve.

Principles

1 The digestion of starch can be quantified and its progress measured.
2 Colorimetry has proved to be a very useful and versatile technique for studying the catalytic activity of a wide variety of enzymes.

Students will probably know from background knowledge that the digestion of starch is catalysed by amylase. The aim here is to measure amylase activity, using the iodine test, and to follow the course of the reaction. Once this has been done and the characteristics of the normal reaction have been established, it is possible to discover one or two properties of the enzyme by modifying the experimental conditions.

Questions and answers

a ***Draw a graph of starch concentration against time and compare it with* figure [P]6. *In what ways are the two graphs similar? How do they differ?***

Ideally the graphs of starch and casein digestion should be similar in that they both should show relatively fast rates of digestion in the early stages of the reaction. Students should be encouraged to give reasons for this. The fact that the curves bend in opposite directions should be noted, but it is unimportant. The course of the reaction is followed by measuring in one case the disappearance of the substrate and in the other the appearance of the product.

b ***Look up the logarithm (base 10) of each starch concentration and then plot the log of starch concentration against time. (Before doing this it is a good idea to express the starch concentrations in mg 100 cm^{-3}. This avoids decimals and the corresponding negative logarithms.) What information does this graph provide which the previous one did not?***

The points should be on or near a straight line, showing that the change in concentration of starch has an exponential relationship with time. This cannot be deduced merely by looking at the previous graph of starch concentration against time. Two faults in procedure commonly prevent these data from producing a straight line on a graph. Firstly, if the initial concentration of starch is too high the colorimeter will not detect significant changes in colour intensity in the early stages of the reaction. Secondly, a 'log plot' will exaggerate any errors in the measurements taken during the final stages of the reaction. If the students' data are largely confined to the last third of the reaction they are unlikely to be sufficiently accurate for a log plot.

c ***Name one phenomenon, not necessarily biological, which shows a similar relationship with time. What is the reason for the similarity between starch digestion and the phenomenon that you have named?***

One example is the cooling curve of a body. Others include radioactive decay and the discharge of a capacitor. All these phenomena are governed by a common principle. With digestion and radioactive decay the rate of decrease is related to the amount of material remaining. It is like water running out of a burette – fast at first, but then getting slower as the head of water decreases. This model is also analogous to the cooling curve, where heat loss is related to the difference in temperature.

d ***How would you modify the above procedure to investigate either the effect of temperature (0° to 100° C) or the effect of the pH of the reaction mixture on starch digestion? Give sufficient details to serve as instructions.***

Note the time (t) for the enzyme–substrate mixture to produce, at 15 °C, one-third of a full-scale deflection of the colorimeter. Set up identical reaction mixtures at different temperatures such as 30 °C, 45 °C, 60 °C, 75 °C, and 90 °C. After exactly t minutes add a sample to the iodine solution and record the intensity of the colour. Repeat this for each temperature. Convert the reading into starch concentrations and plot a graph of these concentrations against temperature. To investigate the effect of pH, set up a similar experiment, keeping the temperature constant, but add acid or alkali to make a range of pH values.

e ***Suggest a hypothesis to explain what happens to the starch when the suspension is mixed with the enzyme extract. What experiments would test this hypothesis?***

The students may well suggest that the enzyme extract catalyses the digestion or hydrolysis of the starch. There are really a number of hypotheses here. The appearance of reducing sugars in the reaction mix, detected by means of Benedict's test, would support the suggestion of hydrolysis. If the reaction has taken place because of catalysis, then the reaction mix will be capable of catalysing the hydrolysis of a second batch of starch after the first has been digested. The catalyst is not consumed in the reaction. If the catalyst is an enzyme, its activity will be destroyed by boiling the extract for 5 to 10 minutes.

INVESTIGATION
5D An enzyme-catalysed synthesis

ITEMS NEEDED

Potatoes, medium-sized 2/group

Glucose solution, 1 %
Glucose 1-phosphate solution, 1 %
Iodine in aqueous potassium iodide solution (see page 122)
Maltose solution, 1 %
Sucrose solution, 1 %
Water, distilled

Beakers, 100 cm^3 4/group
Centrifuges and tubes 1–2/class
Colorimeter with red filter 1/group
Colorimeter tube 1/group
Flask, stoppered, 50 cm^3 1/group
Graph paper
Measuring cylinder, 50 cm^3 1/group
Mechanical homogenizer 1/class or pestle and mortar 1/group
Muslin or nylon stocking
Pipette, dropping 1/group
Scalpel, 1/group
Spatula 1/group
Stopclock or stopwatch 1/group
Syringes, disposable, 1 cm^3 2/group
Syringe, disposable, 5 cm^3 1/group
Syringe, disposable, 10 or 20 cm^3, depending on the size of the colorimeter tube 1/group
Test tubes 7/group
Tile, white 1/group
Water baths, set at 25 °C 1–2/class

(*Study guide* 5.6 'Metabolism, the Laws of Thermodynamics, and the role of enzymes'.)

Sugar solutions. 50 cm^3 of the sugar solutions will be sufficient for eight experiments. If time is short, these solutions should be made up in advance. Store the solution of glucose 1-phosphate at 4 °C for no more than 24 hours.

Potatoes. Those of medium size are most satisfactory. Large potatoes sometimes produce a poor yield of phosphorylase enzyme. Small, 'new' potatoes have such small grains of starch that these are difficult or impossible to spin down.

Principles

1 Virtually all metabolic reactions are catalysed by enzymes. This includes both synthetic as well as degradative reactions. Potato phosphorylase catalyses the synthesis of starch from glucose 1-phosphate.

2 A metabolic pathway, or reaction, responsible for the synthesis of a compound (an anabolic pathway) is rarely a simple reversal of the metabolic pathway, or reaction, which degrades the compound (a catabolic pathway).

3 In the reaction catalysed by potato phosphorylase the energy necessary to ensure that the synthesis of starch can take place is provided by the simultaneous hydrolysis of glucose 1-phosphate.

Questions and answers

a ***Describe the appearance of the mixtures at the end of the investigation. Which of the substrates produced starch after the addition of potato extract?***

All the mixtures may appear a little darker at the end of the experiment. This has nothing to do with the formation of starch but is due to the action of polyphenol oxidase and is the same process that causes apples to turn brown when cut. The mixture containing glucose 1-phosphate should produce starch. If a drop is examined under a microscope, granules (4 to 10 μm in diameter) may be seen.

b ***Compare the graphs obtained in this investigation with that of starch digestion which you drew in answer to question a of investigation 5C. Describe any similarities and differences between them.***

See *figure 20* and the answer to question **c**.

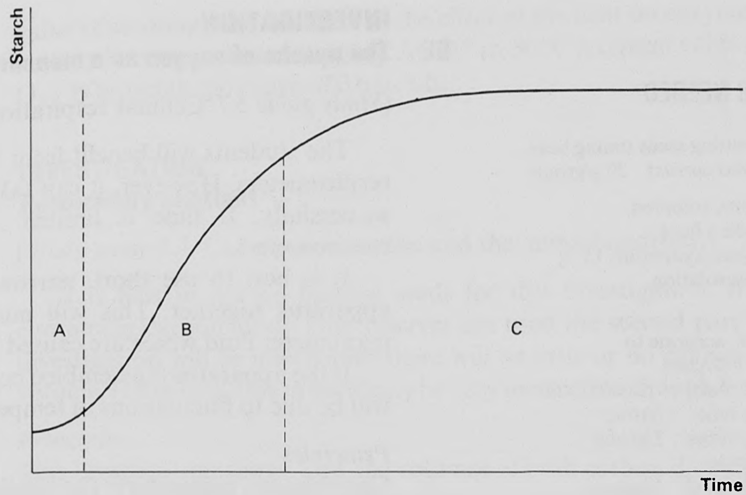

Figure 20
The progress of starch synthesis. (A = lag phase; B = synthesis; C = substrate used up.)

c ***Can you detect from the graphs any differences in the early phases of starch synthesis and digestion. If so, suggest a hypothesis to account for this difference.***

The graph should follow the expected path: a rise in starch concentration to a maximum, at which point all the glucose 1-phosphate has been used up. If sufficient data are gathered early in the course of the reaction the graph may show a curious, initial lag phase. C. S. Hanes (1940) showed that the synthesis was autocatalytic, because the lag phase was decreased by adding small amounts of soluble starch to the reacting mixture of enzyme and glucose phosphate. To propose such a hypothesis of autocatalysis is possibly beyond the capabilities of most students at this stage. However it is worth describing, as it is an example of the way in which graphs may be used to develop hypotheses.

d ***What evidence have you obtained which will enable you to deduce whether or not starch synthesis is a simple reversal of starch digestion?***

The digestion of starch yields maltose and ultimately glucose. Potato phosphorylase fails to catalyse starch synthesis with either sugar as precursor. Instead, the phosphorylated sugar is the required substrate.

e ***Make a list of likely substrates for the potato enzyme which might be tested in addition to glucose, maltose, and sucrose.***

There seems to be little point in trying other non-phosphorylated sugars. Other possibilities include the closely related phosphorylated sugars, such as galactose 1-phosphate or xylose-1-phosphate. Alternatively, glucose with an inorganic phosphate, or glucose with ATP or glucose 6-phosphate may be possible substrates for potato phosphorylase.

INVESTIGATION
5E The uptake of oxygen as a measure of metabolism

(*Study guide* 5.7 'Cellular respiration and the mitochondrion'.)

ITEMS NEEDED

Germinating seeds (mung bean, *Phaseolus aureus*) 20 g/group

Kerosene, coloured, or Brodie's fluid
Potassium hydroxide, 15 % aqueous solution
Water

Balance, accurate to 0.1 g 1–2/class
Basket, metal or plastic, to fit boiling-tube 1/group
Boiling-tubes 2/group
Filter paper
Funnel, small 1/group
Graph paper
Manometer and scale 1/group
Measuring cylinders, 10 cm^3 2/group
Rubber bungs, 2-holed, to fit boiling-tube 2/group
Screw clip 1/group
Stopclock or stopwatch 1/group
Syringe, disposable 1 cm^3 1/group
Thermometer, 0° to 50 °C 1/group
Tubing, glass connecting 1/group
Tubing, rubber connecting 1/group
Water bath, set at 20 °C 1–2/class

The students will benefit from the exercise of assembling their own respirometers. However, it can take a very long time to complete this successfully. If time is limited, provide them with pre-assembled respirometers.

It is best to use short, narrow sections of tubing to connect the apparatus together. This will minimize the wild movements of the manometer fluid which are caused by handling the apparatus.

If the apparatus is assembled correctly, almost all anomalous results will be due to fluctuations of temperature.

Principles

1 Gas changes, particularly the uptake of oxygen, act as indicators of the biochemical activity of organisms and their tissues.

2 Quantitative measurements of gas changes can be achieved through manometry, a historically important technique in metabolic research. Though the Warburg manometer has been most commonly used in research, the respirometer described here is simpler to construct and use.

Questions and answers

a ***When the graph of manometer readings against time is a straight line, what can be said about the uptake of oxygen by the organisms in the respirometer?***

The rate of oxygen uptake is constant.

b ***How much oxygen was absorbed by the organisms? State the amount as millimetres cubed per hour per milligram ($mm^3\ hr^{-1}\ mg^{-1}$) of living material.***

The rates of oxygen absorption depend not only on temperature but also on the state of development of the organism.

c ***How does the rise in temperature affect the rate of oxygen uptake? How can the effect be best expressed?***

The higher the temperature, the greater the oxygen uptake (to a maximum, at which point enzyme damage begins). This can be calculated by using Q_{10}, that is:

$$\frac{\text{rate of oxygen uptake at } 30\,°\text{C}}{\text{rate of oxygen uptake at } 20\,°\text{C}}$$

d ***What rate of oxygen uptake would you expect if you raised the temperature to 40° or 50 °C? Give your reasons.***

The rates of many physiological processes will typically increase by a factor of two with a rise in temperature of 10 °C ($Q_{10} = 2$). However this is only true for temperatures below 40 °C. Above this the Q_{10}

value of seedlings will decrease as the effect of the heat on enzyme activity becomes more damaging. At 40° to 50 °C a typical value of Q_{10} is found in the range of 0.6 to 1.0.

INVESTIGATION
5F Respiratory quotient

ITEMS NEEDED

Germinating seeds (castor oil, *Ricinus* sp. or sunflower, *Helianthus annuus*), 15 to 20 g/group
Kerosene, coloured, or Brodie's fluid
Potassium hydroxide, 15 % aqueous solution
Water

Balance, accurate to 0.1 g 1–2/class
Basket, metal or plastic, to fit boiling-tube 1/group
Boiling-tubes 2/group
Filter paper
Funnel, small glass or plastic 1/group
Manometer and scale 1/group
Measuring cylinders, 10 cm³ 2/group
Rubber bungs, 2-holed, to fit boiling-tube 2/group
Screw clip 1/group
Stopclock or stopwatch 1/group
Syringe, disposable 1 cm³ 1/group
Thermometer, 0° to 50 °C 1/group
Tubing, glass connecting 1/group
Tubing, rubber connecting 1/group
Water bath, set at 20 °C 1–2/class

(*Study guide* 5.7 'Cellular respiration and the mitochondrion'.)

It is best to use oil-bearing seeds for this investigation. If seeds containing carbohydrate food reserves are used the second part of the investigation will be uneventful: there will be little or no change in net volume. (Of course, this in itself may be very instructive, though tedious.)

Principles

1 This investigation shows how the volumes of both carbon dioxide and oxygen may be measured during gaseous exchange.

2 The respiratory quotient provides more information about the respiratory substrate. However, this information must be interpreted with care and, on occasion, may amount to very little.

3 Theoretical values for the respiratory quotient can be calculated from the equations for the complete oxidation of compounds. The following examples are for glucose and the fat tristearin, respectively:

$$C_6H_{12}O_6 + \underline{6O_2} \longrightarrow 6H_2O + \underline{6CO_2} \qquad R.Q. = 1.0$$

$$2C_{57}H_{110}O_6 + \underline{163O_2} \longrightarrow 110H_2O + \underline{114CO_2} \qquad R.Q. = 0.69$$

Questions and answers

a ***Is it possible to deduce from the respiratory quotient what substance, or type of compound, has been respired by the seeds?***

No. The respiratory quotient, on its own, cannot indicate the nature of the respiratory substrate. If an organism is respiring a mixture of different compounds, the resultant respiratory quotient will depend on the proportion of each substance involved.

b ***What respiratory substrate is most likely to be present in the seeds, and what tests could you perform to discover the nature of the substrate? Does the RQ correspond with your expectations?***

If oil-bearing seeds are used, the RQ should be about 0.7.
Students could then carry out food tests (see page 152) on the seeds to discover the nature of their reserves, and see if these tests confirm their numerical results.

c ***What would you expect to be the RQ of a growing culture of yeast?***

The quotient has no real meaning with anaerobic organisms because they absorb no oxygen. As oxygen absorption decreases, so the RQ tends to infinity.

d ***Are question c and its answer in any way relevant to the respiration of germinating seeds? Give your reasons.***

Yes. Many germinating seeds are known to respire anaerobically and their RQs will thus depend not only on the substrates respired but also on the type of respiration; the greater the fraction of anaerobic respiration, the larger the RQ will become.

e ***Suggest three different hypotheses to account for a respiratory quotient of unity (that is, 1.00).***

1 Aerobic respiration of a carbohydrate substrate.
2 Aerobic respiration of the two substrates, one with an RQ below unity and the other with one above unity.
3 Aerobic respiration of a substrate with a respiratory quotient less than unity and anaerobic respiration of another substrate, to give an overall result of 1.0.

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CHAPTER 6 HETEROTROPHIC NUTRITION

A review of the chapter's aims and contents

1 All heterotrophic organisms require an external source of organic food, partly to satisfy their energy needs and partly for growth and the repair of tissues.
2 The energy needs of mammals depend on their body mass, but the relationship is a complex one.
3 Heterotrophs feed on a wide variety of foods, to which they are structurally and behaviourally adapted.
4 Polysaccharides and proteins are polymers of simpler units, hexoses and amino acids. The twenty different amino acids found in proteins result in an almost infinite number of possible structures. This in turn gives rise to the very large number of different enzymes catalysing quite specific reactions.
5 The digestive breakdown of food occurs in a wide variety of situations and involves both extracellular and intracellular hydrolysis.
6 The alimentary canal of mammals has two principal functions – digestion and absorption.
7 The hydrolysis of cellulose poses particular problems. Few organisms secrete cellulase, but rely on the breakdown brought about by symbiotic micro-organisms.
8 The liver and the pancreas together play a crucial role in the regulation of blood sugar.

PART I The *Study guide*

6.1 The need for food

Assumption

1 That students have an understanding of the changing relationship between surface area and volume.

Principles

1 Heterotrophs require a continuous supply of organic material for food.
2 The energy expenditure, and therefore the amount of food needed by a mammal, is related to its body mass, but this relationship is not a simple one.

Students may be familiar with the idea that a mammal's food consumption is not simply related to its body mass. Thus they may know that per unit of body mass a shrew, for instance, consumes a greater amount of food than an elephant. They may attribute this to the greater surface area of the shrew in relation to its body mass. The consequent heat loss from this surface necessitates a large and regular supply of energy in the form of food. This section outlines the development of the idea that the relationship between body mass and food consumption is not as simple as might be assumed at first sight. The idea is developed further by K. Schmidt-Nielsen (1972) in his excellent short book 'How animals work'.

STUDY ITEM
6.11 Food for energy

Questions and answers

a ***List the factors which determine how much food an animal will require to meet its energy needs.***

The body mass and shape of the animal, its level of activity, its body temperature in relation to that of its surroundings, the medium in which it lives (air, water, or soil), and the insulation properties of its body surface will all influence an animal's energy expenditure and hence its requirement for food. The energy content of the food which the animal eats is a further factor which should be mentioned.

b ***Suggest one measure which could fairly easily be taken to obtain an indication of the energy output of an animal.***

The easiest parameter to measure would be the oxygen consumption of the animal. Students may already have measured this with a human subject using a spirometer (see *Practical guide 1*, investigation 2B). Very small animals can be placed in a simple respirometer, such as the one used in investigations 5E and 5F, and their oxygen consumption measured in this way. The principles involved in either of these pieces of apparatus could be modified for other animals. Such a modification would be a good experimental design item for students. An alternative approach to the problem would be to measure the energy intake of the animal by regulating its food intake so as to maintain the animal at a constant mass. The energy content of the food and the faeces would then have to be ascertained using a calorimeter.

c ***Calculate, for each dog, the metabolic rate per kilogram of body mass.***

The metabolic rates of the dogs per kilogram of body mass are as follows:

$A\frac{1174}{3.19} = 368\,\text{kJ per day}$; $B\frac{1795}{6.50} = 276\,\text{kJ per day}$; $C\frac{2617}{9.61} = 272\,\text{kJ per day}$; $D\frac{3515}{18.20} = 193\,\text{kJ per day}$; $E\frac{3796}{19.80} = 192\,\text{kJ per day}$;

$F\frac{4014}{24.00} = 167\,\text{kJ per day}$; $G\frac{4653}{31.20} = 149\,\text{kJ per day}$.

d ***What relationship exists between the metabolic rate per kilogram of body mass and the size of the dog?***

The figures show clearly that the bigger the dog the lower is the metabolic rate per kilogram of body mass. The data could also be shown as a graph with body mass on the x axis and metabolic rate per kilogram of body mass on the y axis.

e ***Calculate the metabolic rate per m² of body surface per day for each dog.***

The calculations give the following figures:

$$\text{A}\,\frac{1174}{0.2423} = 4845\,\text{kJ};\ \text{B}\,\frac{1795}{0.3724} = 4820\,\text{kJ};\ \text{C}\,\frac{2617}{0.5286} = 4951\,\text{kJ};$$

$$\text{D}\,\frac{3515}{0.7662} = 4588\,\text{kJ};\ \text{E}\,\frac{3796}{0.7500} = 5061\,\text{kJ};\ \text{F}\,\frac{4014}{0.8805} = 4559\,\text{kJ};$$

$$\text{G}\,\frac{4653}{1.0750} = 4328\,\text{kJ}.$$

f ***What relationship exists between the metabolic rate per m² of body surface and the size of the dog?***

The data are somewhat ambiguous, but they do suggest that this relationship is closer than that between metabolic rate per kilogram and the size of the dog. If a graph of the data is plotted (this can conveniently be done by using the same axes as the previous graph), it can be seen that there is a slight tendency for the metabolic rate per m^2 of body surface to decrease as the size of the dog increases.

g ***Suggest a reason for this relationship.***

The reason hinted at earlier is the most obvious one. Dogs are homoiotherms and will lose heat to their surroundings through their body surfaces. The surface areas of dogs are therefore likely to have an important influence on their metabolic rates. Another way of viewing this is to consider that surfaces (such as those of cells and intracellular membranes) are closely involved in many aspects of metabolism. Surface area is therefore likely to be closely related to metabolic rate.

h ***Is this information compatible with your answer to g?***

As is suggested above, the relationship appears to be close, but not perfect. □

STUDY ITEM
6.12 Food for the Man-Mountain

Some interesting background information for this Study item is contained in Schmidt-Nielsen's book mentioned above. In it he refers to a paper in the *Annual Review of Physiology* by Kleiber (1967) entitled

'An old professor of animal husbandry ruminates'. In this paper Kleiber suggests that Swift had anticipated modern work, in particular Kleiber's own research, by some 233 years. However, a careful comparison of *Gulliver's travels* with Kleiber's paper reveals some discrepancy between their figures. Swift's calculations are obviously based on the assumption that food requirements are directly related to body mass, and not to the body mass raised to the power of 0.75 as Kleiber suggests. Whether this discrepancy is accidental, or a deliberate attempt by Kleiber to credit Swift with knowledge which he did not in fact possess, is unknown.

Questions and answers

a ***How did the Emperor arrive at the figure of 1728 Lilliputian rations as being an appropriate amount for Gulliver?***

1728 is equal to $12 \times 12 \times 12$, that is, 12^3. If we assume that Gulliver was a man of nearly 6 ft (that is 170 to 180 cm), he was just 12 times the height of a Lilliputian, one of whom was, to use Swift's words, 'not six Inches high'.

b ***What assumptions did the Emperor make in this calculation and to what extent were they justified?***

The Emperor obviously assumed that food requirements were directly related to body mass. We have already seen that this assumption is not justified. As mammals become larger, their food requirements in relation to their body mass become proportionately smaller.

itals

c ***If the Lilliputians had known of Rubner's investigations with dogs of different body masses (see page [S]164), how would the Emperor have adjusted his calculation and how many Lilliputian rations would Gulliver have been given?***

Rubner suggested that the metabolic rate, and therefore the food requirement, of a mammal was determined by its surface area. Gulliver's surface area would have been just 144 times that of a Lilliputian (that is 12×12 or 12^2). He would thus have been given 144 Lilliputian rations.

d ***Calculate how many Lilliputian rations Gulliver* should *have been given.***

In order to determine the food requirement of the larger of two mammals, we must take its body mass in relation to the smaller mammal and raise it to the power of 0.75. In this case, as we have seen, Gulliver's body mass was 1728 times that of a Lilliputian. His food requirement would therefore have been $1728^{0.75}$ Lilliputian rations. With the help of an electronic calculator this comes to about 268. Gulliver should thus have been given 268 Lilliputian rations.

e ***What assumptions are you making in your calculations and to what extent are they justified?***

All of these calculations – and the one used to answer question **d** is no exception – assume that Gulliver was a perfectly scaled up version of a Lilliputian. This is as fantastic as the difference in size. His body mass, being 1728 times that of a Lilliputian, would have to have been supported on legs whose cross-sectional area would have been only 144 times that of a Lilliputian's. In addition, other important assumptions are being made. For example, it is assumed that the levels of activity and the insulation properties of the skin and clothes of both Gulliver and the Lilliputians were similar. Such assumptions may not be justified! □

Many sources of food

Principles

1 There is a wide variation in the sources of food among heterotrophs.
2 The 'feeding' mechanisms, digestive systems, and ways of life of heterotrophs are adapted to their diet.

The range of sources of food of heterotrophs is only briefly mentioned. There is an opportunity here for students to read more widely on the subject. Morton's book (1967), mentioned in the *Study guide*, is a good starting point. Firsthand experience of a range of feeding mechanisms can be obtained on field courses.

STUDY ITEM
6.13 Contrasts in feeding and way of life

Questions and answers

a ***What are the visible differences between the skulls, jaws, and teeth of the two animals? Explain how these are adapted to the food which each animal eats.***

In the dentition of the rabbit, the wide diastema, absence of canine teeth, and molariform premolars which together with the molars are flattened and ridged, are all associated with a diet consisting mainly of plant leaves. In contrast, the cat has no conspicuous diastema, other than spaces to accommodate the large pointed canines. The premolars and molars are sharp, pointed, and laterally flattened for slicing the meat which is the cat's food. The shapes of the jaws are also markedly different, with the fulcrum of the cat's jaw being relatively further from the point of attachment of the temporal muscle; thus maximum leverage is obtained by this muscle in closing the jaw. The area for attachment of the masseter muscle in the cat is relatively small compared with that of the rabbit. These differences reflect the contrast in the emphasis on movement in the two mammals. The cat's jaw movement is predominantly a closing one which slices the food, while that of the rabbit is a side to side movement which grinds the food between the premolars and molars. There are also obvious differences in the skull shape, with the rabbit's skull being proportionately longer in order to accommodate the diastema and grinding molars. Its eye sockets are also placed nearer the sides of the skull and the area for the attachment of the temporal muscles is relatively small.

b ***What is the main difference between the two guts? Relate this to the difference in diet of the two animals.***

The most conspicuous difference is the enlargement of the caecum and appendix in the rabbit to form a fermentation chamber for the microbial breakdown of cellulose. In contrast, the cat's gut is relatively short and lacks such a fermentation chamber. More detailed consideration of cellulose breakdown is given in section 6.6.

c ***From the photographs and from your knowledge of these two animals, comment on any other physical differences in relation to their diet.***

1 The forward pointing eyes of the cat give binocular vision, which is important in judging distances when approaching, and pouncing on prey.
2 The cat's claws are used to secure its prey, while its limbs are adapted to a quick pounce rather than to sustained running.
3 Its keen sight at low light intensities is an adaptation to night hunting.
4 The split lip of the rabbit permits the incisors to be used in cropping grass and other leaves.
5 The other obvious physical features are probably more related to their way of life than to their diet and are considered below.

d ***What differences in way of life exist between these two mammals? How are these differences related to their feeding habits?***

The cat is mainly a solitary night hunter, moving around woodlands and other similar habitats in search of its prey of small mammals (including rabbits) and birds. In the wild its lair is usually quite well protected, as its newly born young are not able to defend themselves. Cats show considerable agility, which is doubtless an advantage in catching their prey. They have a keen sense of smell and a well defined territory, which they clearly mark. These are common features in solitary carnivores. In contrast rabbits, being the prey of carnivores, defend themselves by feeding in groups. At least one of a group of rabbits is likely to see advancing predators and warn the rest, which can then retreat to their burrows. Their dental and other adaptations to feeding do not coincide with defence mechanisms, and hence they rely on the burrow, where the young are able to develop to a quite advanced state before moving out to feed. Their limbs are adapted to rapid running to enable them to reach their burrows before being caught by a predator. □

6.2 The nature of food

Assumption

1 A knowledge of basic organic chemistry derived from previous chapters and from earlier courses.

Principles

1 Polysaccharides are polymers formed by glycosidic condensation of large numbers of monosaccharide units.
2 Glycosidic links can be formed at more than one carbon atom in the hexose ring; hence branching chains can be formed.
3 Proteins are composed of long chains of amino acids, which have a characteristic spatial arrangement that is of crucial importance to their function.

This section introduces the student to the chemical structure of carbohydrates and proteins. Some understanding of this structure is necessary for an appreciation of both the digestive process and the functioning of enzymes. The use of molecular models may be helpful in considering some aspects of structure. At a simpler level, coloured 'poppet' beads may be used to convey the basic ideas of polymerization and the sequence of amino acids in a protein chain. Those students who want, and are able to cope with, a more detailed treatment can refer to some of the suggestions for further reading, in particular the books and booklets by Barker (1968), Phelps (1972), and Phillips and North (1973). The book by Wood and Pickering (1982) is also especially useful in this context. (See the Bibliography.)

STUDY ITEM
6.21 Some properties of carbohydrates

Questions and answers

a ***Why is starch from plant tissue insoluble, although it forms a colloidal solution on boiling?***

In many plant tissues, the starch grains are rendered insoluble by being coated in a layer of cellulose. They are thus effectively removed from solution in the cell and form an ideal storage chemical. On boiling, the cellulose coating is ruptured and the starch forms the familiar, somewhat opalescent, colloidal solution.

b ***From your knowledge of enzymes and of the structure of polysaccharides, why are starch and glycogen frequently and easily hydrolysed, while cellulose is not?***

In cellulose the glucose residues are joined by β-glycosidic links, whereas in both starch and glycogen the linkages are α-glycosidic in structure.

c *If you follow the course of an enzyme-catalysed hydrolysis of starch by testing at intervals with dilute iodine solution, you will find that the results pass from deep blue, through deep reddish brown to pale yellow. What do these results suggest about the hydrolysis of the two components of starch?*

It has already been explained that one of the constituents of starch, amylose, gives a deep blue colour with iodine solution. This constitutes about a quarter of starch. The other three-quarters is amylopectin, which forms a deep reddish brown colour with iodine solution. In the course of an enzyme-catalysed breakdown, it is the amylose which is first hydrolysed, leaving the amylopectin. In time this too is hydrolysed and the only colour remaining is that of the dilute iodine solution. (See also question **d** of investigation 6A.)

d *The disaccharide sucrose is frequently found to be the form in which carbohydrate is moved around the plant. In what ways is it particularly suited to this role?*

Starch, or some other large polysaccharide molecule, would be unsuitable as a transport carbohydrate, as it cannot be passed through cell membranes from one cell to another. A smaller molecule, such as glucose, has the disadvantage that it is more reactive and is the starting point of a number of biochemical pathways in the cell. Sucrose is a small enough molecule to be conveniently passed from one cell to another, yet it is not so likely to be metabolized as it passes through the cell.

e *Glucose and maltose give positive results when boiled with Benedict's solution as a test for reducing sugars. Sucrose gives negative results. Explain why this is so. What results would you expect from lactose?*

In glucose and maltose the reactive reducing group is still exposed and can therefore reduce Benedict's solution. The same is also true of lactose. In sucrose, however, both reducing groups are involved in the link and are therefore unavailable for the reduction of Benedict's solution. □

Proteins

STUDY ITEM
6.22 A multiplicity of polypeptides and proteins

Questions and answers

a *What are the two amino acids involved in the formation of the dipeptide shown in* **figure** [S]137*? Suggest a name for this dipeptide.*

The two amino acids are glycine and alanine, both of which have been mentioned earlier in the *Study guide.* Glycyl-alanine would be an appropriate name for this dipeptide.

b ***How is this dipeptide suited to further condensation reactions with other amino acids?***

The glycine residue still has a free amino group and the alanine residue still has a free carboxylic acid group. Both of these can form further peptide bonds by condensation.

c ***How many different dipeptides can be formed from the twenty amino acids used in the synthesis of proteins?***

20^2 or 400 different dipeptides.

d ***How many different tripeptides can be formed from the same twenty amino acids?***

20^3 or 8000 different tripeptides.

e ***How many different proteins, 141 amino acid units long, can theoretically be formed from the twenty basic amino acids?***

20^{141} or 2.79×10^{183}.

f ***How does your answer to question e relate to what you have learned about enzymes and the wide range of organisms living on the Earth?***

The theoretical possible number of proteins is so vast that it greatly exceeds the number necessary for every different species of organism to have a multiplicity of enzymes, without any one being duplicated in another organism. □

The final part of this section emphasizes the importance of the secondary and tertiary structure of proteins in determining their precise functioning as enzymes.

6.3 Food and diet

Principles

1 The energy needs of heterotrophs are one important factor which determines dietary requirements.
2 Heterotrophs have minimal dietary amino acid requirements in order to synthesize the wide range of proteins needed for metabolism and growth.
3 Heterotrophs have a number of additional dietary requirements, such as vitamins and mineral ions, which are needed for specific metabolic processes.

6.4 Digestion

Principles

1 Digestive breakdown occurs in one form or another in every living organism and is not restricted to the guts of heterotrophs.
2 Digestion may be extracellular or intracellular.

3 Extracellular digestion is necessary in heterotrophs to allow large molecules to pass through cell membranes.

STUDY ITEM

6.41 The universality of digestive processes

Questions and answers

In each case state what is being digested and whether this digestion is intracellular or extracellular. What is the destination of the digested materials?

a ***A maize 'seed', or any other 'seed', contains a store of food. On germination this food is used in the early stages of the seedling's life before it becomes fully autotrophic.***

Food stored in a 'seed' in the form of carbohydrate, protein, and fat is broken down intracellularly and transported to the growing points in the young stem and root. Here it may be laid down as new tissue in growth, or broken down to release energy for anabolic processes. In the case of the maize 'seed' shown, most of the food is stored in the form of starch with a small amount of protein. In other species there may be differing proportions of carbohydrate, protein, and fat.

b ***This blowfly is feeding on the carcass of a dead animal. It pumps saliva onto the flesh and then draws the liquefied food into its stomach.***

The flesh of the dead animal is being digested extracellularly before being taken into the stomach of the fly. Here further digestion may take place, again extracellularly, before absorption. There may in addition be some intracellular digestion during the absorption process.

c ***A human leucocyte engulfs and digests invading bacteria.***

Digestion here is intracellular. The bacteria are engulfed by phagocytosis and lysosomal enzymes are then secreted into the resulting vacuole. Some bacteria are engulfed, but not digested. The products of digestion are partly eliminated from the cell and partly incorporated into the cytoplasm.

d ***A bracket fungus grows on the trunk of a tree.***

Extracellular enzymes secreted by the saprophytic fungus digest the tree. The products of this digestion are absorbed into the tissue of the fungus where they provide energy and materials for growth.

e ***From 111.1 kg (i) this woman slimmed to a mass of 58.5 kg (ii). She did this by taking in less than her daily requirement of food.***

A certain amount of protein, but mainly fat, has been broken down intracellularly. Most of this is used to provide energy for movement, though some will, of course, be used for metabolism.

f *This man is eating a meal.*

The digestive processes which follow the ingestion of the meal are mainly extracellular, though the final stages may be intracellular. A discussion of the processes involved forms the bulk of the remainder of this chapter.

g ***Some plants, such as this Venus fly-trap, live in nitrogen-deficient soils and 'feed' on small animals to supplement their intake of this element.***

The digestion of the insect is extracellular. The products are used for the synthesis of protein in various parts of the plant, especially at the growing points.

h ***In autumn some of the photosynthetic pigments are broken down and withdrawn from the leaves. This leads to a change in leaf colour.***

The breakdown of the pigments is intracellular. Some of the products are retained by the tree after the leaves have been shed; others are lost along with the leaves when they fall.

i ***Even without the help of blowflies like the one shown in b, this carcass will eventually rot away.***

The initial cause of the breakdown will be the hydrolytic enzymes within the cells of the dead animal itself. This will be supplemented by the action of saprophytic micro-organisms which bring about extracellular digestion. The products of this digestion will be absorbed by the micro-organisms, though some may remain in the soil and be absorbed by plants.

Some of the examples discussed above may not normally be considered as digestion. However, in each case the breakdown of organic material involves hydrolytic enzymes and the process is thus essentially digestive. □

Practical investigation. *Practical guide* 2, investigation 6A, 'Digestion by micro-organisms and tissues'.

6.5 The double function of the alimentary canal

Principles

1 The first function of the alimentary canal is a digestive one.

2 Its second function is the absorption of the products of digestion.

The gut as a digestive organ

Practical investigation. *Practical guide* 2, investigation 6B, 'Digestive organs: a model gut'.

STUDY ITEM

6.51 'Experiments and observations on the gastric juice and the physiology of digestion'

The famous story of Beaumont's observations on Alexis St Martin will probably be familiar to many students. In this Study item we look at detailed quotations from Beaumont's writings and consider their significance. Students wanting to pursue the story further should consult the 1959 edition of Beaumont's book.

Questions and answers

a ***In a note after experiment 1 Beaumont says: 'This experiment cannot be considered a fair test of the powers of gastric juice.' Why?***

The experiment was concluded prematurely. Some materials, being partly digested, could have slipped from their silk string under the action of the stomach muscles. St Martin was not completely well, and so conditions may not have been normal. Some students may also point out the lack of a control experiment.

b ***About experiment 1 he also says: 'This experiment is important, in a pathological point of view.' Why is this so?***

Beaumont was able to make observations concerning the effects of indigestion.

c ***In what ways does experiment 3 confirm that the results obtained in experiment 2 are the same as those obtained in the body?***

Experiment *3* is an *in vivo* control for experiment *2*. The results are comparable.

d ***How do you account for the difference in the results obtained from experiments 2 and 3?***

Beaumont gives the answer: 'In the stomach, agitation by muscles removes the digested surface layers, thus exposing undigested layers to gastric juice.'

e ***Make a list of the conditions necessary for digestion in the stomach.***

1 Acid.
2 Mechanical churning.
3 Body temperature.

f *Are these conditions suitable for enzyme activity?*

In terms of the gastric enzymes, yes.

g *What type of food is digested in the stomach?*

☐ Proteins.

The next part of the section deals with a detailed consideration of the digestion of proteins. This is taken as an example of the digestive process. The breakdown of carbohydrates and fats is not discussed in detail, but the same principles apply. Those students who wish to follow through the breakdown of carbohydrates and fats can do so in standard textbooks.

STUDY ITEM

6.52 Secretions of the alimentary canal

Questions and answers

a ***We have seen that many proteolytic enzymes, such as the pepsins and trypsin, are secreted as inactive precursors and only become activated once they are in the lumen of the gut. Suggest a reason for this.***

If they were present in the secretory cells in an active form, autolysis would take place and the cell's own proteins would be hydrolysed.

b ***Why do the cells of the stomach lining which produce hydrochloric acid contain abnormally large numbers of mitochondria?***

The energy required for the secretion of hydrogen ions against a considerable concentration gradient is derived mainly from aerobic respiration. It is worth noting that the hydrogen ion concentration of the secretion from human parietal cells is about one million times greater than that found in plasma. The energy involved in overcoming such a concentration gradient is therefore considerable.

c ***From your knowledge of the factors which affect the rate of enzyme action, suggest a reason for the secretion of large concentrations of hydrogen carbonate ions (HCO_3^-) by the pancreas and small intestine.***

The chyme entering the duodenum has a very low pH owing to the presence of the gastric hydrochloric acid. Unlike the pepsins, most enzymes have pH optima around neutrality. The hydrogen carbonate ions return the pH to approximate neutrality.

d ***What would be the effect of such a deficiency?***

In the absence of enteropeptidase, trypsinogen is not activated to trypsin. Consequently proteins cannot be fully hydrolysed and a severe protein deficiency may result. ☐

The next part of this section considers gut movements. Secondhand data are presented in the form of X-ray photographs. Students may witness gut movements in a small mammal during the course of investigation 6C. An alternative to this first-hand observation is to watch gut movements on film or videotape.

Practical investigation. *Practical guide* 2, investigation 6C, 'Digestion and absorption in the gut of a mammal'.

STUDY ITEM

6.53 The control of gastric secretion

Principles

1 The production of digestive juices by the glands of the alimentary canal is controlled so that they secrete whenever food that is to be digested is present.

2 The co-ordination of secretion is partly under nervous, and partly under hormonal, control.

Questions and answers

a ***Suggest possible mechanisms whereby this gastric secretion could be stimulated.***

The most obvious possibility is that the presence of food in the mouth, or the upper part of the oesophagus, is detected by sensory nerves which, through nervous pathways, stimulate gastric secretion. The stimulus could also be the sight or smell of food. An alternative mechanism which could account for this observation is that the presence of food in the mouth, or oesophagus, results in a hormone being secreted into the blood, which in turn causes gastric secretion.

b ***What does this suggest about the mechanism of stimulation of gastric secretion?***

The mechanism is clearly nervous and not hormonal. In fact Pavlov demonstrated that the dog need not even be fed. The sight and smell of food is sufficient to stimulate gastric secretion. This cephalic phase of gastric secretion does not appear to be as important in humans as it is in dogs.

c ***What do these results suggest about a second mechanism for stimulating gastic secretion?***

The Heidenhain pouch secretes hydrochloric acid (shown on the graph as H^+) when it has no nervous connection with the rest of the stomach. It would appear that the stomach is secreting a hormone which passes to the pouch via the blood and causes this secretion of H^+. This hormone is called gastrin.

d ***What factor additional to those you have suggested above appears to be involved in the control of gastric secretion?***

Gastric secretion is inhibited by the presence of hydrochloric acid at this concentration.

e ***From the results of the experiments we have discussed, describe the mechanism for controlling gastric secretion in the dog.***

Eating food initially causes gastric secretion via the vagus nerve (the cephalic phase of secretion). The presence of food in the stomach results in the production of a hormone which maintains gastric secretion (the gastric phase). Excess hydrochloric acid inhibits further secretion. (There is a further intestinal phase of gastric secretion which is probably due to the release of gastrin from the mucosa of the upper part of the small intestine. Evidence for this is not presented here.)

f ***How could you determine, in human subjects, whether increased gastrin levels in the blood lead to increased gastric secretion?***

Levels of gastric secretion could be measured before and after the injection of gastrin into the blood stream. □

6.6 Mammals and cellulose digestion

Assumption

1 A knowledge of the structure of cellulose and other polysaccharides which has been gained from section 6.2.

Principles

1 In spite of widespread dependence on cellulose as a source of food, only a very few animals are able to synthesize cellulase.
2 Cellulose is difficult to hydrolyse owing to the presence of β-glycosidic links between the glucose units.
3 Many animals harbour symbiotic micro-organisms in their guts, which produce cellulases.

The bulk of this section is concerned with a description of the digestion of cellulose in ruminants and the rabbit. Students who wish to pursue this in more detail are referred to Morton (1967) and Schmidt-Nielsen (1975). (See the Bibliography.)

STUDY ITEM
6.61 Cellulases in vertebrates and invertebrates

Questions and answers

a ***From your knowledge of the structure of polysaccharides, suggest why cellulases are uncommon in the animal kingdom, whereas amylases are secreted by representatives of many phyla.***

The links between the glucose units in cellulose are β-glycosidic, whereas those between the glucose units in amylose are α-glycosidic. Quite different forms of enzyme would be needed to hydrolyse these links. Those hydrolysing β-glycosidic links do not appear to have evolved in a widespread way among heterotrophs.

b ***Suggest a function for this sodium hydrogen carbonate.***

It is used to neutralize the acids produced by the anaerobic bacteria in the rumen.

c ***What differences have you observed between the faeces of horses and those of cows? Account for these differences in the light of what you know of the feeding habits and digestive processes of the two animals.***

Horse faeces contain conspicuously more undigested fibrous material than those of cows. Horses do not 'chew the cud' as cows do. The bulk of their digestion of cellulose takes place in a greatly enlarged caecum.

d ***What other differences, not observable with the naked eye, would you expect to find between the faeces of the horse and the cow?***

Tests for cellulose would reveal a much higher proportion of this polysaccharide in the horse's faeces than in those of the cow. The high proportion of organic material in horse dung is one reason why it is much sought after by gardeners as compost.

e ***Suggest further investigations which could be carried out with these two molluscs to help to determine whether or not they secrete cellulase.***

It would be necessary to obtain samples of gut which were completely free of micro-organisms. One way of doing this might be to grow the molluscs in a sterile environment after washing the eggs in a bactericidal solution. This technique is the one used in the investigations of the silverfish described in the next part of this Study item. Another possibility is to use sections of gut which have been rinsed in a bactericidal solution. If in either of these two cases cellulase activity were detected, it would be likely that it originated in the mollusc itself.

f ***Is this sufficient evidence to suggest that silverfish secrete a cellulase?***

Yes. It would seem that they do. The treatment of the eggs and the aseptic conditions in which the silverfish were kept preclude digestion by micro-organisms. Yet the appearance of radioactively labelled carbon in the carbon dioxide exhaled by the insects shows that digestion of cellulose has taken place. The silverfish themselves must therefore have brought about this digestion.

g ***Describe the relative roles of the termite itself, its gut bacteria, and its gut flagellates, in the digestion of cellulose.***

The role of the termite itself is to find food, and to masticate and ingest it. The flagellates are clearly necessary for any chemical breakdown of the cellulose. That these flagellates (*Trichomonas* in particular) are capable of hydrolysing cellulose has been given further support by their culture over a number of years outside the bodies of termites. In such conditions cellulase activity continues. The role of the gut bacteria in the termite is not clear from the evidence presented here, other than the presumption that they are unable to break down the cellulose in the form in which it enters the termite gut. Termites thrive on wood in spite of its low nitrogen content. It has been suggested that the bacteria are nitrogen-fixing species and that the termite obtains much of its dietary nitrogen from this source.

h ***What further experiments would you recommend to confirm your answer to g?***

Experiments similar to those described above for the molluscs would give additional confirmation. To these could be added the technique of isotope labelling described for the silverfish. □

6.7 Absorption

Principles

1 The surface area for absorption of food by a large organism, such as a human being, is greatly increased by macroscopic, microscopic, and sub-microscopic foldings of the intestinal membrane.
2 The blood supply to the villi maximizes the rate of absorption of the end-products of digestion.
3 Absorption from the lumen involves both passive and active processes.

Students may like to use the data given to calculate the total absorptive surface area of the human gut. Of the total length of the gut (4.5 m), somewhat in excess of 3 m consists of the small intestine, which has a diameter of about 1 cm. If we assume that the intestine itself, the villi, and the microvilli are all cylindrical in shape, the arithmetic is relatively easy, especially if an electronic calculator is used. A mathematical model of this increase in absorptive surface area is provided by a three-dimensional version of the snowflake curve which may be familiar to students following mathematics courses. The biological implications of it are explored by Carter *et al.* (1981). Students may also get firsthand experience of microscopic intestinal structure from investigation 6D.

Practical investigation. *Practical guide* 2, investigation 6D, 'The fine structure of the intestinal wall'.

STUDY ITEM

6.71 **Mechanisms for absorption from the small intestine**

Questions and answers

a ***What relationship is shown between molecular size and rate of absorption?***

The data suggest little relationship. Indeed the smaller pentoses are absorbed more slowly than the hexoses.

b ***Suggest a reason for the difference in the rates of absorption of glucose and galactose by living and by poisoned intestine.***

Cyanide is known to inhibit respiratory breakdown. The fact that the rates of absorption of glucose and galactose are markedly slowed under these conditions suggests that this is an active process requiring the expenditure of energy by the absorbing cells. The large number of mitochondria in the epithelial cells has already been remarked on.

c ***Suggest a reason for the difference in the rates of absorption of glucose and galactose when compared with that of fructose.***

Fructose is not actively absorbed; thus, when the absorption mechanisms for glucose and galactose are poisoned, all three hexoses are absorbed at the same rate – by passive diffusion.

d ***On the basis of these results and those summarized in table [S]26, comment on the absorption of glucose from the small intestine.***

The active absorption of glucose depends upon the presence of sodium ions (Na^+). It has also been shown that drugs which inhibit the active transport of ions (the sodium pump) also inhibit the absorption of glucose. It is worth noting at this stage that glucose and galactose have similar structural formulae (as is shown in figures (S)124 and (S)131). Fructose, on the other hand, has a somewhat different structure. The sodium-dependent carrier involved in absorption is specific to the glucose/galactose configuration. □

6.8 The role of the liver

Principles

1 A vital function of the liver is to regulate the blood sugar level.
2 The pancreatic hormone, insulin, plays an essential part in this control mechanism.

The story of our developing knowledge of the role of the pancreas and insulin in the regulation of the blood sugar level is a fascinating one which is still far from complete. Although the mechanism of interaction between insulin and the level of blood sugar has been largely elucidated, the genetic and other causes of diabetes are still not clear. (See *Study guide II*, Chapter 24.)

STUDY ITEM

6.81 The control of blood sugar

Questions and answers

a ***How can you account for the differences in the shapes of the two graphs?***

Apart from the use of sugar for respiration, it could be converted to another substance, stored, or excreted. Hence the actual blood sugar level does not rise to the level expected.

b ***What assumptions must be made in order to plot the graph of the expected blood sugar level?***

That all the available sugar in the meal was digested, absorbed into the blood stream, and utilized at the rate expected.

c ***If the amount of sugar in the blood is not as high as you would expect, where is it likely to be in the body?***

In some organ or tissue through which blood flows. It could be there as glucose or in a changed form. Alternatively, it could have been excreted by that organ.

d ***If your answer to question c were treated as a hypothesis, how would you test it?***

Test the blood for sugar both before it enters and after it leaves the organ or tissue suspected.

e ***In what ways do the graphs provide evidence that sugar is stored somewhere in the body and not destroyed?***

Each graph shows a steady blood sugar level before and after feeding. If no compensation is made, the blood sugar level would drop, because the sugar would have been used in respiration. Thus we can form the hypothesis that the sugar is stored, and then released gradually to maintain the level.

f ***What other evidence would you require, to be sure that a particular organ stored sugar?***

Test the level of sugar in the organ before and after a meal. This should be done in conjunction with the procedure outlined in the answer to question **d**.

g ***What experiments would have to be performed to establish that the pancreas controls the blood sugar level?***

Remove the pancreas from a mammal and note the effects on the blood sugar level.

h ***If you were to continue the research beyond this stage what questions would you next ask?***

Does the pancreas itself store sugar? What part of the pancreas regulates the sugar level? What happens if extracts of the pancreas are injected into an animal (1) with its pancreas removed, and (2) with its pancreas intact? Many other suggestions may be made by students and these should be discussed on their merits.

i ***Which of the two hypotheses mentioned earlier do these experiments support? Give your reasons.***

That the islets of Langerhans produce an internal secretion. The acinar tissue was degenerate and exhausted, and was unlikely to be able to modify the blood. The effect must come from other parts of the pancreatic tissue.

j ***Does the secretion which controls the level of blood sugar pass through the pancreatic duct?***

No.

k ***What is the chemical nature of the active ingredient of the pancreatic secretion which controls blood sugar? Give the evidence which leads you to this conclusion.***

The evidence from the experiment on 10th September suggests that it is a protein which is hydrolysed by the active pancreatic juice.

l ***Why was the extract of degenerated pancreas filtered?***

To show that it was a substance made by the cells, and not the cells themselves, which reduces the blood sugar level.

m ***Why do you think the experimenters made haemoglobin estimations?***

To show that the fall in the blood sugar level was not due to dilution of the blood.

n ***It was found that the extract did not cure diabetes. It had to be given regularly to prevent the symptoms returning. Explain this fact.***

It supplied the missing factor, but did not restore the islet cells to activity. □

Practical investigation. ***Practical guide 2*, investigation 6E, 'A microscopic investigation of the liver'.**

PART II The *Practical guide*

The investigations related to this chapter appear in *Practical guide 2, Chemical reactions in organisms.*

Food tests. There is no separate investigation on food tests, though some are incorporated in investigations 6A and 6B. If students are not familiar with such tests from earlier courses, they can be incorporated at any convenient stage. Outlines of suitable tests can be found in Revised Nuffield Biology (1975).

INVESTIGATION
6A Digestion by micro-organisms and tissues

ITEMS NEEDED

Barley, germinating seeds 2–3/group
Locust guts, fresh, dissected into portions 1/group
Yeast suspension

Amylase solution, 0.5 %
Iodine in aqueous potassium iodide solution (see page 122)
Starch/agar in Petri dishes (with lids) 2/group
Water, distilled
Water, distilled, sterile

Cork borer (No. 4) 1/group
Forceps 1/group
Incubator, set at 25 °C 1/class
Mounted needle 1/group
Pipette, dropping 1/group

(*Study guide* 6.4 'Digestion'.)

Starch/agar. (2 g agar and 1 g starch to 100 cm^3 distilled water, sterilized in autoclave. This is sufficient for six plates.)
Starch/agar plates. These are much more convenient for assaying amylase than a starch suspension. The technique can be modified and extended in all sorts of ways. The plates can be used semi-quantitatively to estimate the activity of amylase by measuring the diameter of the cleared zone. These plates could be used to investigate amylase activity in the mouse gut (see investigation 6C).

Principles

1 A wide variety of organisms with totally different kinds of nutrition produces enzymes which will hydrolyse starch.

2 Hydrolysis of macromolecules such as starch can generate intermediate breakdown products.

Questions and answers

a ***Unchanged starch forms a deep blue colour with iodine solution. Which organisms or other items have a colourless zone around them?***

All the items suggested in the procedure, except water, should show a clear zone. The need for a control should be obvious.

b ***Are such zones in the agar only very close to the items or do they extend well away from them?***

They extend beyond the items in all directions. This is important as evidence of a diffusable agent at work. From their work in Chapter 5 students should appreciate that this is an enzyme or mixture of enzymes.

c ***What can you say about the chemical composition of the clear zones of agar? What further tests could you perform on these plates to confirm your ideas?***

All that can be said of the clear zones is that they contain no starch. These zones could be tested to see whether they contain the products of starch hydrolysis. Agar can be removed from the clear zones by means of a cork borer and tested with Benedict's reagent for the presence of reducing sugar. Portions of unaffected starch/agar should also be tested in the same way.

d ***What other colours have you observed besides blue? (Look particularly at the boundary between the blue and clear zones.) How could you account for other colours?***

Some items, particularly germinating cereal grains, produce clear zones with a purple fringe or periphery. The colour suggests that starch may be digested in at least two ways and perhaps the purple represents an intermediate breakdown product of starch. This is, in fact, due to amylopectin and is referred to in question c of Study item 6.21.

e ***In the light of your answers to questions a, b, and c, relate this investigation to the process of digestion. Is there any significance in the similarity of the effect of animal and plant tissue and micro-organisms on starch?***

The significance of the digestion of starch for all organisms lies in the breakdown product. Benedict's test would show this to be a reducing sugar. Since sugars have smaller molecules than polysaccharides, they diffuse more rapidly, particularly through certain membranes. Digestion in the gut precedes absorption and this may also be so with micro-organisms. Young plants use the food stored in seeds; this has to be transported around the germinating seedling.

INVESTIGATION
6B Digestive organs: a model gut

(*Study guide* 6.5 'The double function of the alimentary canal'.)

ITEMS NEEDED

Locust, adult, freshly killed 1/group

Benedict's solution
Iodine in aqueous potassium iodide solution (see page 122)
Starch suspension, 1 % (see page 123 but use 10 g starch)
Water, distilled

Beaker, 250 cm^3 1/group
Boiling-tubes or test-tubes, depending on diameter of Visking tubing 4/group
Bulldog clips or paper clips 2/group
Bunsen burner 1/group
Dissecting dish, with wax layer 1/group
Forceps 1/group
Gauze 1/group
Mat, heatproof 1/group

(continued)

Principles

1 Differentially permeable membranes, such as Visking tubing, are impermeable to macromolecules, such as starch. The digestion of the macromolecules yields breakdown products that can diffuse through the membrane.

2 Models can be very helpful in investigating natural phenomena. Here, the Visking tubing represents the real gut wall, which is difficult to handle experimentally.

Pieces of gut produce small amounts of enzyme and so the volume of substrate used should not exceed that suggested in the instructions. The volume of water outside the bag should be kept to a minimum. If the Visking tubing will fit inside a test-tube, this should be used.

The investigation need not be limited to examining the digestive activity of the locust gut. This could be replaced by portions of gut from a freshly killed mouse. In this case the amylytic activity of different regions of the gut could be examined.

ITEMS NEEDED *(continued)*

Mounted needles or scalpels 2/group
Pins, dissecting
Pipette, dropping 1/group
Scissors 1/group
Tile, white 1/group
Tongs 1/group
Tripod 1/group
Visking tubing, 45 cm/group
Watch-glass 1/group
Water bath, set at 25° to 30 °C 1/class

Questions and answers

a ***Iodine forms a blue compound with starch; Benedict's solution forms an orange precipitate when heated with reducing sugars. In which samples did you find starch and in which did you find reducing sugars?***

Expected observations:

	Macerated gut + starch suspension		*Starch suspension alone*	
	inside bag	*outside bag*	*inside bag*	*outside bag*
Iodine test	blue	yellow	blue	yellow
Benedict's test	orange precipitate	orange precipitate	no change	no change

b ***From these observations alone, is there any evidence that gut tissue changes starch to reducing sugars? Explain your answer.***

No. Reducing sugars may have been present in the gut all the time. From the previous exercise it is known that starch is altered by locust gut, and by other tissues too.

c ***How could you use the starch / agar plates from investigation 6A to add to this evidence?***

The plates can be taken from the cold store and portions of the clear zones can be tested with Benedict's solution for the presence of reducing sugars.

d ***Consider the answers to question a. What do these observations and the properties of Visking tubing suggest to you about the problem of digestion for a living locust feeding on starch-filled leaves?***

If the properties of the cellulose bag in any way resemble those of animal gut, then we have a simple model of digestion/absorption. Starch cannot penetrate the membrane, but, if it is converted to reducing sugars, the latter can then do so. The cellulose bag thus simulates the gut of a locust that has eaten starch; the water outside the bag represents the surrounding tissue of the locust's body.

INVESTIGATION
6C Digestion and absorption in the gut of a mammal

(*Study guide* 6.5 'The double function of the alimentary canal'.)

ITEMS NEEDED

Mouse or rat, freshly killed 1/group

Amino acids, mixture in standard solution
Ninhydrin solution or spray (*TAKE CARE*)
Ringer's solution (mammalian)
Solvent for chromatogram
Water, distilled *(continued)*

Ringer's solution (mammalian). Dissolve 0.3 g calcium chloride (anhydrous), 0.25 g potassium chloride, and 8.5 g sodium chloride in 1 dm^3 distilled water.
Amino acids, mixture in standard solution. Prepare a standard solution containing a mixture of three amino acids as markers for TLC. Select from the list below three amino acids with widely differing Rf values.

ITEMS NEEDED *(continued)*

Beakers, total volume 1.5 dm^3
Chromatographic plates, thin layer 3/group
Dissecting dish, with wax layer 1/group
Dissecting instruments
Filter paper
Melting point tubes, drawn out by hand, or other fine capillary tubes 2/group
Microscope, binocular L.P. 1/group
Microscope slides (or watch-glasses) 6/group
Oven, set at 110 °C 1/class
Pins, dissecting
Pipettes, dropping 2/group
Specimen tubes and bungs, for chromatography 3–6/group
Specimen tubes, small 6/group
Syringe, 1 cm^3, plastic, disposable 1/group
Thermometer, 0° to 50 °C 1/group
Water bath, set at 37 °C 1–2/class

Dissolve 0.1 g of each in 100 cm^3 of solvent (propan-2-ol and water, mixed in the ratio of 1 to 9).

cysteine	0.08	serine	0.27	tryptophan	0.50
lysine	0.14	glutamic acid	0.30	methionine	0.55
arginine	0.20	threonine	0.35	valine	0.60
histidine	0.20	alanine	0.38	phenylalanine	0.68
aspartic acid	0.24	proline	0.43	isoleucine	0.72
glycine	0.26	tyrosine	0.45	leucine	0.73

These Rf values are for paper chromatography with the same solvent system as the one used in this investigation. The Rf values for TLC should be roughly similar. (Rf values are generally much less reliable with TLC than with paper chromatography, since they depend on a number of factors, such as the quality and thickness of the absorbent.)

Solvent for chromatogram. Mix butan-1-ol, glacial ethanoic acid, and water in the proportions 80:20:30. Shake and allow to settle, then separate the upper organic phase (this is best done in a separating funnel). Provide each group of students with 20 cm^3 of this upper phase (freshly made). (*TAKE CARE* in the preparation of this solvent: in particular, glacial ethanoic acid is highly corrosive.)

The use of thin layer strips makes the whole operation possible within the time limits of a laboratory period. In order to economize on thin layer plates, cut the strips to a size that can easily be fitted into a specimen tube. It is probable that the complex mixture of the digestive products in the extracts will produce a streak of stain rather than discrete spots. The individual compounds will probably not be fully resolved unless two-dimensional chromatography is used.

Any small mammal is suitable for this investigation; mice are often the easiest to obtain, but rats are equally suitable and may be better for some stages. Students will get more from this investigation if they themselves dissect the mice or rats. However, it is possible to look for gut movements in one or two mice dissected in front of the class. Different groups can take different sections of the gut from these mice in order to analyse the contents by chromatography. If mice are used for the chromatographic analysis, students must take care not to dilute too much the extract of the gut's contents.

Intestinal activity decreases rapidly with time even though fresh, warm Ringer's solution is continually applied to the dissection. If all the members of a class start dissecting at the same moment, it is likely that by the time they examine the gut linings under the microscope, there will be little, if any, movement of the villi. One way of overcoming this difficulty is to kill one or two mice later in the practical session and get a few students who have watched the dissection to proceed directly and quickly to this stage of the exercise.

Principles

1 Movements of the gut, like contractions of a frog's heart, can be observed after the animal has been killed. They are more complicated than the simplified accounts of peristalsis given in most textbooks.

2 It is possible to show by chromatography that a greater variety of amino acids exists in the intestine than in the stomach. Evidence of this kind leads to hypotheses about the functions of different parts of the alimentary canal.

Questions and answers

a ***Which parts of the alimentary canal appeared to move and how do you account for the movement?***

In the living mammal the whole gut is capable of moving, but under experimental conditions the small intestine is the most likely part to be seen to move. The movement is worm-like, presumably owing to the muscle in the gut wall itself, which is arranged in a manner similar to that in earthworms.

b ***It is common knowledge that food passes along the alimentary tract from mouth to anus. Do the gut movements appear to move food in this direction? If not, what do the movements appear to do to the food inside the gut?***

Three types of movement may be seen:
1 Pendular movement, which is due to the contraction and relaxation of the longitudinal muscles.
2 Segmentation, which is caused by the contraction and relaxation of the circular muscles.
Both these types of movement help to mix the contents of the gut.
3 Peristalsis, which is caused by rhythmic contractions of the circular and longitudinal muscles, which move the food along the gut.

c ***Does the inside surface of the gut appear to be adapted to a digestive or an absorptive function, or both? Apply the question to each part of the gut that you examined, and state the reasons for your conclusions.***

The digestive function cannot be related to the internal surface of the gut as seen by the naked eye or a hand lens; microscopic examination is necessary. Absorption depends on, among other things, the surface area and this can be related to visible features. Unlike the stomach and caecum, the small intestine has a large surface area compared with the volume of digested food contained within it. The internal surface of the ileum and duodenum is like 'fur' in that it has many small projections (villi). By increasing the surface area these make the intestine a more efficient absorber. This feature is not found in the oesophagus, stomach, caecum, or rectum, though their inner surfaces are found to be ridged or folded.

d ***The solution rising up the thin layer plate separates various products from the digestion of proteins. Ninhydrin forms coloured compounds with amino acids. From your observations, is there any evidence that proteins have been digested?***

A larger number of spots, or a longer streak, may be seen on chromatograms of the contents of the ileum than on those of the contents of the stomach. Digestion breaks down protein molecules into their constituent amino acids, and so an increase in the number of free amino acids suggests that digestion has taken place.

e ***Describe how the chromatogram of the gut extract compares with that of the mixture of pure amino acids. Explain the differences which you have observed.***

The pure amino acids should have been resolved into distinct, well separated spots. If the extract of the gut's contents contains products of the digestion of proteins, it is likely that these will form a streak up the thin layer plate. In this case, the technique described will not resolve the spots of individual compounds, simply because there are too many of them too close together. Two-dimensional chromatography or electrophoresis is often used to separate such complex mixtures.

f ***Which parts of the gut appear to be chiefly responsible for absorbing digested food? (Confirm your answer by reference to a textbook.) It would be reasonable to assume that absorption occurs in the last section of the gut, when the food is completing its journey through the alimentary tract. How do you account for the fact that this is not so?***

The duodenum and ileum are the portions of the alimentary canal from which the digestive products pass into the blood stream. The question is asked in order to stimulate interest in the position and function of the caecum, colon, and rectum. Does any digestion take place behind the ileum? It is said that the caecum contains bacteria that break down cellulose. Clearly, this is a much larger organ in animals like rabbits, which eat much more cellulose, than in rats or mice. If digestion does occur in the caecum, then where are the products absorbed? An attempt to answer this question can lead to an understanding of the significance of coprophagy in rabbits and certain other mammals. The practice occurs to a lesser extent in rats and mice. (See also page 200 of the *Study guide.*) Efficient absorption can only take place in the presence of a large amount of water. Yet in terrestrial animals the water too must be absorbed. The absorption of digested products must therefore take place first, followed by the absorption of water.

ITEMS NEEDED

Ileum of rat or mouse, T.S., prepared (if possible, provide slides with a variety of stains: for example P.A.S., H. and E., and Azan) 1/1

Graticule, eyepiece 1/1
Hand lens 1/1
Micrometer stage 1/1
Microscope, monocular 1/1

INVESTIGATION
6D The fine structure of the intestinal wall

(*Study guide* 6.7 'Absorption'.)

Principles

1 A thorough investigation of the relationship between the structure and function of an organ cannot be conducted at the level of the macroscopic structure alone. It will inevitably lead to questions which can only be answered satisfactorily by microscopic and even molecular studies.

2 Two-dimensional sections must be interpreted in terms of the three-dimensional structure. (A transverse section of ileum is much easier to orientate in relation to the whole organ.)

3 The structures that are visible in a prepared section are determined as much by the staining methods used as by anything else.

If the appropriate sections are available, this investigation can be extended to an examination of other parts of the alimentary canal, such as the duodenum and stomach. An atlas of histology should be available to the students for reference during the practical session.

Questions and answers

a ***What is the approximate length of a typical villus in your section of the ileum?***

0.5 to 1.0 mm. It is important that students appreciate the order of magnitude of these structures.

b ***Explain why many villi appear in the section as islands of tissue with no connection to the wall of the ileum. (See* figure [P]17b*).***

Villi are flexible structures which will stand at a variety of angles to the wall of the ileum. Although the 'islands' do appear unconnected, their connections exist in another plane not taken by the section.

c ***Make a simple sketch of your section to record the distinct layers of tissue in the wall of the ileum. Indicate on your sketch the arrangement of muscle fibres in the two layers of muscle.***

d ***Examine the orientation of muscle fibres in the inner and outer layers of muscle. Which layer possesses longitudinal muscle fibres? What effect will the contraction of the inner and outer layers of muscle have on movements of the intestine?***

The outer layer of muscle possesses longitudinal fibres. These appear in cross-section in a transverse section of ileum. Contraction of these fibres will decrease the length of the ileum and cause an increase in its diameter. Contraction of the circular muscle causes the diameter of the gut to decrease and its length to increase in consequence.

e *Does the diagram in* figure [P]17a *and* b *represent a longitudinal or transverse section of ileum? Explain your answer.*

Transverse section. The longitudinal muscle fibres in the outer layer appear in cross-section. Students must take care when comparing slides with illustrations in books to distinguish accurately between longitudinal and transverse sections or much confusion will occur.

f ***If your section is stained by P.A.S., mucus and cells producing it (goblet cells) will show up clearly, stained deep pink. Comment on the frequency and distribution of the goblet cells. What part do you think mucus plays in the action of the alimentary canal?***

Numerous goblet cells are distributed fairly evenly throughout the epithelium of the ileum. Mucus, being slimy, is most likely to act as a lubricant, which facilitates the movement of digested food and protects the villi from physical damage due to friction.

g ***Can you identify any other secretory cells? If so, record their position on a simple, outline sketch.***

Other secretory cells in the crypts of Lieberkühn are not so easy to identify; figure (P)17 and a good illustrated textbook or atlas of histology are probably essential aids here.

h ***Where in the wall of the intestine are blood vessels situated? Refer to*** **figure [P]18,** ***which is a photomicrograph of an injected specimen. Through what structures must digestive products pass to reach the blood stream?***

Figure (P)18 shows capillaries to be centrally situated within each villus, and not immediately next to its surface. Products of digestion must, therefore, pass through the membranes and cytoplasm of the epithelial cells, through or around the intervening cells, and finally through the endothelial cells of the capillary. The shortest distance travelled through the villus is much greater than the thickness of epithelia in the alveoli of the lungs.

i ***Look at*** **figure [S]153b,** ***an electronmicrograph of a minute portion of the inner surface of a human ileum. Estimate the length of the microvilli in this figure from the magnification (× 40 000) of the electronmicrograph. Explain the relationship of the epithelial cells which line the ileum to:***
1 the villi, and
2 the microvilli.
What is the significance of the villi and microvilli for the function of the ileum?

In the electronmicrograph the villi appear to be about 30 mm long. Their actual dimensions are therefore:

$$\frac{30 \times 10^{-3}}{40\,000}\,\mathrm{m} \simeq 0.7 \times 10^{-6}\,\mathrm{m} = 0.7\,\mu\mathrm{m}$$

While the surface of the villus is covered by thousands of epithelial cells, the microvilli are folds in the plasma membrane of an epithelial cell. Students must be quite clear about the great difference in size between microvilli and villi. This will help to prevent confusion between these structures. Together, these structures produce a vast surface area for absorption.

j ***Mannose and glucose are absorbed across the wall of the ileum at very different rates. Glucose passes five times more rapidly than mannose. Yet they are both hexoses (C_6 sugars) and are therefore very similar compounds. What kind of biological mechanism could distinguish between such similar molecules? Where in the wall of the ileum might this mechanism be located?***

Mannose and glucose are stereoisomers with identical relative molecular masses and similar overall molecular dimensions. Students may suggest the model of a 'molecular sieve', as presented in investigation 6B to account for differences in the permeability of Visking tubing towards glucose and starch. This cannot apply here. The high degree of discrimination exhibited by the wall of the ileum in absorbing the products of digestion is reminiscent of the way in which enzymes react differentially with a variety of potential substrates. The 'specificity' of absorption could arise if the product of digestion had to bind to a complementary 'carrier protein' before it could enter the villus. A protein that is 'designed' to bind molecules of glucose may not bind mannose so tightly. The location of such proteins in the plasma membrane of epithelial cells, perhaps on the microvilli, will be an obvious suggestion, though by no means the only possibility.

INVESTIGATION
6E A microscopic investigation of the liver

ITEMS NEEDED

Liver, prepared section, stained with haemotoxylin and eosin (H. and E.), (sections of pig's liver show the lobular structure most clearly) 1/1

Graticule, eyepiece 1/1
Hand lens 1/1
Microscope, monocular 1/1

(*Study guide* 6.8 'The role of the liver'.)

Principles

1 Hepatocytes are arranged in the liver so that each one receives its supply of blood from the hepatic artery and hepatic portal vein. Products secreted by each hepatocyte leave the liver either by the hepatic vein or by the bile duct.

2 Hepatocytes are centres of intense metabolic activity. Hence the tissue is well supplied with blood.

3 Each hepatocyte carries out all the functions of the liver; there is no division of labour.

It is not possible to deduce the functions of the liver from an examination of its microscopic structure. The student should have some idea of the variety of functions before doing this investigation.

A major problem in understanding the liver is the difficulty of relating the cross-section of a lobule to the three-dimensional structure of the liver. Some idea of the size of various structures will help. The procedure suggests that certain dimensions should be measured using an eyepiece graticule. This will not be necessary if the students know the width of the field of view of their microscopes. In this case it will be possible to make estimates, which are perfectly adequate, without using a graticule.

Reference to figure (P)19 will help to relate the appearance of the cross-sections to the overall lobular structure. It may also help students to show them a simple model of several hexagonal pencils held together in a bunch by an elastic band. The pencil leads would represent the central vein of each lobule. Portal tracts may be imagined as running in between the pencils parallel to their leads. If appropriate photomicrographs are available (for example, in an atlas of histology), they should be on display.

Questions and answers

a ***How many vessels have you distinguished in the portal tracts? What are they, and how do they differ from each other?***

Each portal tract should contain three vessels: hepatic portal vein, hepatic artery, and bile duct. The bile duct may be distinguished by its cuboidal endothelium. The portal vein and artery should be distinguished by the thickness of their walls. (Portal tracts also contain lymph vessels, though these are not normally visible.)

b ***Describe the arrangement of hepatocytes inside a lobule. Record the diameter of a lobule and the width of a sinusoid.***

Hepatocytes are arranged in rows which more or less radiate out from the centre of the lobule. There are gaps, called sinusoids, in between the rows of hepatocytes. Lobules of the human liver, may be 1 mm in diameter; sinusoids are typically 10 to 20 μm across.

c ***What fills the sinusoids between the rows of hepatocytes in the living animal? As it is not possible to examine the living tissue, you could only deduce the answer by examining the structures that connect with a sinusoid.***

Sinusoids are filled with blood. Their connections with the central vein of a lobule may be visible. Their connections with blood vessels in the portal tract will be more difficult to establish. These connections can be studied by injection and corrosion techniques.

d ***Does blood flow from the outside of a lobule inwards, towards the central blood vessel, or in the opposite direction? Explain how you arrive at your answer.***

Blood flows from the outside of a lobule towards its centre. This may be deduced from the fact that branches of the hepatic artery are in the portal tracts, while at the centre of each lobule there is a vein.

e ***List the structures or vessels that blood must pass through in travelling from the hepatic portal vein to the hepatic vein.***

1 Hepatic portal vein.
2 Branch of hepatic portal vein in a portal tract.
3 Sinusoid.
4 Central vein.
5 Hepatic vein.

f ***From your observations and by examining* figure [P]21, *can you tell whether the hepatocytes and the blood are in direct contact or are kept separate from one another?***

Sinusoids are lined with endothelium, which is continuous with the endothelium of the blood vascular system. In sinusoids this tissue is highly perforated. It is presumed that the blood plasma can pass directly into the 'space of Disse' between the endothelial cells and the hepatocytes.

g ***What evidence can you obtain from examining the prepared section and the electromicrograph in* figure [P]21, *to suggest that the metabolism of the hepatocyte is very active?***

The cytoplasm of the hepatocyte is packed with organelles. The numerous mitochondria suggest a high turnover of energy. The extensive rough endoplasmic reticulum points to active protein synthesis. A well developed Golgi apparatus is evidence for rapid packaging of secretory products. The microvilli of the cell membrane (visible in the space of Disse) suggest extensive transport across the membrane. The very rich blood supply to the liver also suggests that the organ is anything but quiescent.

h ***Is there any evidence from the appearance of hepatocytes to suggest that these cells might be grouped into different kinds, according to the functions which they carry out?***

One hepatocyte looks very much like another. There is no evidence from their appearance of any division of labour among hepatocytes.

i ***Can you find any evidence on your slide to suggest how bile, that has been secreted from a hepatocyte, could be channelled into a bile duct?***

Sections of liver stained with H. and E. will not show the bile canaliculi that permeate between the hepatocytes. These drain into the bile ducts and must be revealed by other histochemical methods.

j ***List the ways in which the composition of blood in the hepatic vein might differ from that of blood in***
1 the hepatic portal vein during digestion and absorption of a meal, and
2 the hepatic artery.

1 During digestion and absorption of a meal, blood in the hepatic portal vein carries more
sugars,
amino acids, and
lipids
than blood in the hepatic vein.
2 Blood in the hepatic artery carries more
oxygen,
excess amino acids,
lactic acid, and
haemoglobin (dissolved in the plasma).
Blood in the hepatic vein carries more
urea, and
plasma proteins.
The net balance between absorption and secretion of substances such as glucose and lipids depends on the nutritional state and activity of the animal. This balance is influenced by hormones such as insulin and adrenaline.

k ***An examination of the microscopic structure of the liver helps us to understand how the liver works. Despite this, it does not tell us what the liver actually does. What type of investigation would be most useful in establishing the functions of the liver?***

Studies which have measured the influence of the liver on the chemical composition of blood have been particularly important. These have been carried out in several ways. The liver may be removed completely (hepatectomy) followed by measurement of the levels of certain blood components for as long as the animal survives. Blood samples can be collected from the blood vessels which supply and drain the liver, and their compositions can be compared. The liver can be isolated from its normal blood supply and perfused with fluids of known composition. The influence of the liver on this composition can be readily detected.

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CHAPTER 7 **PHOTOSYNTHESIS**

A review of the chapter's aims and contents

1 Photosynthesis is the most important and fundamental biological process. The continuation of life on this planet depends on the maintenance of effective photosynthesis. The aim of this chapter is to emphasize the importance of this process and to provide an up-to-date résumé of its biochemistry.

2 The emphasis is on the unique light-harvesting processes and the mechanisms which convert light energy into chemical energy for the reduction of carbon dioxide. Carbon photoreduction is presented as a cyclical series of interconversions, which simultaneously provide the photosynthetic product and regenerate the carbon dioxide acceptor.

3 The chloroplast is introduced as the complete photosynthetic unit in higher plants. The major features of its structure that are related to its photosynthetic ability are described and illustrated by electronmicrographs.

4 CAM and C_4 photosynthesis are introduced as adaptations to survival in arid and tropical climates. The characteristics of photorespiration are described.

5 Quantitative aspects of the biochemistry of photosynthesis and the chloroplast structure are illustrated in examples and Study items.

6 Our detailed knowledge of photosynthesis has developed since the time of Priestley and continues to do so today. The current understanding is presented with a historical perspective.

PART I The *Study guide*

7.1 Photosynthesis and the biosphere

Assumption

1 A knowledge of respiratory gas exchange gained from earlier chapters.

Principles

1 Photosynthesis is crucial to the life of animals as well as plants on this planet.

2 The balance of the atmospheric gases oxygen and carbon dioxide is a delicate one, which can be irrevocably damaged by the destruction of ecosystems.

Photosynthesis involves complex light-harvesting and endothermic reactions. Chlorophyll *a* is the essential light-harvesting pigment required for the process and, with other accessory pigments, is found in all photosynthetic membranes. The details of the photosynthetic process differ in bacteria, most algae, and higher plants; the accessory pigments also differ. In this chapter photosynthesis is described as it occurs in the leaf cells of a higher plant.

Recent research has shown that because sugars are quantitatively insignificant products of photosynthesis the classical equation for photosynthesis

$$6CO_2 + 6H_2O \rightarrow C_6H_{12}O_6 + 6O_2$$

provides an inadequate description of the process as it is now understood. It is also not particularly helpful to consider photosynthesis as a reversal of respiration. In principle and in detail, photosynthesis is an entirely different process. It is far less confusing to the student if the principles of the processes are taught totally separately and the essential and unique role of light in photosynthesis is emphasized.

The bulk of this introductory section deals with photosynthetic gas exchange and productivity on a world scale and is intended to emphasize the importance of photosynthesis for the continued maintenance of life.

Practical investigation. *Practical guide* 2, investigation 7A, 'The interaction of plants and animals'.

7.2 The sites of photosynthesis

Principles

1 The structure of a leaf is closely correlated with its function as a photosynthetic organ.

2 The structure of the chloroplast as the photosynthetic organelle can be resolved by electronmicroscopy and is similarly related to its function.

STUDY ITEM

7.21 The leaf as a photosynthetic organ

Questions and answers

a ***From your knowledge of their shape and structure, suggest how green plants make the most of available sunlight.***

In order that a plant may make the most of any available sunlight it must present a large surface area of leaf to the incident light. It is not surprising therefore that leaves should usually be flat, thin, rather rigid structures fixed at an angle to the branch or twig which bears them. Most plant stems are positively phototropic, that is to say, they grow towards the light. In addition, most leaves are able to position themselves so that the upper epidermis (adaxial surface) is about 90° to the incident light, and the positioning of leaves in a plant canopy often represents a beautiful and efficient arrangement for optimal absorption.

It is also useful to point out the interesting orientations which are adopted by leaves in their natural habitats. The leaves are generally held at an angle to the petiole so that they are well positioned to absorb the incident light. Leaves are also often arranged in a canopy or on a single branch so that they do not directly overlap each other but form a mosaic in which each leaf is exposed to the light. Mosaic arrangements are best seen in plants growing in the shade and good examples are ivy and periwinkle. In forests, the whole canopy is an extensive three-dimensional mosaic in which every niche that light penetrates is filled with a leaf. An extreme example is seen in tropical rain forests, where the trees are frequently like open umbrellas with all the branches at the top of the tree. Leafy climbers and low shrubs occupy the second layer and there is also an extensive ground cover of numerous plants, many of which have broad fleshy leaves.

The rigidity of the leaf is an important feature and assists in the efficient capture of light. The veins not only conduct water, minerals, and small organic molecules, but also support the softer, more spongy leaf tissues. A useful exercise to illustrate the importance of the midrib for the leaf's rigidity can be carried out using a thin sheet of A4 paper. Hold the paper by the middle of the short side and note that it flops down. Now fold the paper in half along the long axis and hold it up again. The sheet of paper (or leaf) no longer flops, but behaves as a more rigid sheet or leaf which can be orientated as a single unit.

b ***Examine* figure [S]158a *and* b *and suggest how the leaf is adapted for efficient exchange of gases.***

The other main requirement for photosynthesis is the efficient exchange of gases, that is to say, the inward diffusion of carbon dioxide and outward diffusion of oxygen. Gaseous exchange must be under close control in order to prevent the leaf from becoming dehydrated – a leaf which is structurally adapted for the greatest possible diffusion of carbon dioxide must also face the threat of water loss. The large intercellular air spaces and the consequent large surface area exposed to the air, coupled with the presence of stomata, permit maximum gas exchange.

c ***How is the water loss kept to a minimum in the leaf section shown in* figure [S] 158b*?***

The leaf has evolved a nearly waterproof, waxy cuticle to overcome this problem. This cuticle is punctured by pores or stomata which open in the light to permit the exchange of carbon dioxide and oxygen and close in the dark to minimize water loss when photosynthesis has stopped.

d ***Examine* figure [S]158b *and* c *and suggest how the cells and chloroplasts are arranged for maximum photosynthetic activity.***

Below the upper epidermis of the leaf are one or more layers of palisade mesophyll cells which contain chloroplasts, the chlorophyll-containing organelles which carry out photosynthesis. The tight packing and elongated structure of the palisade mesophyll cells enable solutes and gases to move rapidly from one cell to another. Beneath the palisade mesophyll cells are the spongy mesophyll cells, which are rather more lobed and contain fewer chloroplasts. The irregular shape of the spongy mesophyll cells and the numerous air spaces between them allow carbon dioxide to diffuse to the exposed surfaces and so to the bounding envelope membranes of the chloroplasts, which lie in a single layer just inside the cell membrane.

The irregular shape of the spongy mesophyll cell facilitates diffusion, since high ratios of surface area to volume increase the rates of gaseous diffusion. □

Practical investigation. *Practical guide* 2, investigation 7B, 'The structure of a leaf'.

The chloroplast

Students may have already used the light microscope to observe chloroplasts in leaf cells and will appreciate that the greater power of the electron microscope is needed to resolve the internal structure of the chloroplast. The preparation of tissue for observation in the electron microscope involves potentially destructive procedures. Thus it is important to know about the treatments, so that their effects on the final photographic image in electronmicrographs such as those in figures (S)158d and (S)160 can be appreciated.

Leaf tissue is first treated with a chemical fixative glutaraldehyde which serves to cross-link the proteins and stabilize them. It is particularly effective in preserving the membrane's structure. The fixed tissue is next 'stained' in a solution containing heavy metal atoms, most usually osmium tetroxide (OsO_4). Some of the cellular components adsorb more of the osmium salt than others. The osmium atom is a large one and its presence in the tissue effectively prevents the bombarding electrons from passing through the area of the cell in which it has been deposited. After dehydration the 'stained' tissue is embedded in resin, and thin sections are cut and placed in the microscope. The dehydration is required to remove water which would also impede the passage of the electrons; resin-embedding is required so that very thin sections can be cut. A photographic film is placed below the section in the microscope and electrons which pass through the section fog this film. The exposed areas of film appear as white areas on the final photographic print: those which were not exposed to electrons, that is, those corresponding to areas of the section through which the passage of electrons was impeded, appear black. The black and white contrasts allow different structures in the cell to be distinguished. The print reflects the patterns of osmium accumulation in the cell and merely differentiates areas which readily adsorb the salt from those which do not. However, the patterns are very consistent and this consistency provides the basis for the belief in the value of electronmicrographs in giving information about cell structure. For example, membranes and ribosomes always accumulate a lot of osmium; cell walls and starch grains generally adsorb very little.

It is generally held that the consistent patterns of heavy metal accumulation reflect the various patterns of cell structures, such as membranes, ribosomes, and cell walls, which are formed from assemblies of different macromolecules. By the analysis of these patterns many new structures have been discovered. In the case of the chloroplast, the double nature of the chloroplast envelope, the partitioned structure of the grana, and the existence of chloroplast ribosomes have all been discovered by the interpretation of electronmicrographs.

Different phases of photosynthesis take place in different compartments of the chloroplast and this can be demonstrated experimentally after the chloroplasts have been isolated from the leaves. The leaf tissue is chopped up so that the chloroplasts are released from the cell and they are separated from the other cellular constituents by differential centrifugation. Isolated intact chloroplasts carry out *all* the photosynthetic reactions, provided that they have an adequate supply of carbon dioxide and inorganic phosphate and that they are illuminated. Intact chloroplasts can be swollen and broken by changing the water potential of their suspending medium by dilution. The thylakoid system floats out of the broken chloroplasts and may be separated from the ribosomes and stroma by centrifugation. Illuminated, separated, photosynthetic membranes carry out all the light-harvesting and energy-transduction processes of photosynthesis; but effective photoreduction of carbon dioxide will only occur when the stroma is also present within the envelope of the intact chloroplast.

STUDY ITEM

7.22 The size and structure of a chloroplast

Principles

1 A large electronmicrograph is presented to the student for detailed observation and measurement.

2 The relative sizes of structures related to photosynthesis are examined and a larger scale visual analogy demanded.

Questions and answers

a ***Use the micrograph to calculate the actual length, width, and area of the chloroplast section. Use a ruler or squared paper to measure the dimensions.***

The length and width of the chloroplast section should be measured with a ruler (in millimetres). The actual size of the section can then be calculated by reference to the scale at the bottom of the electronmicrograph, that is, the bar on the micrograph represents 1 μm in real life. The chloroplast's area can be measured by tracing around the outline of the chloroplast section on to transparent paper and then on to squared paper. The squares should be of known dimension, for example, 1 mm squares. (The chloroplast section is about 5 μm long and 1.5 μm wide.)

b *Assume that when the section is rotated about its long axis this represents the 3-dimensional shape of the chloroplast. Calculate the approximate volume of the chloroplast.*

The choice of an appropriate method to determine the volume is difficult because of the shape of the chloroplast section. One way to calculate its approximate volume is to assume that the chloroplast is ellipsoidal in shape; then its volume $= \frac{4}{3}\pi ab^2$, where 'a' is its width and 'b' its length. Alternatively, it could be assumed the central part of the chloroplast is a cylinder and the ends are half-spheres. Other methods of calculation can be devised, but the difficulty of calculating the volume of cellular organelles with any precision is clearly illustrated.

c *Calculate the mean number of lamellae per granum. Describe how you distinguished between granal (appressed) and non-granal (non-appressed) membranes. By reference to* figure [S]159, *explain why you would expect the limits of a granum to be unclear in some thin sections.*

The number of lamellae per granum varies between 6 and 12, with a mean number of about 10. The difficulty in making these observations is to decide where the edge of a granum occurs. The lamellae in some of the grana cannot be as clearly seen as they can in other grana. This is because the grana lie at angles to each other in the stroma and the lamellae can only be clearly seen in those grana in which the piece of section falls at right angles to the granal stack. It would be legitimate to exclude all those grana which cannot be clearly seen from your observations, but the proportion which have been excluded should be clearly stated and their general features, in comparison with grana which have been included, should be described. This particular electronmicrograph is very unusual because so many of the constituent granal stacks can be clearly seen. Only about 1 in 5000 pictures shows this type of chloroplast structure, but these are the ones generally chosen for demonstration purposes.

d *Identify the double envelope of the chloroplast, the grana, the stroma, the plastoglobuli, and the chloroplast ribosomes in the electronmicrograph.*

Reference to the labels in figure (S)160 will give the correct identifications. It is interesting to point out that the ribosomes in the cytoplasm appear to be larger than those in the chloroplast. In the top lefthand corner of the micrograph part of the nuclear double membrane and nucleoplasm can be seen. The cell wall is at the bottom of the picture.

e *Devise a method for calculating the number of ribosomes per unit cytoplasmic volume.*

The concentration of ribosomes in the cytoplasm can be calculated by adopting an ecological technique. The number of ribosomes inside a square or rectangle of known dimensions is counted. This is repeated several times for squares placed in different areas of the cytoplasm. The important and somewhat subjective choices are the dimensions of the square, the number of samples, and the areas of the cytoplasm which are comparable.

f ***By reference to the dimensions given in* figures [S]158 *and* 160, *calculate the ratio of the following: diameter of plastoglobulus: length of chloroplast: length of palisade cell: thickness of leaf: length of leaf.***

The size of each of the diagrams can be measured and their relative sizes calculated by reference to adjacent drawings. The ratio required is approximately 1:10:80:300:10 000.

g ***Construct an analogy from the sizes of everyday objects which corresponds to the ratios calculated in answer to question f. Assume that the plastoglobulus is the size of a tennis ball.***

One such appropriate analogy would be of a leaf cell with a lecture theatre which will hold 150 people. On the same scale an average student in the lecture theatre would be the size of a chloroplast and a plastoglobulus the size of a tennis ball. The dimensions of the building which contains the lecture theatre can be calculated to represent a small piece of leaf tissue and the size of the town in which the building is placed, the size of the whole leaf. The extra space not occupied by people in the lecture theatre represents the cell vacuole: its large dimensions are immediately apparent from this analogy.

h ***Turn back to page [S]134. What could you use to represent a mitochondrion in your analogy?***

☐ A rugby ball would be of an appropriate size in the above analogy.

7.3 The mechanism of photosynthesis

Principles

1 There are three main stages in the photosynthetic process: light harvesting, energy transduction, and reduction of carbon dioxide.
2 The oxygen evolved in photosynthesis is derived from water.
3 Our current understanding of photosynthesis has developed over the last two centuries and has involved scientists working in many countries and using a variety of techniques.

STUDY ITEM
7.31 The source of oxygen in photosynthesis

Principle

1 Ruben's and Kamen's classical experiment provides conclusive evidence that the oxygen evolved in photosynthesis is derived from water and not from carbon dioxide.

Questions and answers

a ***What does this stage of the experiment suggest about the origin of the gaseous oxygen evolved?***

Clearly the oxygen did not originate from the carbon dioxide, which had been supplied with its oxygen radioactively labelled.

b ***What can you deduce from these results about the source of the oxygen evolved? Explain your reasoning.***

The oxygen clearly must have come from the water which was the only source of ^{18}O.

c ***How can you modify the summary equation for photosynthesis shown above in the light of Ruben's and Kamen's evidence?***

The equation may now be written:

$$\text{carbon dioxide} + \text{water} + \text{light} \xrightarrow{\text{chlorophyll}} \text{organic compounds} + \text{oxygen} + \text{water}$$ □

Practical investigation. *Practical guide* 2, investigation 7C, 'The evolution of oxygen'.

When the physiology of photosynthesis is being described, the concept of limiting factors is often introduced. This was conceived by F. F. Blackman during his studies of photosynthesis in Cambridge in the 1920s. The concept has a wide biological application, since biological processes are characteristically complex and involve a series of constituent reactions, and each reaction generally responds independently to changing conditions. The slowest reaction will always limit the rate of the biological processes and different reactions will be limiting under different conditions.

The example of George, Mary, and Peter packing the air-fresheners illustrates the principle of limiting factors. The concept is also illustrated by the answers to the questions in Study item 7.32, which is based on the photosynthetic rates under the changing conditions of temperature and light intensity, as shown in the experiments recorded in figures (S)162 and (S)163.

STUDY ITEM

7.32 Limiting factors in the rate of photosynthesis

Principles

1 The rate of photosynthesis in any given set of conditions is limited in practice by one of these conditions.

2 Experimental evidence supports the concept of limiting factors.

Questions and answers

a ***Which of these plant species has the highest rate of photosynthesis?***

Maize has the highest rate of photosynthesis, as shown on the graph.

b ***Does this species have the highest rate of photosynthesis at* all *light intensities?***

Maize does not, however, show the highest photosynthetic rate under *all* the conditions tested. When the light intensity is less than 200 μEi m^{-2} s^{-1}, sunflower and rye-grass have slightly higher photosynthetic rates than maize. This diagram draws attention to a common feature of C_4 plants, that is, they perform less well than C_3 plants at low light intensities. However, at this stage the characteristics of C_3 and C_4 pathways have yet to be elucidated. Reference can again be made to this point at an appropriate stage later.

c ***What limits the rate of photosynthesis of all three species in the section of the graph marked P?***

It can be seen that for all three plants a rapid increase in the rate of photosynthesis occurs with an increase in light intensity. Hence in this section of the graph light is clearly the limiting factor.

d ***What limits the rate of photosynthesis of all three species in the section of the graph marked Q?***

In sunflower and rye-grass, beyond a certain light intensity, further increases in light do not significantly increase the rate of photosynthesis – the curve reaches a plateau. This is because the photochemical reactions are no longer rate-limiting. Therefore, in order to increase the photosynthetic rate we must increase the rate of the reaction which is limiting. The same is true of maize, except that in this case the curve reaches a plateau at a much higher level and at a higher light intensity. The differences in the light responses between the tropical plant (maize) and the temperate plants are significant. As we have seen maize is an example of a C_4 plant, characterized by possessing an additional biochemical pathway which initially catalyses the fixation of the carbon dioxide into a 4-carbon compound. The C_4 pathway of photosynthesis will be discussed further in section 7.5, but plants like maize, which have this pathway, show a larger increase in photosynthetic rate at higher light intensities than C_3 plants.

e ***At what temperature does increasing light intensity have the greatest effect on the rate of photosynthesis in cord grass?***

In cord grass the greatest effect of light intensity is at 30 °C. This is because at 30 °C, temperature limits photosynthesis to the least extent.

f ***What are the relative rates of photosynthesis at 5 °C, 15 °C, and 25 °C at an intensity of 500 μEi m^{-2} s^{-1} (equivalent to about 25 per cent sunlight)?***

The rates are 12, 55, and 90 ng CO_2 cm^{-2} s^{-1} at 5 °C, 15 °C, and 25 °C respectively. The Q_{10}, that is, the increase in rate for each 10° rise in temperature, can also be calculated for 5 °C/15 °C and 15 °C/25 °C.

g ***Explain why the increase in the rate of photosynthesis between 0 and 500 μEi m^{-2} s^{-1} is greater at 30 °C than at 5 °C in cord grass.***

At 5 °C the temperature limits photosynthesis, so that increases in light intensity have less effect.

h ***The rate of photosynthesis at 5 °C does not increase at high intensities of light. What is the biochemical explanation for this lack of response?***

The rate of photosynthesis at high intensities of light does not increase at 5 °C because at this low temperature the temperature-sensitive enzyme reactions of the carbon reduction pathway proceed very slowly. Although increasing light intensity could increase the light-harvesting and electron transfer reactions (which are relatively unaffected by temperature), photosynthetic rates do not increase because the reduction of carbon dioxide is limited. □

The data presented in figure (S)163 show that the photosynthetic rate can be increased at high light intensities if the temperature is increased. This is because the enzymic reactions which fix carbon dioxide become rate-limiting at high light intensities. The increase in temperature speeds up the enzymic reactions and increases the photosynthetic rate. Note that the temperature has no effect on the photosynthetic rate at low light intensities – the curves all go through the same points – because the photochemical reactions which are rate-limiting at low light intensities are insensitive to temperature. At high light intensities the rate of photosynthesis can also be increased by increasing the concentration of carbon dioxide in the atmosphere. High concentrations of carbon dioxide increase the rate of the enzymic reactions involved in the fixation of carbon dioxide.

The light-harvesting reactions are described in some detail, since they are unique and basic to a proper understanding of photosynthesis. The concepts are new and exciting, and are unfamiliar because they are not encountered in other branches of biology. They should be introduced as the vital part of photosynthesis. The unravelling of the complexities of the light-harvesting process challenges the ingenuity and imagination of experimental scientists because of its great importance in understanding the mechanisms of energy conversion.

The principles of pigment absorption will be familiar to the students who have studied physics. A few simple demonstrations using coloured solutions and a selection of complementary filters should be helpful to those who have not studied the attributes of visible light before.

Chlorophyll *a* is the main light absorbing pigment in photosynthesis. The study of the chemistry of the chlorophyll molecule helps to explain both its properties of light absorption and the specific orientation that it adopts in the photosynthetic membrane. The insert in figure (S)165 shows the orientation of the chlorophyll molecule in the lipoprotein matrix of the thylakoid membrane.

Various carotenoid pigments are known to act as light-harvesting pigments in some algae. Their role in absorption is only a minor one in higher plants, where they protect the chlorophylls from photo-oxidation.

Practical investigation. *Practical guide* 2, investigation 7D, 'Leaf pigments'.

The pigment molecules must be precisely orientated in the photosynthetic membrane if they are to continue to function. The concept of a mosaic arrangement of membrane components is introduced. Like many other biological membranes, photosynthetic membranes have a very specific composition and their component molecules are precisely orientated in relation to each other. The continuing function of the membrane depends on the maintenance of this complex structure. It is not possible to illustrate in one model all the different aspects of membrane structure and the interactions of the constituent compounds, and so several different ones (figures (S)166, (S)167, and (S)168) are provided. They should be studied together, because each model emphasizes a different aspect of the photosynthetic membrane. Figure (S)166 emphasizes the relationship between the larger light-harvesting complexes and the smaller reaction centre complexes. Figure (S)167 illustrates the functional dependence of the electron transfer reactions on the light-harvesting reactions. Figure (S)168 shows the three-dimensional arrangement of the individual carrier molecules and their orientation in relation to the other membrane components.

The successful completion of the light-harvesting and energy transduction phases of photosynthesis results in the reduction of $NADP^+$ and the phosphorylation of ADP to ATP. The relationship of these events to the other events occurring in the membrane is also illustrated in the model in figure (S)168. Frequent cross-reference to figures (S)166 to (S)168 and to the functional descriptions in the *Study guide* will enable students to appreciate the complexities and interactions involved in membrane function.

The importance of light energy in 'pushing' the electron against the natural thermochemical gradient should be emphasized. Electrons effectively become associated with increased electronegativity in response to illumination. This enables them, subsequently, to move with the thermochemical gradient, and this in turn allows phosphorylation and $NADP^+$ reduction to occur. It can be very confusing to draw analogies with respiratory electron transport in the mitochondrion and this should be avoided.

Practical investigation. *Practical guide* 2, investigation 7E, 'The reducing activity of chloroplasts: "the Hill reaction"'.

7.4 The reduction of carbon dioxide

Assumption

1 That students have an understanding of the light-harvesting and energy transduction phases of photosynthesis in generating ATP and NADPH.

Principles

1 The Calvin pathway of carbon dioxide photoreduction is cyclical.
2 The cycle is dependent on light for a continuous supply of NADPH and ATP.
3 Carbon dioxide photoreduction occurs simultaneously with light harvesting and energy transduction.

Carbon dioxide and water are used in photosynthesis and oxygen is evolved. The product of the photoreduction of carbon dioxide is triose phosphate, a sugar containing three carbon atoms. During active photosynthesis most of the triose phosphate molecules not required for the regeneration of the acceptor move out of the chloroplast into the cytoplasm. In the cytoplasm triose phosphate enters the cellular metabolic pathways and becomes incorporated into sucrose and other carbohydrates, amino acids, and proteins and also fatty acids and lipids. Eventually, the photosynthesized carbon is incorporated into numerous complex macromolecules including nucleic acids and the co-factors required for many cellular syntheses. It should be emphasized that the continuing growth and development of a green plant depends on a continuous supply of photosynthesized carbon and it is no exaggeration to describe the whole plant as the 'product of photosynthesis'.

Occasionally some of the triose phosphate produced by carbon dioxide photoreduction is converted within the chloroplast to starch and deposited in the chloroplast stroma. A diagram showing the balance between the alternative fates of triose phosphate is given in *figure 21*.

The three possible fates of triose phosphate are:

1 To be fed back into the Calvin pathway to provide newly regenerated acceptor molecules of RuBP.

2 To be passed through the envelope into the cytoplasm. This process is exceedingly rapid and takes place several times faster than the rate of photosynthesis, provided that an adequate concentration of inorganic phosphate ions is present in the cytoplasm. The transport of triose phosphate across the chloroplast envelope depends on the operation of a protein translocator, which is an integral part of the inner envelope membrane of the chloroplast. The transport of triose phosphate can only occur in combination with a counter current of inorganic phosphate ions.

3 To be utilized for starch synthesis in the chloroplast. Starch provides a temporary store of carbohydrate which can be hydrolysed when required.

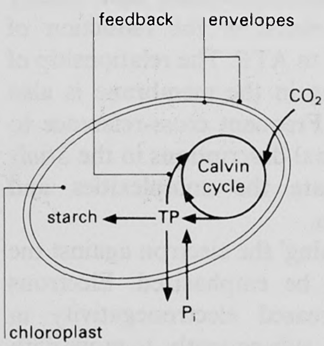

Figure 21
The fates of triose phosphate (TP). *Based on Walker, D. A. and Edwards, G. E.,* C3, C4 mechanisms, and cellular and environmental regulation of photosynthesis, *Blackwell Scientific Publications, 1983.*

The concentrations of phosphate ions in the cytoplasm and in the chloroplast stroma provide a sensitive control mechanism which determines the fate of the triose phosphate molecules. With the help of the diagram on the left it is a useful exercise to consider what will happen when the chloroplast is present in a low phosphate environment. The efflux of triose phosphate will be inhibited and, provided the Calvin pathway is fully saturated, more triose phosphate molecules will be available for starch synthesis. (This additional starch synthesis has been demonstrated in isolated chloroplasts and also in leaf cells fed with mannose, which becomes converted to mannose phosphate, with the result that the cytoplasmic inorganic phosphate pools are depleted.) In contrast, the situation where phosphate concentrations are particularly high can also be considered. In this case the efflux of triose phosphate will be favoured; little will be available for starch synthesis. It can be shown that little triose phosphate leaves the chloroplast in the period immediately following the transfer from dark to light: all the molecules are needed to restore the steady state levels of the Calvin cycle intermediates, which have been depleted in the dark.

It should be clear from the text that it is no longer strictly correct to refer to the 'dark' and 'light' phases of photosynthesis. This is because the activity of several of the enzymes of the Calvin pathway is modulated by light. It cannot be emphasized too frequently that the light-harvesting, energy transduction and carbon reduction phases of photosynthesis occur simultaneously. The differences in the time scale of the different phases of photosynthesis are shown in table (S)27. It is interesting to translate this time scale into the relative equivalent in seconds, minutes, days, and years. The analogy brings out the differences very dramatically.

The cycle consists of four consecutive events: carboxylation, phosphorylation, reduction, and regeneration of the acceptor. (Regeneration of the acceptor also involves an additional phosphorylation step.) The product of the cycle is one molecule of triose phosphate for every three molecules of carbon dioxide which are photoreduced. The carbon balance of the cycle and the sequence of sugar phosphate interconversions are shown in figure (S)170 where the sugars are described by the number of carbon atoms which they contain.

STUDY ITEM
7.41 Experimental evidence for cyclical pathways

Principles

1 The first stable product of carbon dioxide assimilation is phosphoglyceric acid from which, after further phosphorylation and reduction utilizing the ATP and NADPH formed in the earlier stages of photosynthesis, triose phosphate is formed.

2 Triose phosphate is the precursor of other organic compounds formed in the plant.

3 Triose phosphate also passes through a cycle of regeneration from which ribulose bisphosphate is formed. This ribulose bisphosphate takes part in further carboxylation.

Questions and answers

a ***Why was an algal suspension rather than a leaf used for these experiments?***

The algal suspension was a suspension of single cells which could be maintained in a steady state, so that all the cells behaved in a rather similar way when the conditions were altered. Because of diffusion problems and the heterogeneity of its structure, leaf tissue is less easy to handle. Sampling a cell suspension is also much easier.

b *How do these results support the hypothesis that phosphoglyceric acid is a very early product of carbon dioxide reduction?*

Phosphoglyceric acid is the most rapidly labelled compound and therefore is the earliest product.

c *The graph shows a fall in the level of radioactivity found in sugar phosphates after approximately two minutes of photosynthesis. Why should this occur?*

Some of the labelled carbon (^{14}C) is leaving the cycle and becoming incorporated into malate and alanine.

d *Why are the levels of PGA and RuBP in a 'steady state' in the light?*

The cycle is operating effectively in a steady state and so the labelling in each intermediate is constant, although carbon is flowing through the cycle. When the flow of carbon is altered, the proportion labelled in the different intermediates also changes, for example, when light is removed or the concentration of carbon dioxide is reduced.

e *Why is there a rapid change in the levels of radioactivity when the illumination is switched off?*

Regeneration of the acceptor RuBP from PGA does not occur in the dark, since regeneration requires ATP and NADPH generated in the light. PGA synthesis continues for a short time in the dark after a period of illumination, until the pool of RuBP generated in the light is exhausted.

f *Why does this change take the form of an increase in PGA and a decrease in RuBP?*

The formation of PGA from RuBP and of RuBP from PGA are cyclical events so that changes in one component inevitably affect the other. The light-dependent events of the cycle follow the carbon dioxide fixation reaction, which produces PGA.

g *How do the results provide evidence that PGA is converted to RuBP?*

The production of PGA and that of RuBP are affected in opposite directions when conditions are changed.

h *Why does the level of PGA fall?*

Carboxylation is also reduced and fewer molecules of PGA are synthesized.

i *Why does the level of RuBP rise?*

The labelled molecules of RuBP cannot become carboxylated, since insufficient carbon dioxide is available; so the pool size of labelled RuBP increases in relation to that of PGA.

j ***Explain how the oscillations in PGA and RuBP shown in both graphs* b *and* c *support the hypothesis that a cyclic series of reactions is involved in carbon dioxide reduction.***

The oscillations are in opposite directions. The answers to questions **d** to **i** also support this conclusion. In the experiments in which he fed $^{14}CO_2$ to photosynthesizing algal cells, Calvin also analysed the labelling of the individual carbon atoms in the sugar molecules which had been synthesized. The labelling was uneven in the different carbon atoms and from the distinctive labelling patterns of the different triosė, pentose, and hexose sugars Calvin was able to suggest the enzymic reactions which might be involved in the sugar interconversion reactions. The analysis of sugars with four and with seven carbon atoms supported his hypothesis. Algae and leaf tissue were then examined to see if they contained the necessary enzymes to catalyse the reactions which were proposed. All the enzymes shown in the scheme below have now been identified (see *figure 22*).

☐

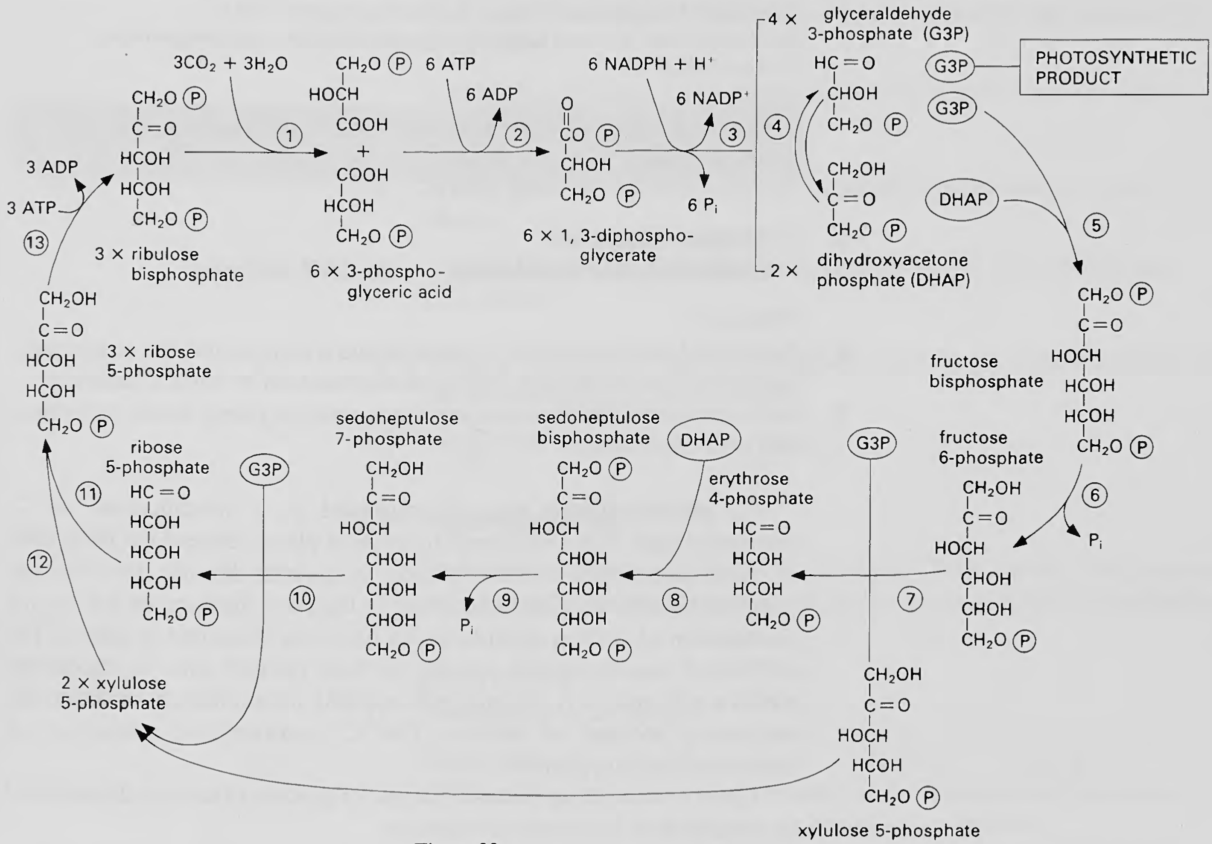

Figure 22
The carbon pathway of photosynthesis: the Benson–Calvin pathway. The diagram shows the fate of the six triose phosphate molecules synthesized during the photoreduction of three molecules of carbon dioxide. The enzymes involved in the pathway are: 1, ribulose bisphosphate carboxylase; 2, phosphoglycerokinase; 3, triose phosphate dehydrogenase; 4, phosphotriose isomerase; 5, aldolase; 6, fructose bisphosphatase; 7, transketolase; 8, aldolase; 9, sedoheptulose bisphosphatase; 10, transketolase; 11, phosphopentose isomerase; 12, phosphopentose epimerase; 13, phosphopentokinase.

Some of Calvin's data are shown in table 11. A very useful exercise is to work out how labelled ^{14}C from the $^{14}CO_2$ flows through the cycle intermediates during the first few seconds of the operation of the cycle in the light, as shown in table 11.

Carbon atom in sugar or PGA	*PGA*	*Fructose*	*Sedo-heptulose*	*Ribulose*	*Sedoheptulose from soyabean* 5s	0.4s
1, carboxyl	82	3	2	11	2	0 (assumed)
2, alpha	6	3	2	10	4	0 (assumed)
3, beta	6	43	28	69	30	33
4		42	24	5	29	8
5		3	27	3	31	49
6		3	2		4	0 (assumed)
7			2		2	0 (assumed)

Table 11
The radioactivity distribution in compounds from a flow experiment by Bassham J. A. and Calvin M. *Scenedesmus obliquus*, 5 second exposure to $^{14}CO_2$.
Based on Bassham, J. A. and Calvin, M., The path of carbon in photosynthesis, *Prentice-Hall*, 1957.

Practical investigation. *Practical guide* 2, investigation 7F, 'The production of starch by leaves'.

7.5 C_4 photosynthesis and
7.6 Crassulacean acid metabolism — the CAM pathway

Principles

1 Additional pathways exist in some plants which permit the temporary storage of carbon dioxide before its assimilation by the C_3 pathway.

2 Such additional mechanisms are widespread in plants found in tropical and arid conditions.

C_4 photosynthesis may be regarded as a modification of C_3 photosynthesis. It is widespread in tropical plants. Indeed the formation of triose phosphate via the fixation of carbon dioxide into ribulose bisphosphate in the Calvin pathway is the only mechanism for the *net assimilation* of carbon dioxide which has been identified in plants. The additional mechanisms for fixing carbon dioxide into C_4-molecules which are found in C_4 plants and in CAM plants merely facilitate the temporary storage of carbon. The C_3 pathway is present in all photosynthesizing plants.

CAM is interesting because the initial capture of carbon dioxide and its assimilation are separated in time.

Practical investigation. *Practical guide* 2, investigation 7G, 'Carbon fixation in CAM plants'.

STUDY ITEM

7.61 Gas exchange and water loss

Principles

1 In C_4 plants the assimilation of carbon dioxide and its subsequent entry into the Calvin pathway are separated in different parts of the leaf. Stomata are open for the intake of carbon dioxide and for water loss during the day and are closed during the night.

2 In CAM plants the assimilation of carbon dioxide takes place during the night when the stomata are open. The entry of carbon dioxide into the Calvin cycle happens during the day, when the stomata are closed.

Questions and answers

a ***Identify A or B as the pattern for a CAM plant and give reasons for your choice.***

B is the CAM plant because the stomata are open only at night.

b ***Give your reasons for identifying the other graph as the pattern for a C_4 plant.***

A is the C_4 pathway. In C_4 plants all the assimilation of CO_2 occurs during the day, when the stomata are open.

c ***Which pattern would describe a C_3 plant? Give reasons for your choice.***

A, since C_3 and C_4 plants have similar patterns of water efflux and CO_2 influx.

d ***Why do the levels of water output follow the same pattern as those of the movement of carbon dioxide?***

☐ They reflect the stomatal movements.

7.7 Photorespiration

Photorespiration should not be confused with aerobic respiration enhanced in the light. The two processes are quite distinct biochemically.

STUDY ITEM

7.71 Competition for an enzyme

Principles

1 Carbon dioxide and oxygen compete for the active site on the same enzyme, ribulose bisphosphate carboxylase/oxygenase.

2 The action of the enzyme depends on the relative concentration of the two gases.

Questions and answers

a **From your knowledge of the mechanism of enzyme action, what conditions will favour the carboxylation pathway?**

High concentrations of carbon dioxide will favour collisions between the enzyme and this gas; this results in carboxylation of ribulose bisphosphate.

b **What conditions will favour the oxygenation pathway?**

Relatively high concentrations of oxygen will favour oxygenation of ribulose bisphosphate. □

STUDY ITEM

7.72 A comparison between C_3, C_4, and CAM plants

Principle

1 C_3, C_4, and CAM plants differ in their anatomy, morphology, and ecology as well as in their biochemistry and physiology.

Question and answer

ital

a **Draw up a four-column table with the column headings 'Feature', 'C_3 plants', 'C_4 plants', and 'CAM plants'. Complete the table as far as you can for the following features, which should be entered in the first column of the table: habitat; leaf anatomy; initial carbon dioxide acceptor; first-formed product of carboxylation; number of processes of carbon dioxide fixation; rate of photosynthesis; photorespiration; rate of water loss; time of stomatal opening; type of chloroplast.**

Table 12 shows some of the characteristics of the three groups of plants. □

Feature	*C_3 plants*	*C_4 plants*	*CAM plants*
Habitat	Do well in temperate climates.	Do well in high light intensities, high temperatures, and semi-arid environments.	Do well in very arid environments.
	Usually moderately productive.	Often highly productive.	Usually very poorly productive.
Leaf anatomy	Lack bundle sheath cells.	Possess bundle sheath cells.	Lack bundle sheath cells.
Initial carbon dioxide acceptor	Ribulose bisphosphate (5-carbon sugar).	Phosphoenol-pyruvate (3-carbon acid).	Phosphoenol-pyruvate in dark; ribulose bisphosphate in light.
First-formed product of carboxylation	Phosphoglycerate (3- carbon acid).	Oxaloacetate (4-carbon acid).	Oxaloacetate (4-carbon acid).
Number of carbon dioxide fixation processes	Only one main CO_2 fixation process.	Two main fixation processes separated in space.	Two main fixation processes separated in time.
Rate of photosynthesis	Mostly moderate.	Often rapid.	Usually slow.
Photorespiration	Present.	Absent.	Sometimes repressed.
Rate of water loss	Lose water readily.	Lose water less readily.	Conserve water effectively.
Time of stomatal opening	By day.	By day.	By night.
Type of chloroplast	Usually only have one type of chloroplast.	May have two distinct types of chloroplast.	Usually only have one type of chloroplast.
Other features not discussed in this chapter	Photosaturate at about 1/5 full sunlight.	Do not readily photosaturate.	May not photosaturate.
	Have relatively high CO_2 compensation points.	Have relatively low CO_2 compensation points.	Have high affinity for CO_2 by night.
	Discriminate against $^{13}CO_2$.	Discriminate against $^{13}CO_2$ less readily than C_3.	Discriminate usually like C_4.
Examples	Typically temperate species such as spinach, wheat, and potato.	Typically semi-tropical species such as sugar cane, maize, and amaranth.	Typically succulent species such as cacti and stonecrop.

Table 12
The descriptions above are generalizations. For example, not all C_4 plants display exceptionally high rates of photosynthesis, and some C_3 species, such as sunflower, apparently photosynthesize as well as C_4 species, despite the handicap of photorespiration. CAM plants may also change their behaviour according to their environment, behaving more like C_3 species if given adequate water.
Based on Walker, D. A., Energy, plants and Man, *Packard Publishing, 1979.*

PART II The *Practical guide*

The investigations related to this chapter appear in *Practical guide 2, Chemical reactions in organisms.*

INVESTIGATION
7A The interaction of plants and animals

(*Study guide* 7.1 'Photosynthesis and the biosphere'.)

ITEMS NEEDED

Asellus sp. or *Gammarus* sp. or other small live freshwater animals
Elodea sp. or other live freshwater plants
Terrestrial plants, collections of live shoots
Woodlice or other small, live, terrestrial animals

Hydrogen carbonate indicator solution
Water, distilled

Aquaria, glass or transparent plastic 2/group
Balance, accurate to 1 place of decimals 1/class
Beakers, $100\,cm^3$ (for holding plant shoots) 5 or 6/group
Cotton thread
Lamps (150 watt) 2/group
Pipette, dropping 1/group
Plates, glass, to cover aquaria 2/group
Scissors 1/class
Test-tubes or specimen tubes (about 2 cm diameter) 4/group
Time switch, mains 1/class
Tubing, Visking, or watch-glasses
Vaseline or silicoset rubber

Bicarbonate indicator solution

0.1 g	cresol red
$20\,cm^3$	ethanol
0.84 g	sodium hydrogen carbonate (Analar)
0.2 g	thymol blue
$1\,dm^3$	water, distilled

Dissolve the thymol blue and cresol red in the ethanol. Filter, as dyes from some sources leave a sediment when solutions are prepared. Dissolve the sodium hydrogen carbonate in about $900\,cm^3$ distilled water. Prevent dirt or dust from getting into the water as this will affect the final colour. Add the filtered dye and make the volume up to exactly $1\,dm^3$. This stock solution is ten times as strong as that normally used. Dilute at the time of use.

It is quite easy to introduce so many animals that the indicator, having turned yellow in the dark, never returns to red in the light.

A single group of students should not be expected to set up both parts of this investigation. The two parts can be assigned to different groups and their results shared among the whole class.

Principles

1 The compensation period of a community depends not only on the type of plants present, but also on the balance of plants and animals. Introducing more animals to the community increases its equivalent compensation period.

2 Animals not only produce carbon dioxide continuously but do so to a greater extent than plants, mass for mass. This has implications for the relative proportions of animals and plants supported in a 'balanced' community.

Questions and answers

a ***By what means can you ensure that the quantities of plants in both containers are equal (step 3)?***

By weighing them.

b ***Is it better to stand the plants in soil or water (step 3)? Give your reasons.***

In water. Soil contains micro-organisms which respire and so affect the carbon dioxide content of the air.

c *Should steps be taken to maintain a constant temperature in the containers and if so, why?*

Yes. Temperature affects the colour of the indicator; comparisons made at different temperatures are invalid.

d *The change in colour of the hydrogen carbonate indicator is gradual. It is difficult to assess accurately the time it takes for it to become a certain shade of red again. What steps can you take to make the measurements more precise?*

If colour is judged by eye, the amount and quality of light entering the indicator must be the same for each observation. The use of some form of colorimeter is preferable. Arrangements can be made to withdraw samples of indicator at intervals and measure their optical density in a colorimeter. Whichever method is adopted, the thickness of the sample must be the same whenever an observation is made.

e *Is the size of the container important in relation to the number of plants used? Would you expect different results using large containers with a few plants?*

No. The compensation period is the time required for plants to absorb whatever carbon dioxide may have been produced in the dark. In theory, the size of the container is of no consequence. In practice, a few plants would have little effect on the atmospheric composition in a large container and it would therefore be difficult to detect changes in carbon dioxide.

f *What is the proportion of animals to plants used in step 4 as determined by fresh mass?*

This will vary depending on the animal and plant material used.

g *Was the indicator restored to its red colour when the container was illuminated (step 9)? From this observation state whether the community contained too many animals for continued survival or whether it could accept more.*

If the compensation period is nearly a whole day, even under intense illumination, there are probably more animals present than can be supported by the plant community. If compensation never occurs, then there are too many animals present. It must be remembered that only carbon dioxide is being considered here. 'Support' will depend on several factors, some of them, such as food, far more pressing than carbon dioxide.

h *What proportions of animals and plants, by mass, form a balanced microaquarium community in terms of the carbon dioxide concentration (step 14)?*

An exact figure cannot be expected from this technique.

i ***Suppose that you wanted to set up a balanced aquarium community. Would steps 10 to 14 above form a useful method for determining the proportion of animals and plants to be used?***

They would help, if the aquarium were to be sealed and illuminated continuously (both are unlikely conditions). Information about other factors, such as the supply of food, is more important; thus the realistic answer to the question is 'no'.

INVESTIGATION
7B The structure of a leaf

ITEMS NEEDED

Leaf, *Elodea* sp., fresh 1/1
Leaf sections, prepared T.S. of a monocotyledon (for example, lily, *Lilium* sp. or maize, *Zea mays*) 1/1
a dicotyledon (for example, privet, *Ligustrum* sp. or lilac, *Syringa* sp.) 1/1
a xerophyte (for example, marram grass, *Ammophila arenaria*) 1/1

Microscope, monocular 1/1
Microscope slides and coverslips
Razor blade, single-sided, in holder (if sections are to be cut) 1/1
Scissors, fine 1/1
Tissues, lens 1/1
Watch-glass 1/1

(*Study guide* 7.2. 'The sites of photosynthesis.')

Students should be encouraged to make accurate drawings of the sections which they examine. However, these should be plans which show just the distribution of tissues rather than the cellular details. This may be a suitable occasion to introduce the distinction between C_3 and C_4 metabolism, particularly if slides showing the specialized 'bundle sheaths' are available. This is examined in more detail in section 7.5 of the *Study guide*.

Principles

1 The structure of a leaf is adapted to its function of photosynthesis.
2 The diversity of shapes among leaves can mask their basic similarities of structure.
3 The typical laminate leaf form is an adaptation to ensure that cells containing chloroplasts are exposed to sunlight, carbon dioxide (or hydrogen carbonate ions), and water.

Questions and answers

a ***Which of the structural features that you have observed are found:***
1 in only the dicotyledonous leaf;
2 in only the monocotyledonous leaf;
3 in both types of leaf?

1 The dicotyledonous leaf is asymmetric in relation to its upper and lower surfaces: the palisade mesophyll is next to the upper epidermis and the stomata are often more frequent in the lower epidermis.

2 The monocotyledonous leaf has a regular distribution of vascular tissue in its cross-section. This arises from the parallel arrangement of vascular bundles in the leaf.

3 Epidermis, spongy photosynthetic tissue, vascular tissue, and stomata are structures found in both types of leaf.

These points are illustrated in *figure 23*.

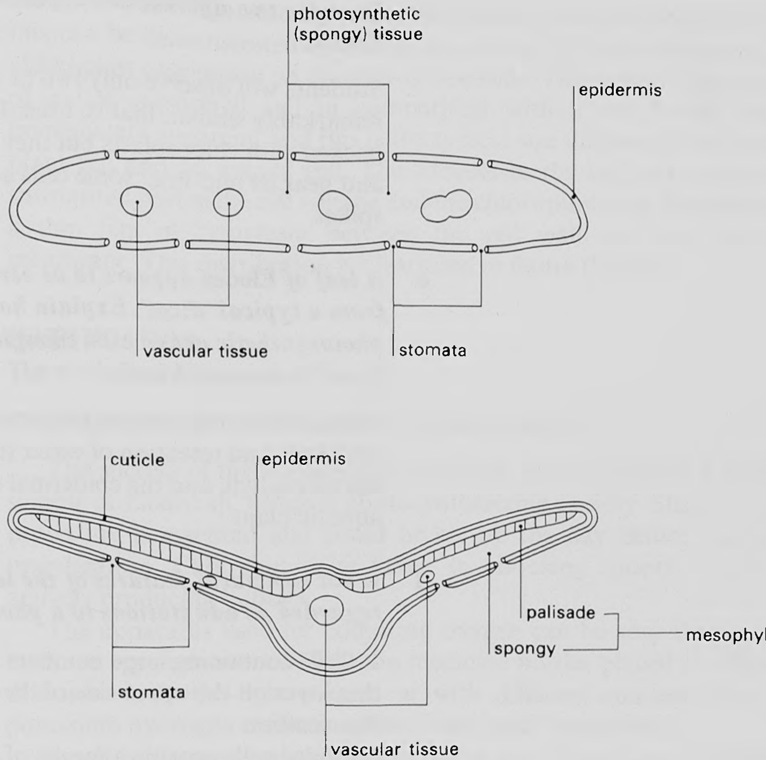

Figure 23
A plan drawing of two kinds of leaf in transverse section.

b ***To what extent can you suggest explanations for the differences between the structures of the monocotyledonous and dicotyledonous leaves?***

The distribution of 'veins' in a leaf is likely to be related to their function as supporting structures. The parallel arrangement of veins supports a long narrow leaf. Leaves of monocotyledonous plants receive equal illumination on both sides, since they are supported more or less vertically. They do not, therefore, show the distinct upper and lower sides that are obvious in leaves from dicotyledonous plants.

c ***Which features of the leaf from the xerophyte contrast with the other leaves which you have examined? Try to explain why the xerophyte should possess such structures.***

Several features of the xerophyte's leaves are concerned with the retention of water: its thick structure (a lower ratio of surface area to volume); the thick cuticle; its sunken stomata; the fact that the stomata may be confined to one surface of the leaf; the epidermal hairs surrounding the stomata.

d *Describe the different cell types that you have found in the leaf of* Elodea.

Students will observe only two or three cell types. Most cells are remarkably similar, that is, brick-like in shape. The majority are packed with chloroplasts but they are colourless at the edges of the leaf and near its mid-line. Some cells at the edge may project as small spikes.

e *A leaf of* Elodea *appears to be very simple when compared with one from a typical 'dicot'. Explain how it can be an efficient photosynthetic organ even though it lacks some of the structures found in an aerial leaf.*

Many of the structures in the aerial leaf are related to gaseous exchange and retention of water (for example, guard cells, air spaces in the mesophyll, and the epidermal cuticle). These are not needed in an aquatic plant.

f *What structural features of the leaves examined so far can be regarded as adaptations to a photosynthetic function?*

1 Cells containing large numbers of chloroplasts may be clustered in a thin layer on the upper side of the leaf if this receives more illumination.
2 Guard cells provide a means of entry and exit for gases.
3 Spaces in the spongy mesophyll allow diffusion of gases.
4 Vascular tissue provides water and support.
5 The high ratio of surface area to volume and the small distance between the surfaces favour the rapid exchange of gases by diffusion between photosynthesizing tissue and the environment.
Note: **1** to **5** apply to aerial leaves; only **5** applies to *Elodea.*

Leaves vary considerably in structure, rigidity, and cuticle development, and it is useful to handle a variety of leaves and to chop, dissect, and squash them in a variety of ways to examine the internal tissue. Moss leaves are extremely good for illustrating chloroplasts in cells because they are only a single cell thick. Leaves chopped on a glass slide with a sharp one-sided razor blade provide a mixture of mesophyll cells, vein tissue, and chloroplasts in suspension. These may be observed under a $\times 40$ lens of a microscope. Thin hand sections can also be cut, if the leaves are placed in a slit in a piece of pith or slice of carrot. Soft leaves such as those of broad beans, spinach, peas, and dandelions can be stripped of their epidermis to reveal the underlying cells. The leaf should be held between the first finger and thumb of the left hand and the surface layer torn from right to left at an angle of 45°. The upper epidermis should come away with a few green mesophyll cells adhering to it. A small piece of epidermal tissue (about 5 mm $\times$ 5 mm) with its adjacent mesophyll cells can be mounted in a drop of 0.3 mol dm^{-3} sucrose solution, covered with a coverslip, and then observed.

A particularly effective method of isolating chloroplasts is to chop the soft leaves finely. Chloroplasts are the same size as red blood cells and this can be demonstrated by mounting a drop of blood alongside the chloroplast suspension on the microscope slide. The extremely large size of a leaf mesophyll cell in comparison with a red blood cell is immediately apparent, and this is the typical size differential between a plant cell and an animal cell. The vacuole in the leaf cell occupies a substantial part of the cell volume and the chloroplasts are distributed in a thin film of cytoplasm between the cell wall and the vacuolar membrane. This distribution is illustrated in figure (S)158c.

INVESTIGATION
7C The evolution of oxygen

ITEMS NEEDED

Elodea sp., shoot, fresh 1/group

Potassium hydrogen carbonate solution, 1.0 %

Aquarium tank 1/class
Beaker, 250 cm^3 1/group
Capillary, glass, for collecting oxygen 1/group
Graph paper
Lamp (bright light source) 1/group
Ruler 1/group
Scale, about 5 cm 1/group
Stopclock or stopwatch 1/group
Syringe, plastic, 1 cm^3 1/group
Thermometer, 0 °C to 50 °C 1/group
Tube, specimen or test-tube 1/group
Tubing, plastic

(*Study guide* 7.3 'The mechanism of photosynthesis'.)

The success of this investigation depends upon obtaining a healthy sample of *Elodea* sp. which is photosynthesizing rapidly. Stage 2 in the procedure is essential and could be set up the day before the main investigation. Care should be taken in choosing shoots which are actively producing bubbles.

The apparatus used for collecting oxygen can be very simple. For instance, equal masses of *Elodea* in medicine bottles placed at different distances from the light source, or with different concentrations of potassium hydrogen carbonate, have been used successfully.

Small bright sources of light are better than larger ones. Projector bulbs have produced better results than large bulbs of equivalent power.

Principles

1 Photosynthesis occurs in aquatic plants, where the oxygen may be evolved in bubbles.

2 The rate of photosynthesis depends on the intensity of the light source up to a maximum.

3 The principle of 'limiting factors' may be investigated further by varying either the concentration of hydrogen carbonate or the temperature, while maintaining a high and constant intensity of light.

Questions and answers

a ***State any relation between gas production and light intensity which has been demonstrated by your results.***

The amount of gas collected per unit of time is inversely proportional to the square of the distance from the light source. The graph, ideally, should be linear, unless light is not the only limiting factor.

b ***How would you confirm, experimentally, that the light intensity decreases with the square of the distance?***

By means of a light meter. Some consideration should be given to how reliably light meters can be calibrated over a wide range of light intensities.

c *What would you expect the composition of the gas in the bubble to be? How would you determine this?*

The gas collected is unlikely to be pure oxygen. Its composition can be analysed by using solutions of potassium hydroxide and potassium pyrogallate and a capillary 'J-tube'. (See Revised Nuffield Biology, *Text 2 – Living things in action*, chapter 4.)

d *What changes can occur in a bubble of gas as it rises from a piece of pondweed to the surface of the water? How might these changes affect your results?*

As a bubble of oxygen rises through the water, some of the oxygen tends to dissolve and nitrogen, already present in the water, comes out of solution into the bubble. The composition of the gas collected therefore depends partly on the metabolism of the plant and partly on the contents of the water.

INVESTIGATION
7D Leaf pigments

ITEMS NEEDED

For extraction of leaf pigments:
Leaves of land plants (collection of green, variegated, and red leaves sufficient to supply each group with a handful of each type)

Propanone, A.R.

Electric grinder (1/class) or pestle and mortar (1/group) and sand, acid washed
Spatulas

For method A:
Beaker, 250 cm^3 1/group
Test-tubes, large 2 or 3/group

For method B:
Sodium sulphate, anhydrous, A.R.

Beakers, 100 cm^3 2/group
Connector, T-piece, plastic 6/class
Filter paper
Flask, round-bottomed, small, fitted with bung and glass tube (2/group) or vacuum desiccator
Funnel, filter 2/group
Petri dish or watch-glass 1/group
Vacuum pump 1/class
Vacuum tubing, rubber

For chromatographic analysis:
Leaf pigment extracts

Petroleum ether, A.R., boiling range 80 °C to 100 °C
Propanone, 90 % *(continued)*

(*Study guide* 7.3 'The mechanism of photosynthesis'.)

Propanone, 90%. Propanone: distilled water. 9:1. (*TAKE CARE:* propanone is highly flammable.)

If A.R. analytical grade, reagents, and solvents are not available do not attempt extraction method B.

Clean glassware is essential.

Grinding is more efficient if leaves have been stored in a deep freeze.

A quick method for preparing the pigment solution is as follows:

Homogenize the leaves in a blender with a small volume of ethanol.
Grind in a pestle and mortar with sand for 10 minutes.
Decant and filter.

If it is necessary to connect several flasks to a vacuum pump at the same time, use plastic T-piece connectors and vacuum tubing as in *figure 24*. Have good screw clips available to close off openings not in use.

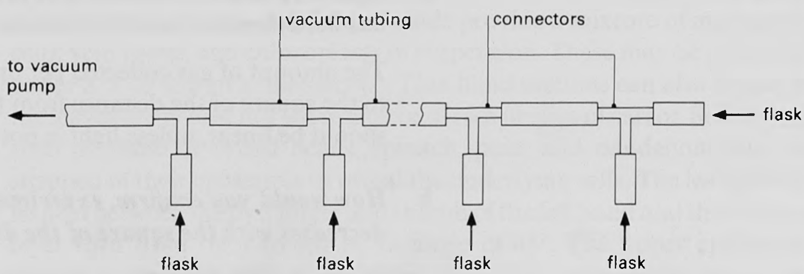

Figure 24
A method of connecting several flasks to a vacuum pump simultaneously.

ITEMS NEEDED *(continued)*

Capillary tube, fine, or micro-pipette, or fine melting point tube 3/group
Measuring cylinder, 10 cm^3 1/group
Pencil 1/group
Ruler 1/group
Scissors 1/group

For method A (paper chromatography):
Containers with lid for chromatographic separation (tall jars or gas jars) 2 or 3/group
Paper, chromatographic strips, to fit container, Whatman No. 1 2 or 3/group

For method B (thin layer chromatography):
Chromatographic plates, thin layer, to fit inside specimen tube 2/group
Specimen tubes and bungs 2/group

Principles

1 Two pigments, chlorophyll *a* and chlorophyll *b*, are always present in the leaves of plants.

2 Other pigments may be common, while some appear to be peculiar to particular species.

3 Plant pigments can be separated rapidly and easily by chromatography.

Questions and answers

a ***What is the essential difference between methods A and B of preparing the extract, and why does method B produce better results?***

Method B attempts to remove all the water from the solution of pigments (steps 5, 6, and 7). Dry solutions yield much better separations with the organic solvents in chromatography. After evaporation of the solvent (step 6), the pigments can be dissolved in a very small volume of propanone; this gives a suitably concentrated solution for spotting onto the chromatogram.

b ***Do all the pigment extracts contain the same variety of components? If not, give details.***

and c ***Are some pigments common to all the extracts?***

Ordinary leaves usually contain five pigments in the following order:

pigment	*colour*	*Rf*
carotene	yellow	0.95
phaeophytin	grey	0.83
xanthophyll	yellow	0.71
chlorophyll *a*	blue-green	0.65
chlorophyll *b*	green	0.45

The Rf values refer to the paper chromatography system described in the procedure.

Chlorophylls *a* and *b* are present in all the extracts.

d ***What conclusions can you draw from your answers to questions b and c concerning the role of pigments in photosynthesis? Try to find out, from textbooks, what function is performed by plant pigments.***

Chlorophylls seem to be essential, though this cannot be inferred from this practical work alone. Other pigments may also function in photosynthesis. There is a tendency to regard chlorophyll as the only pigment of importance. This investigation merely establishes the variety of pigments in leaves. It should lead on to the questions: 'How can it be proved whether a particular pigment does or does not function in photosynthesis?' and 'What are the advantages of a variety of photosynthetic pigments?'

INVESTIGATION
7E The reducing activity of chloroplasts: 'the Hill reaction'

ITEMS NEEDED

Leaves from green cabbage, lettuce, spinach beet, or broad bean plants (2 to 3 weeks old)

hyphen

Chloride/phosphate buffer ($0.5\ mol\ dm^{-3}$ KCl)
2,6-dichlorophenol-indophenol (DCPIP)
Phosphate buffer, pH 6.5, ($0.07\ mol\ dm^{-3}$)
Sucrose/phosphate buffer ($0.3\ mol\ dm^{-3}$ sucrose)

Aluminium foil
Balance 1/class
Beaker, $250\ cm^3$ 1/group
Board or tile, for chopping 1/group
Boiling tube 2/group
Centrifuges, bench 2/class
Centrifuge tubes 4/group
Knife, for chopping 1/group
Labels or chinagraph pencils
Lamp, preferably 75 to 100 watt (a microscope lamp will do) 1/group
Magnetic stirrer 1/class
Measuring cylinder, $50\ cm^3$ 1/group
Microscope, monocular 1/group
Microscope slides and coverslips
Muslin or stocking material
Pestle and mortar 1/group
Rack, test-tube 1/group
Scissors 1/group
Stopclock or stopwatch 1/group
Syringes, plastic disposable, $1\ cm^3$ 3/group
Syringe, plastic disposable, $5\ cm^3$ 1/group
Test-tubes 5/group

(*Study guide* 7.3 'The mechanism of photosynthesis'.)

Phosphate buffer, pH 6.5 ($0.07\ mol\ dm^{-3}$)
2.8 g Na_2HPO_4 (anhydrous M.W. 141.97) and
6.4 g KH_2PO_4 (anhydrous M.W. 136.09) made up to $1000\ cm^3$ with distilled water.
Sucrose/phosphate buffer ($0.3\ mol\ dm^{-3}$ sucrose)
51.4 g sucrose made up to $500\ cm^3$ with phosphate buffer.
2,6-dichlorophenol-indophenol (DCPIP)
0.06 g DCPIP made up to $100\ cm^3$ with phosphate buffer.
Chloride/phosphate buffer ($0.5\ mol\ dm^{-3}$ KCl)
3.7 g KCl made up to $100\ cm^3$ with phosphate buffer.

Plant material	*Amount ground in $40\ cm^3$ sucrose/phosphate buffer*
green cabbage	10 g
lettuce	50 g
spinach beet	20 g
broad bean plants (2 to 3 weeks old)	20 g

Table 13

Table 13 gives a rough guide to the amounts of plant material that are suitable. However, the activities of extracts from one type of leaf can vary considerably. For instance, extracts from green cabbage are often very active and need diluting. If at all possible prepare a sample extract before the lesson and assay its activity to determine whether it will require dilution. If this is not possible, warn the students that stage 10 may add significantly to the length of this investigation. *Even without this problem allow $1\frac{1}{2}$ hours to complete the work.*

It is essential that all solutions, tubes, and other apparatus, including the pestle and mortar, are thoroughly chilled beforehand and kept in melting ice until the dye reduction stage.

Assumptions
That students have a knowledge of the following:
1 The overall equation for photosynthesis and how this compares with the overall equation for respiration.
2 The principle of limiting factors.
3 The differentiation of organelles within eukaryotic cells.
4 The organization of enzyme-catalysed reactions into metabolic pathways.

Principles
1 Metabolic pathways are investigated by analysing the chemistry of intermediate reactions and by establishing the location of these reactions within the cell.
2 From the work of R. Hill and R. Scarisbrick in 1940, it is clear that when isolated chloroplasts are illuminated, they can reduce certain

oxidizing agents and produce oxygen. The retention of photosynthetic activity by isolated chloroplasts is only partial, since they cannot reduce carbon dioxide.

Students will need to understand that a reducing agent is required in photosynthesis to convert carbon dioxide to carbohydrate. The reagent 2,6-dichlorophenol-indophenol (DCPIP) is used to detect the reducing agent.

It is important to emphasize that the ultimate source of reducing activity in photosynthesis is hydrogen in the water molecule. Though chloroplasts do evolve oxygen released from water in the Hill reaction, this is technically difficult to detect.

Finally, at some stage the relation between the Hill reaction of isolated chloroplasts and the other photosynthetic reactions in the intact leaf should emerge (see the answer to question **d** below).

Questions and answers

a ***All the solutions contain phosphate salts which buffer at pH 6.5 and the sucrose/phosphate buffer contains 0.3 mol dm^{-3} sucrose. Explain the importance of each of these ingredients.***

Enzyme reactions often have an 'optimal pH' and this is equally true of metabolic pathways. It is important to maintain the pH close to this optimal level.

When organelles are extracted from cells into a buffer solution they can easily burst because of excessive uptake of water ('osmotic shock'). This is prevented by ensuring that the extraction medium is more or less iso-osmotic with the contents of the organelles. In this investigation 0.3 mol dm^{-3} sucrose serves this purpose.

b ***1 Describe the changes in colour that took place in tubes 1 to 4.***
2 Explain how the controls help you to arrive at any conclusions.

1 Tubes 1, 2, and 4 are controls and should not change colour. The colour of the suspension in tube 3 should change until it matches the colour of tube 2.

2 Tube 1 shows that DCPIP does not decolorize when exposed to light. Tube 2 provides a reference for the colour of the sediment extract in the absence of DCPIP. Tube 4 shows that there is no colour change in the absence of light. It is therefore possible to infer that loss of colour in tube 3 is a direct result of the illumination of the sediment extract.

c ***Compare the results in tubes 3 and 5, that is, the reactions with sediment and supernatant extracts. Which extract has produced more reducing activity?***

If the intact chloroplasts have sedimented during centrifugation, the reducing activity in the sediment extract will be greater than in the supernatant. (The efficiency of the centrifugation can cause some variation in this result.)

d *Explain why the enzymes which generate the reducing activity should be found mainly in one extract and not in the other. The results of your microscopic examination should help here.*

It should be possible to see many more chloroplasts in the sediment extract than in the supernatant. This would suggest that the enzymes which produce the reducing activity are confined to the chloroplasts.

e *The rate of photosynthesis in intact green leaves can be limited by any one of the following factors:*
1 Light.
2 Temperature.
3 Carbon dioxide.
One of these should have little effect on the production of reducing power in the leaf extract. Explain why.

Carbon dioxide will have no influence on the Hill reaction, which is a light-driven transfer of electrons from water to non-physiological oxidants. It demonstrates that the photochemical evolution of oxygen can be separated from the reduction of carbon dioxide.

f *Describe how you might extend this investigation to show*
1 how the production of reducing power depends on light intensity, and
2 how the production of reducing power varies with the wavelength of the light.

Students will probably have little difficulty in devising procedures in which the intensity and wavelength of light are varied. It is more difficult to control other variables. When light intensity is the experimental variable it is important to control temperature. When wavelength is the experimental variable it is important to control both light intensity and temperature.

INVESTIGATION
7F The production of starch by leaves

ITEMS NEEDED

Circaea lutetiana (enchanter's nightshade), or
Impatiens walleriana (busy Lizzie), or
Nicotiana sp. (tobacco), or
Pelargonium sp. (geranium)
(healthy potted plants which have recently been well illuminated)

Ethanol
Glucose solution, 5 % aqueous
Iodine in aqueous potassium iodide solution (see page 122)
Water

Beaker, 100 cm^3 1/group
Beaker, 250 cm^3 1/group

(continued)

(*Study guide* 7.4 'The reduction of carbon dioxide'.)

Pelargonium sp. works well but may need longer than 48 hours to destarch.

Steps 1, 2, 3, and 4 can be avoided in class if the students are provided with destarched plants and a previously degassed solution of glucose. If the solution is to be degassed by boiling, do this in advance to allow enough time for it to cool down.

Principles

1 Though starch is produced by photosynthesis there are other, non-photosynthetic pathways producing starch.
2 Starch synthesis may serve as a good indicator of photosynthesis but it is not an essential part of the process.
3 Starch synthesis cannot take place when neither photosynthesis nor

ITEMS NEEDED *(continued)*

Bunsen burner 1/group
Cork borer (8 to 10 mm bore) 1/group
Filter pump (optional) 1/class
Flask (and bung), conical for filtering, thick walled, with side arm (optional) 1/class
Flask, stoppered 1/class or 1/group
Forceps 1/group
Gauze 1/group
Labels
Mat, heat proof 1/group
Syringe barrels, with bungs, 20 cm^3 2 to 4/group
Syringes, disposable plastic, 20 cm^3 3/group
Taps, plastic, to fit syringe nozzles 2/group
Tile, dimple 1/group
Tripod 1/group
Vacuum tubing to connect flask and pump (optional) 1/class

respiration are possible. A source of energy is indispensable (see investigation 5D).

Questions and answers

a ***In which conditions did leaf discs produce starch?***

Starch synthesis occurs in illuminated leaf discs and darkened discs exposed to air. It does not occur in dark, anaerobic conditions and can be less efficient with inverted leaf discs.

b ***What are the leaf discs deprived of when they are immersed in the solution in step 7?***

Air – that is, nitrogen, oxygen, carbon dioxide, and the rare gases.

c ***Under what conditions, if any, did your leaf discs synthesize starch in the dark? Propose a hypothesis to account for this result. How would you test your hypothesis?***

To produce starch in the dark, leaves require oxygen. Set up discs floating on glucose solution as before, but in an atmosphere of nitrogen. If no starch is formed, the hypothesis is supported.

d ***Do the results of the investigation affect the validity of the starch test as an indicator of photosynthetic activity? If so, give details.***

Yes. The elementary notion of photosynthesis:

$$\text{carbon dioxide} + \text{water} + \text{light} \xrightarrow{\text{chlorophyll}} \text{starch} + \text{oxygen}$$

must be modified to:

$$\begin{array}{c} \text{carbon dioxide} + \text{water} + \text{light} \xrightarrow{\text{chlorophyll}} \text{x} + \text{oxygen} \\ \qquad\qquad\qquad\qquad\qquad\qquad\qquad\quad \downarrow \\ \qquad\qquad\qquad\qquad\qquad\qquad\qquad\quad \text{starch} \end{array}$$

In many plants photosynthesis may always result in starch, but starch may be formed by other pathways as well.

INVESTIGATION
7G Carbon fixation in CAM plants

ITEMS NEEDED

Plants (at least two of each species), CAM and non-CAM

Carbon dioxide
Phenolphthalein indicator
Sand, fine quartz, washed
Sodium hydroxide solution of known molarity, *e.g.* 0.1 mol dm^{-3}
Standard pH solutions, *e.g.* pH4 and pH7 (available in tablet form)
Water, distilled

Balance 1 or 2/class
Beakers, 100 cm^3 2/group
(continued)

(*Study guide* 7.6 'Crassulacean acid metabolism – the CAM pathway'.)

Plant
Examples of suitable CAM plants are: *Crassula argentea* (jade plant), *Bryophyllum daigremontianum* (good luck plant), *Kalanchoë* sp. and *Echeveria* sp. Several of these succulents are easy to propagate or are available from suppliers of house plants. Their leaves are large; a small specimen of *C. argentea* may yield 30 g of leaves. Examples of suitable non-CAM plants are *Hedera* sp. (ivy) and any species of grass.
Carbon dioxide
Carbon dioxide gas can be obtained from its solid form or from a gas cylinder, or soda water sparklet.

ITEMS NEEDED (*continued*)

Burette, 50 cm^3 1/group
Centrifuges, bench 2 or 3/class
Centrifuge tubes 2/group
Container, light proof (to hold plants) 1/class
Flasks, conical, 50 cm^3 2/group
Measuring cylinder, 50 cm^3 1/group
Muslin or stocking material (fine gauze)
Pestle and mortar 1/group
pH meter 1/group
Scissors 1/group
Syringe, plastic disposable, 10 cm^3, or pipette, 10 cm^3 1/group
Test-tube 1/group
Tile, white (for titration) 1/group

Phenolphthalein indicator
100 cm^3 ethanol, 95 %
0.5 g phenolphthalein
Dissolve the phenolphthalein in the ethanol.

Sodium hydroxide solution, 0.1 mol dm^{-3}
4 g sodium hydroxide
1 dm^3 water, distilled

Sodium hydroxide of known molarity
With a standard solution of 0.1 mol dm^{-3} sodium hydroxide the end point of the titrations may be reached with fairly small volumes (for example, 1 to 3 cm^3). If it is felt that unskilled students would benefit from handling larger volumes, use 0.02 mol dm^{-3} sodium hydroxide. (*TAKE CARE*: sodium hydroxide is corrosive.)

Principles

1 Photosynthesis is a complex process involving several different metabolic pathways.
2 Certain plants fix carbon dioxide in the dark. In this case it is possible to study carbon dioxide fixation separately from the other stages of photosynthesis.
3 By exchanging gases in the dark, certain plants can limit water loss and survive in dry habitats.

It is not necessary to measure the pH of the extract and also titrate it with sodium hydroxide solution. These measurements yield essentially the same information. However, if a pH meter is available it will be useful for most students to experience the two methods.

The extract prepared from these plants can be clarified by filtration with Whatman No. 1 filter paper. This is not as fast as centrifugation, unless it is carried out with a filter pump and Buchner funnel.

The key to the experiment is the comparison of the acidity of extracts from 'light' and 'dark' treated plants. However, if the work is to be completed within an hour each student, or group of students, will probably prepare only one extract. Success in comparing their results will depend upon them agreeing to follow a 'standard procedure'. Even so, systematic errors will be very hard to eliminate with students who are inexperienced in titration. On the other hand a stable pH meter is probably easier to operate in a standardized way and may give more consistent readings. If possible, the class results should be pooled and averaged.

'Crassulacean acid metabolism', which is summarized in *figure 25*, is very common, occurring in 25 or so plant families (including two ferns and a gymnosperm). The cactus *Opuntia* sp. and succulents such as stonecrops (*Sedum* spp.) are archetypal CAM plants.

The dark fixation of carbon dioxide in CAM species does not replace the normal C_3 metabolism. Both operate but at different times of the day. After malic acid has been produced in the dark (see the scheme above) it is stored in the cell vacuole. When it is light the malic acid is decarboxylated, so regenerating carbon dioxide in the cytoplasm. The carbon dioxide can then be fixed and reduced in the normal way via the Calvin cycle.

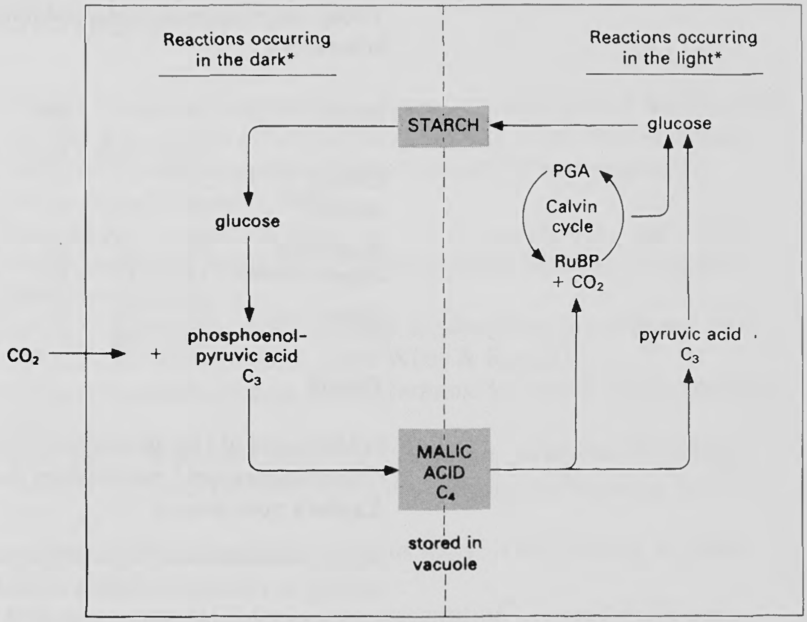

Figure 25
A summary of Crassulacean acid metabolism.
PGA: phosphoglyceric acid. RuBP: ribulose bisphosphate.
* There is, of course, no connection between these reactions of CAM, which occur in the light or dark, and the misleading terms 'light and dark reactions' described in most textbooks. This could be a source of confusion for students. Another problem may arise with students who have learned about 'C_4 plants'. It may be necessary to draw a clear distinction between 'C_4 metabolism' and 'CAM'. They are quite distinct adaptations. 'C_4 metabolism' is probably a device to minimize 'photorespiration', which is an increasing problem at high temperatures. 'CAM' is more simply a device for water conservation.

Questions and answers

a ***Which extract (from 'light' or 'dark' treated plants) contains a higher concentration of acids?***

The extract from 'dark' treated plants.

b ***Is it possible to decide whether the measured difference in acidity between the two extracts is significant?***

It is possible to obtain a reliable measure of the difference between the acidity of the two types of extracts and determine its significance. This depends, however, on having a sufficient number of measurements for each extract to permit a statistical analysis of significance (for example, the standard error of the difference). If only two or three measurements for each type of extract have been made then such a test is not possible. Even so, it should be noted that measurements of pH with a stable pH meter can readily be made to an accuracy of ± 0.1 pH units. Differences of 0.3 or more pH units are likely to be significant.

Table 14 gives some examples of readings obtained in a school laboratory.

Species	*Titre* (cm^3)		*pH*	
	Light	Dark	Light	Dark
Crassula	2.0	4.3	6.1	5.0
argentea	1.9	4.3	6.1	5.0
Bryophyllum	0.8	4.3	6.0	4.7
daigremontianum	1.6	4.5	6.0	4.7
Echeveria sp.	0.9	3.4	5.9	4.8
	1.0	3.4	5.9	4.8

Table 14

c ***Is the result of this investigation consistent with the scheme for Crassulacean acid metabolism that was outlined in the introduction? Explain your answer.***

In Crassulacean acid metabolism carbon dioxide is fixed in the dark, leading to the accumulation of acid in cell vacuoles. This acid is consumed during subsequent light periods. The only result consistent with this scheme is a higher concentration of acid in the extract from the 'dark' treated plants.

d ***What advantage might this type of metabolism give to a plant growing in a dry habitat?***

A CAM plant will fix carbon dioxide at night and can keep its stomata closed during the day. This may give it a considerable advantage over C_3 plants as these might suffer severely from water loss in a dry habitat.

e ***When water is readily available, CAM plants can be at a disadvantage in competition with non-CAM plants. Give reasons for this disadvantage.***

The typical CAM plant will have a succulent morphology which reduces the transpiration rate. This will lead to a number of possible disadvantages if water is plentiful: less efficient gaseous exchange, reduced uptake of minerals, and less efficient cooling of its leaves. CAM itself may not be a disadvantage to many, perhaps most, succulents in which it occurs. Such plants commonly fix carbon dioxide by day, via the normal C_3 route, when conditions are moist. (See Moore, 1981.)

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PART TWO CONTROL AND CO-ORDINATION IN ORGANISMS

In references to figures and tables, **'S'** denotes the **Study guide**.
'P' refers to the **Practical guide**.
Example: 'figure (S)2'.

□ denotes the end of a Study item.

CHAPTER 8 THE PLANT AND WATER

A review of the chapter's aims and contents

1 This chapter surveys the relationship between plants and water.
2 There was a gradual development of knowledge from the work of Hales, who used simple techniques, to the present day understanding of water movement into, through, and out of plants.
3 The energy for the movement of water and solutions in a continuous system is defined in terms of the chemical potential of the solution. Water potential is defined, and this is the term used throughout these books when considering the movement of water.
4 The mechanism by which water can reach the top of tall trees, and the properties of water on which this depends, are examined. The role of water in the life of the plant in general is considered briefly.
5 A mechanism has developed in plants by which a relatively stable water content is maintained – an example of homeostasis – a recurring theme in these books.

PART I The *Study guide*

8.1 By what mechanisms does a plant obtain, translocate, and conserve water?

Assumption

1 That students are aware of the basic structure of a flowering plant.

Principles

1 An important innovation in biological studies in the seventeenth and eighteenth centuries was the introduction of quantitative methods by scientists. It was a reflection of a change in the philosophy of scientists.
2 This change led to major discoveries such as those relating to water movement in plants.
3 Root pressure and transpiration play a part in the passage of water into and through a plant.
4 Water can move in a detached branch in both longitudinal directions.

STUDY ITEM
8.11 *Vegetable staticks*

Practical investigations. *Practical guide 3*, investigation 8A, 'Is the water content of a plant constant or does it fluctuate?'; investigation 8B, 'Do roots affect the rate of water input into land plants?'; investigation 8C, 'Is the rate of water input (and output) affected by environmental conditions around the aerial portions of the plant?'; investigation 8D, 'What is the role of the leaf in the loss of water vapour from a cut shoot?'.

The series of experiments described here, undertaken by Stephen Hales early in the eighteenth century, shows an acute appreciation of experimental method and considerable technical skill. The experiments laid the foundations of our knowledge of water movement in plants and led naturally to later investigations such as those of Böhm and of Dixon and Joly.

They provide the background for the practical investigations listed above.

Students will need to read the quotations carefully in order to extract the information asked for, as the language and style which Hales used to communicate scientific information are very different from those of today.

Questions and answers

a ***What terms would we use for the words 'imbibed' and 'perspired' used by Hales?***

The water 'imbibed' is the input and that 'perspired' is the output. 'Imbibition' constitutes an inflow and the term 'perspiration' has been replaced by 'transpiration'.

b ***What conclusions can be drawn from the results he describes?***

'Perspiration' (output) is greatest in light, warm, and dry conditions; it is least in the dark and damp.

c ***Are Hales's conclusions reasonable?***

Yes, as we can assume that Hales had already experimented on the input of water at the 'great end'.

d ***Does this experiment show that water can move in both directions in a plant stem?***

Yes; it demonstrates that water can move in both longitudinal directions.

e ***What can you infer, from the results of this experiment, about those properties of the tissues through which water passes in the plant?***

When the end, *i*, was clear of water, the mercury began to fall. This caused a sucking back effect and Hales noted '... air bubbles passing more freely down to *i*...'. This meant (since the 'great end' was sealed off) that water channels acted as open tubes.

f ***From these results, what part of the plant would you expect to provide the mechanism by which water passes through the plant?***

The leaves. Hales noted that at dusk '...the perspiration of the leaves decreased, and consequently the imbibing of the water at *i* abated...'.

g ***Is root pressure a force or the result of other forces acting in the system?***

The phenomenon described as root pressure is the result of an osmotic inflow across the differentially permeable root membranes. This is effected by the presence of solutes in the xylem sap, because of metabolic pumping of solutes into the xylem. Therefore, metabolic inhibitors, low temperatures, and shortage of oxygen reduce root pressure. So will the withholding of inorganic nutrients from a plant.

h ***Is root pressure alone enough to account for the passage of water up a plant such as a vine?***

Although root pressure alone could be responsible for the rise of water to above 21 feet (640 cm) in the vine, as described by Hales, this (as he also mentions) occurs only at certain times of the year. The 'ascent of sap' in general is only partly due to root pressure because, as soon as plants begin to transpire, the positive root pressure in the xylem gradually changes into a negative pressure or tension (see Study item 8.31) and positive pressures can no longer be measured in the xylem of a transpiring plant.

□

8.2 Imbibition and osmosis in seeds and seedlings

Practical investigations. *Practical guide 3*, investigation 8Ha, 'Water movement by imbibition into a non-living physical system and into live seeds' and 8Hb, 'Increase in the mass of seeds in different soils and in known solutions'; investigation 8I, 'A demonstration of the pressure produced during imbibition', investigation 8J, 'Osmotic water movement into a non-living system and into living plant tissue', and investigation 8K, 'A demonstration of the osmotic input into seedlings, producing root pressure so that guttation droplets are extruded at special points on the leaves'.

Principle

1 Plant water relations can be described in the same terms as those used by physical scientists.

Water potential

The term water potential, ψ (psi), is used throughout these texts to describe the water status of cells, tissues, or organisms. The energy available for movement of particles in a continuous system originates in differences in chemical potentials. The difference between the chemical potential of pure free water at s.t.p., and the chemical potential of water in a system, is called the water potential of the system. The water potential of pure free water at s.t.p. is zero. If solutes are dissolved in water, its water potential is lowered. If water is exposed to a tension, that is, to suction or negative pressure, its water potential also becomes lower than zero. In plants, water potential usually assumes a negative value because sap contains solutes and is often under tension.

The direction of net diffusion of water is from a region of higher water potential to one of lower water potential; one of the advantages of this terminology is that it enables imbibition, osmosis, and water translocation to be considered within the same basic concepts.

Students may have some difficulty in handling negative values. For instance they may find it difficult to appreciate that -0.05 MPa is a higher value of water potential than -5.0 MPa.

Questions and answers

a ***If a solution is said to have a $\psi_s = -0.1$ MPa, what does this value represent?***

It represents the force with which water will flow into that solution from a body of pure, free water.

b ***Since water movement during osmosis as well as during imbibition is down a gradient in water potential, what is the distinguishing feature between the two processes?***

The distinguishing feature is the presence of a differentially permeable membrane in an osmotic system. This allows a hydrostatic pressure to develop and this counteracts osmotic flow.

c ***By reference to the process of osmosis, explain how root pressure develops in a rooted plant. (Note: there can be no positive pressure in a cut shoot because there is no membrane at the cut end.)***

The many cell membranes in the plant root, starting with the root hair, taking in the several layers of cortex cells and finally the endodermal cells, collectively act as an osmotic membrane. This separates the dilute soil solution from the more concentrated sap in the xylem. Thus, there will be an osmotic flow of water into the xylem via this composite membrane, and a hydrostatic pressure will build up in the xylem cylinder as the height of the sap column rises.

8.3 The cohesion theory of water movement in the plant

Principles

1 Physical models can be used to investigate biological systems.

2 The continuity of a column of water in the plant water system is maintained by cohesive and adhesive forces.

3 Neither the tension (negative pressure) which develops as a result of transpiration, nor root pressure due to osmotic inflow of water into roots, can account for the ascent of water to the top of tall trees; nor can a combination of both.

4 The cohesion theory is offered as an explanation of the rise of water in the xylem vessels of trees, shrubs, and herbaceous plants that transpire.

STUDY ITEM

8.31 The rise of water in a plant

Practical investigations. ***Practical guide 3*****, investigation 8E, 'Translocation paths for water in plant stems', investigation 8F, 'The anatomy of a stem and how water moves up', and investigation 8L, 'Water movement against gravity in a physical system and in plants'.**

Questions and answers

a ***What does this demonstration show?***

A column of water, evaporating at one end, can raise a column of water or mercury above normal barometric height, demonstrating that some sort of tension (negative pressure) is being exerted on the water/mercury column.

b ***What forces hold the column of water and mercury together?***

Apart from atmospheric pressure plus tension forces, there must be some sort of cohesive force involved. This will hold the water column together and be in continuous contact with the mercury.

c ***Describe another simple demonstration of the action of this force.***

Any demonstration of surface tension will do. For example, place a needle on a filter paper on the top of a beaker of water. Press the paper gently down into the water. The needle floats on the water surface because of the cohesion of the water molecules (surface tension).

d ***Construct a drawing of the type of apparatus you would expect Dixon and Joly to have used when repeating Böhm's experiment, but replacing the porous pot with a pine twig.***

See *figure 26*.

e ***Combine the findings of Hales, Böhm, and Dixon and Joly into a general theory which might explain the rise of water in a plant. What further evidence is required to confirm the theory?***

Cohesive forces between the water molecules hold the continuous columns of water together. Thus, when water transpires from the leaf, a

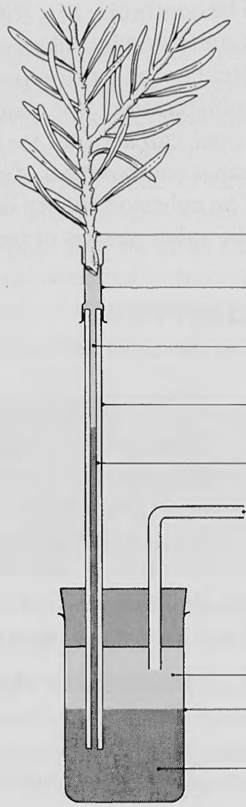

Figure 26
Apparatus possibly similar to that which Dixon and Joly used.

high enough tension in the xylem sap is set up and causes a mass movement of water throughout the xylem.

Further evidence required

1 Evidence that the cohesive strength of the xylem sap is adequate.

2 Proof that the sap columns are continuous. (Much of the evidence on these two points is indirect and indeed there is some doubt about its validity. The only really adequate evidence which exists in support of the theory is results of experiments which suggest that the xylem sap is under a tension. This will have developed on account of evaporation in the leaves and water loss from the leaves. Discussion with students might consider the path taken by the water in the leaf.)

f ***How does transpirational water vapour loss cause a reduction in plant water potential?***

☐ See the statement made in the answer to question **d** on page 218.

8.4 What role does water play in the life of a plant?

Principle

1 As well as acting as a solvent and being essential for photosynthesis and other plant functions, water has a role in the mechanical support of plants.

STUDY ITEM

8.41 (Essay) *(J.M.B.)*

Explain how the properties of water relate to its various roles in flowering plants.

☐ This will provide students with a useful summary of the work so far.

8.5 Stomatal control of the water content of plants

Principles

1 Plants have evolved a mechanism which largely controls the rate of diffusion of water vapour into the atmosphere.
2 This results in a relatively stable water content (homeostasis).
3 The physiology of stomata depends on a number of inter-related factors.

Question and answer

a ***The stomata control the rate of water vapour diffusion out of leaves. Could there be an equally effective mechanism controlling a plant's water content situated somewhere else, for instance in the roots, the petioles, or the mesophyll cells?***

No; control in the roots would lead to the drying out of the whole plant, in the petioles to the drying out of leaves, and in the mesophyll to the drying out of the epidermis. Only a control in the vapour phase will do.

8.6 Mineral nutrition and salt intake

Principle

1 Mineral salts can move into and within the plant only as solutes in water.

STUDY ITEM

8.61 Van Helmont's willow

Principles

1 Incorrect assumptions can lead to false hypotheses and conclusions.
2 An investigator's approach to a problem, and his interpretation of it, are limited by the available knowledge.
3 Prediction is an important aspect of scientific investigation.

This classic experiment of van Helmont provides an example, both of the careful use of the experimental method and the dangers of using false assumptions when drawing conclusions. It is also interesting as a historical document, being one of the first biological experiments in the modern tradition.

The careful control of experimental variables, the quantitative approach, and an implicit reliance on the principle of the conservation of mass are aspects of van Helmont's work which are important qualities of a good experiment and typical of modern methods.

It is suggested that it be used as a vehicle for a discussion on the experimental method.

Questions and answers

a ***What hypothesis was van Helmont testing in this experiment?***

That plant material is derived from water alone.

b ***What results do you think he might have predicted in advance of doing his experiment?***

He might have predicted that all water entering the plant minus the water leaving it would be converted into plant material. His account suggests he predicted that no change would occur in the soil and other material, apart from the plant, as a result of adding water. If he had taken the former prediction and been led to measurements of water input and output he might have drawn different conclusions. This is worth discussing.

c ***To what extent was van Helmont justified in drawing his conclusions from the results of his experiment?***

Van Helmont's results can be interpreted differently in terms of modern knowledge, but it should be realized that this was not available to him. Given the limitations of his information, his conclusions can be said to be justified. He can, however, be criticized for not attempting to measure water input and output directly and for neglecting the loss in mass of the soil. His work illustrates the extent to which the solution of a problem is limited by the scope of our knowledge and our techniques. This point is as valid today as it was in van Helmont's time.

d ***Clearly van Helmont's conclusion is not one that you would agree with in the light of your studies of plants. For what reasons would you reject his conclusions?***

Van Helmont clearly assumed that nothing entering a plant from the air could add to its mass and bulk. He was not aware of photosynthesis and Priestley's discovery of a relationship between the air and plants was still over a century in the future. Nor did van Helmont think it possible that, in parallel with a process whereby material was added from the air, other material, namely inorganic salts, could be obtained from the soil. We would reject this hypothesis because it is based on false assumptions.

e ***Many plants cannot obtain enough nitrogen from their environment although the atmosphere contains 79 % nitrogen and the soil solution a fair amount of nitrates. Do you know of another mechanism by which nitrogen becomes available to some plants?***

Students will probably already be familiar with the role of nitrogen-fixing bacteria in the root nodules of leguminous plants. □

PART II The *Practical guide*

The investigations related to this chapter appear in *Practical guide 3, Cells, tissues, and organisms in relation to water.*
Teachers may find it convenient to organize at least some of the investigations in this chapter as a circus of experiments, depending on the previous background of the students. In this case, although not every student will carry out each investigation, the details of the experiments and the results of all of them should be presented for all the students to have.

INVESTIGATION
8A Is the water content of a plant constant or does it fluctuate?

ITEMS NEEDED

Seedlings, 15 cm high, of one of the following: French bean, broad bean, pea, or tomato 6/group

Oven 1/class
Scissors or scalpels
Transparent plastic hood (optional) 1/group
Weighing machine 1/class

(*Study guide.* Study item 8.11 '*Vegetable staticks*'.)

Growing the seedlings. A suitable time before they are needed (leave time for them to acclimatize for one week), sow single seeds in small pots of peat or Vermiculite. At least 6 comparable seedlings should be available per group. All the seedlings should be watered daily. Three days before the practical class is to take place, cease watering three of the seedlings but do not allow them to wilt too badly. The other three seedlings should be watered daily. All the plants should be exposed to good illumination.
For the variation on this experiment, withhold water from all the seedlings for three days before the practical class, then water three pots and place a transparent plastic hood over these seedlings for three hours.

Principles

1 The water content of plants varies – that is, it is in a dynamic and not a static state within a plant.

2 If a plant is dried at a suitable temperature (80 °C) only water is removed and the water content of a plant can be determined.

Question and answer

a ***What can you conclude from this investigation about the water content of plants and the factors which affect it?***

That the water content fluctuates, according to how much water is supplied to the roots and, if the optional exercise was carried out, according to the rate of vapour loss by transpiration.

INVESTIGATION
8B Do roots affect the rate of water input into land plants?

ITEMS NEEDED

Seedlings grown as in the previous investigation 6/group

Liquid paraffin
Water *(continued)*

(*Study guide.* Study item 8.11 '*Vegetable staticks*'.)

Preparation of the seedlings. Seeds should be sown as for the previous investigation. All the seedlings should be watered daily.

To remove the material in which the seedlings were grown from the roots, immerse them in water and gently swirl them around. It is not

ITEMS NEEDED *(continued)*

Beakers 6/group
Graph paper
Holders, wire
Scissors or scalpels
Stopclock or watch
Top-pan weighing machine

necessary to remove more material than will come off by swirling.

When setting up the shoots without roots avoid air blockages by cutting off the roots under water. Also avoid getting liquid paraffin on the cut stems, by adding it after the shoots have been put in the water.

Principles

1 Plants transpire water vapour so that the rate of water vapour loss from the plant can be determined by weighing, at regular intervals, a whole plant together with its container.

2 The effect of the roots on the water input can be measured by comparing two similar groups of plants, where one group has had its roots removed.

Questions and answers

a ***Does the presence of roots significantly affect the rate of water vapour loss?***

It would be necessary to use plants with a comparable leaf area before any conclusion could be made.

b ***Under the conditions of your experiment, was the rate of loss in mass constant for the two different assemblies?***

It is likely to be so.

c ***If some or all of the leaves were removed, what effect would you predict this would have on the rate of water vapour loss?***

This should decrease the rate of water vapour loss.

d ***How would you establish that the loss in mass was, in fact, due to loss of water in the form of vapour?***

You could pass a dry air stream over the plant and then over a known mass of anhydrous calcium chloride, and then weigh again and find the increase in mass; this would be the water given off by the plant.

INVESTIGATION
8C Is the rate of water input (and output) affected by environmental conditions around the aerial portions of the plant?

(*Study guide*. Study item 8.11 '*Vegetable staticks*'.)

ITEMS NEEDED

Leafy shoot 1/group

Tap water

Beaker, 250 cm^3 1/group
Fan 1/class
Filter paper
Heater, electric, or Bunsen burner 1/class
Plastic bag 1/group
Potometer 1/group
Scalafix tape or rulers
Scalpel (or razor blade) 1/group

(continued)

Setting up the potometer. It should be possible to provide as many potometers of this type as required. Other versions can, of course, be used. (See *figure 27*.)

Instructions are given in the *Practical guide* so that the students can set up the potometer for themselves.

The potometer must be set up carefully so that data can be obtained by the students.

It is most important to plunge the cut end of the shoot immediately into water and to cut off another 3 cm under water. It is also important to

ITEMS NEEDED *(continued)*

Stopclock or stopwatch 1/group
Syringe with needle, $2\,cm^3$–$5\,cm^3$ 1/group
Transparent plastic hood 1/group or 1/class

select a suitable diameter of stem which will fit securely into the rubber bung. Badly fitting shoots are a common source of error. The slanting cut and the fitting of the cut end into the rubber bung must be carried out under water and the bark must be removed for about 3 cm up from the cut end.

To measure the movement of the bubble a 30-cm ruler could be held horizontally using a retort stand and clamp, or a piece of Scalafix tape could be fixed to the horizontal capillary tubing.

Readings can usually be taken at intervals of 15 or 30 seconds as appropriate. Under the conditions being investigated, the readings over 5 to 10 minutes should be regular. At least 10 to 15 readings are required for each experimental condition. However, widely varying rates of water input under the same conditions will be found, even with shoots from the same plant. It may be helpful to use a dilute solution of methylene blue (1 % or less) in place of water in the beaker. This makes the bubble easier to follow.

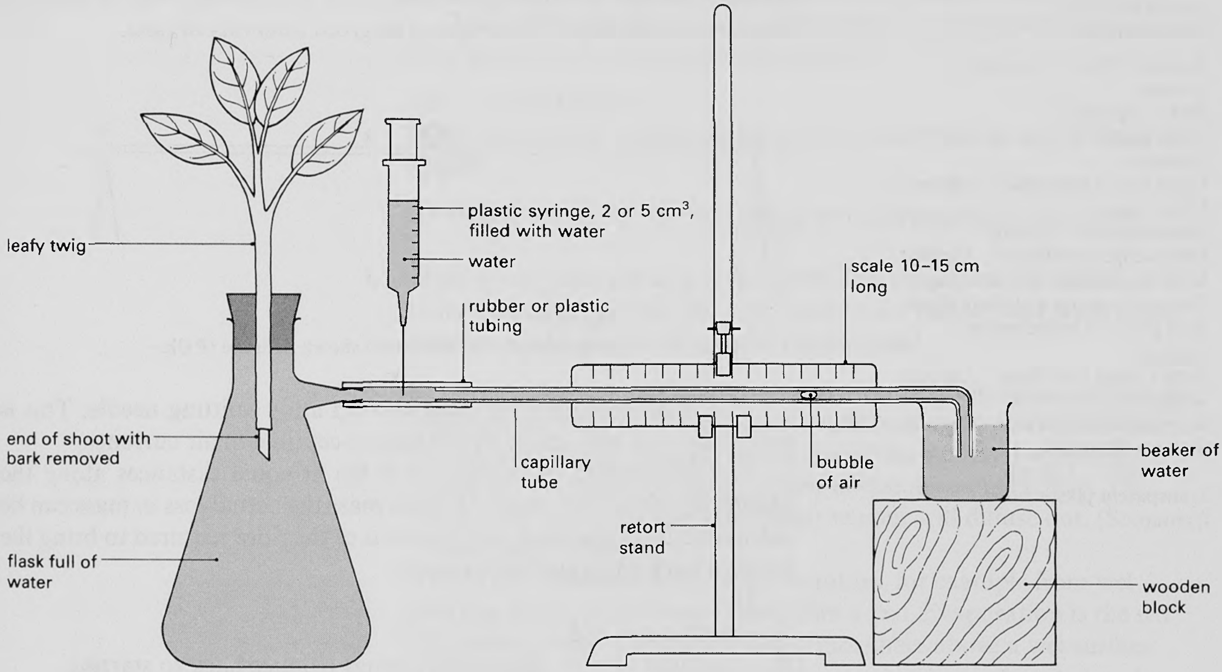

Figure 27
The experimental arrangement for measuring the rate of water input into the cut shoot of a woody plant.

Principles

1 A potometer is used to measure the rate of water input into a plant. The rate of input is obtained by timing the movement of an air bubble along a water-filled tube.

2 The effect of various environmental factors surrounding the aerial portion of the plant on the rate of input can be investigated with this technique.

Questions and answers

a ***How much water passed through the shoot in specified laboratory conditions per unit time?***

This will vary with the plant material and the laboratory conditions.

b ***Under which conditions does water flow vigorously into the shoot?***

It flows most vigorously in the light, in dry air conditions, when the temperature is increased, and in a gently moving air stream.

INVESTIGATION
8D What is the role of the leaf in the water vapour loss from a cut shoot?

ITEMS NEEDED

Fresh leaves

Liquid paraffin
Petroleum jelly

Beaker, 250 cm^3 1/group
Cotton
Fan 1/group
Filter paper
Forceps
Glass spring (optional) 1/group
Graph paper
Microbalance 1/group
Microscope, monocular 1/group
Microscope slides and coverslips
Potometer set up with leafy shoot as in previous investigation 1/group
Retort stand and clamp 1/group
Scalpel or razor blades
Stopclock or stopwatch 1/group
Syringe, disposable, 2 cm^3–5 cm^3 1/group
Transparent plastic hood 1/group

(*Study guide*. Study item 8.11 '*Vegetable staticks*'.)

Microbalance. As well as the microbalance illustrated in *figure 28*, a glass spring can also be used quite effectively to monitor loss in mass by measuring the length of the spring at given intervals of time.

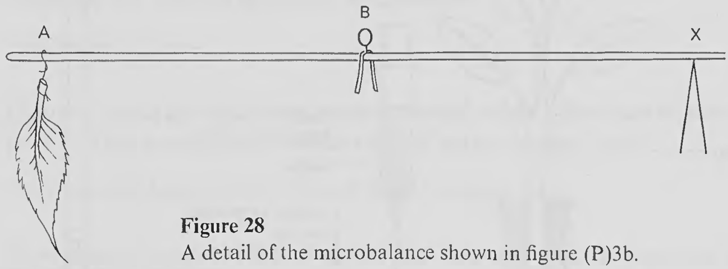

Figure 28
A detail of the microbalance shown in figure (P)3b.

The microbalance illustrated uses an alloy knitting needle. This is preferable to a milk straw as it is less susceptible to air currents.

Notches should be made with a file at equal distances along the needle. By the use of riders of known mass the actual loss in mass can be calculated from the mass and positions of the rider required to bring the balance back to its starting position.

If

A = position of leaf
B = position of rider mass which brings balance back to starting position
X = fulcrum
p = mass of rider
q = loss of mass by leaf

Then

$q \times$ distance AX = $p \times$ distance BX. From this q can be calculated.

Riders of known mass can be made from lengths of wire, such as fuse wire. Calculate the mass per cm of wire and cut off suitable lengths.

Assumption

1 That students can set up a microscope correctly.

Principle

1 Following the measurement of water input, the students use various procedures to investigate the importance of leaves in water vapour loss.

This investigation into the role of leaves in water vapour loss suggests several different experimental methods.

1 Finding the effect of initially open or closed stomata, by using leaves from an illuminated plant and from one that has been in the dark.
2 Testing the effect of removing leaves on water input.
3 Comparing change in leaf mass with that of drying paper.
4 Finding the relationships of loss in mass of leaves to their surface area.
5 Effect of waterproofing leaves.
6 Relationship of water vapour loss to the presence of stomata.

These different methods of investigation could be allocated among class groups as numbers and time permit.

Questions and answers

a ***What part do leaves play in the rate of water vapour loss from a shoot?***

The leaf is a major area from which water vapour loss takes place.

b ***Does a leaf function as a simple physical evaporator of water, or is there any evidence that the process of water vapour loss from a leaf involves a physiological control mechanism?***

Since stomata have variable apertures these investigations provide the evidence, not only on atmospheric conditions as does evaporation from a wet surface, but also on the size of the stomatal apertures, which are the paths for water vapour diffusion out of the leaf. The greater the apertures the faster water vapour will diffuse out. (See *Study guide I*, Chapter 1.)
This is the difference between evaporation, for example from wet blotting paper, and transpiration from a leaf. Evaporation is the net diffusion of water vapour into the atmosphere above a wet surface. Transpiration is the evaporation of water inside an almost closed space, practically saturating it with water vapour, plus the outward diffusion of this vapour through paths of variable conductance.

c ***Does the rate of transpiration from the surface of a leaf depend on its surface area?***

Within limits, yes.

d ***Is the loss of water vapour from a leaf linked with the presence or absence of stomata?***

Yes.

e ***From the results of investigations 8B, 8C, and 8D, summarize what has been established about the input and output of water in plants.***

This should be written as a short essay based on the findings of the three investigations.

INVESTIGATION
8E Translocation paths for water in plant stems

(*Study guide* 8.3 'The cohesion theory of water movement in the plant'; Study item 8.31 'The rise of water in a plant.)

ITEMS NEEDED

Fresh leafy shoot from a plant with a translucent stem, *e.g.* busy Lizzie (*Impatiens walleriana, holstii*) 1/group

Eosin or methylene blue, 1 % aqueous solution
Water

Beaker, $50\,cm^3$ 1/group
Brush 1/group
Dish for collecting sections 1/group
Fan 1/class
Hand lens or microscope, binocular 1/group
Light source 1/class
Microscope, monocular 1/group
Microscope slides and coverslips
Razor blades (new)
Retort stand and clamp or stake 1/group
Scissors
Syringe, large (or bulb pipette), $10\,cm^3$–$50\,cm^3$ 1/group

Assumption

1 That students are aware of the basic structure of a vascular plant.

Principles

1 The movement of a dye solution up the stem can be followed and it is assumed that water moves in a similar fashion.

2 The specific tissues where dye solution is found can then be located.

This investigation can be carried out by individuals or groups. It may well be an experiment which students have covered previously, in which case emphasis could be placed on investigations based on question **c**.

If students have not prepared sections of plant stems before, expect to spend some time over this technique. It is essential to use sharp, new razor blades. Emphasize that thin whole sections of stem are unnecessary and that pieces of $0.2\,cm \times 0.2\,cm$ of tissue are good enough, provided they are fairly thin (20–30 μm).

Questions and answers

a ***To which specific tissue of the stem is the dye confined?***

To the xylem.

b ***Which anatomical structures are found to be stained?***

The empty spaces bounded by thick walls (vessels and tracheids). Note that there are no air spaces.

c ***In your earlier investigations you may have determined some of the conditions under which shoots lose water vapour rapidly. Devise and if possible carry out a further experiment to find out whether the rate at which a dye solution has passed up the stem of a cut shoot is related to the environmental conditions around it.***

One form of suitable experiment would be to set up a shoot in the dye solution under conditions where transpiration is heightened by warm, light, dry conditions in a gently moving air stream and then to measure the rate at which the dye solution moves up the stem. The rate must be compared to that in a shoot of similar size set up under conditions where the rate of transpiration will be low. This experiment should show that there is a relationship between rate of input of dye solution and transpiration.

INVESTIGATION
8F The anatomy of a stem and how water moves up.

ITEMS NEEDED

Fresh or preserved herbaceous stem for sectioning 1/1
Stems, 2–3 mm diameter, dry, old and fresh, young: *e.g.* snowberry, lilac, japonica, and one conifer, treated in macerating fluid

Benzene-1,3,5-triol (phloroglucinol)
Hydrochloric acid, concentrated
Water

Brush 1/1
Dish for collecting sections 1/1
Graticule micrometer, eyepiece 1/group
Micrometer stage
Microscope, monocular 1/group
Microscope slides and coverslips
Mounted needles 2/1
Pipettes, dropping
Projector, 35-mm slide 1/class
Razor blades
Slides, 3.5 mm × 3.5 mm, of T.S. of young dicotyledonous stem

(*Study guide* 8.3 'The cohesion theory of water movement in the plant'; Study item 8.31 'The rise of water in a plant'.)

Macerating fluid. Mix, when required:
1 part by volume glacial ethanoic acid and
1 part by volume hydrogen peroxide (20 vols).

Immerse shavings, splinters, or thick sections of woody stems and boil under reflux for one hour or keep at 60 °C for one day. Shake vigorously and wash material with water; neutralize with a little ammonium hydroxide. The macerated tissue can be stored in 70 % ethanol. *TAKE CARE* during this procedure: the liquids are corrosive.

Principles

1 The distribution of tissues in a stem can be studied by the use of transverse sections and macerated tissue.
2 Lignified tissue is identified by a microchemical test.
3 Water translocation tissue can be seen to correspond with xylem elements.

Again, do not let students spend too much time cutting sections. It is important that they realize the limitations of studying only a transverse section of a stem. If time permits, they could try cutting longitudinal sections. Interpretation of stem structures is not easy; 35 mm × 35 mm transparencies of stems can be used to help with anatomical identification of stem tissues.

The work investigates capillarity as a possible mechanism to explain the passage of water up a plant. This should be linked with *Study guide* section 8.3, 'The cohesion theory of water movement in the plant', which considers other possibilities.

Questions and answers

a ***What tissue areas possess cells with lignified walls?***

Parts of the xylem, such as vessels, tracheids, and fibres. The latter may also be present in other parts of the stem.

b ***How is the xylem suited to its function of water translocation?***

Xylem contains tubular structures with cellulose walls. Water moves both in their lumen and in the fine spaces within the walls.

c [Students have been asked to calculate the height to which water would rise by capillarity in a tube of uniform bore.] ***Is it sufficient to explain how water can reach to the top of a plant?***

This calculation should demonstrate that the force of capillarity is not enough to explain the rise of water to the top of tall plants.

d ***Do the results of any of your earlier investigations involving the effect of roots and leaves on water movement through the plant help to explain how it may occur?***

This should be used as a review of the investigations carried out on the water relations of plants and it should be linked with the *Study guide* material in sections 8.2 and 8.3. It should include an explanation similar to the following statement.

Water flows from regions where its energy content is highest, that is, regions of high water potential, to regions where it is lower. As water vapour is transpired, the water remaining in the leaves is at a decreasing water potential because of adsorption and more concentrated sap. So water from the petiole and stem at higher water potential will flow into the leaves, thereby transmitting the reduction in water potential right down to the roots. The differences in water potential between leaves and roots (soil) can become very large, for instance 1.0 MPa, which is enough to raise water 100 metres. Provided the water columns can withstand such (negative) pressure differences, water will move up to the tops of tall trees (Dixon and Joly).

INVESTIGATION
8G The xylem pathway

ITEMS NEEDED

Broad bean plants grown to the 3 leaf stage in compost
2 or 3/group

Eosin, 0.05 % solution in tap water
Rose Bengal, 0.05 % solution in tap water

Beaker, 100 cm^3 or 250 cm^3 1/group
Fan heater (optional)
Razor blades (single edged) or scalpel blades
Scissors
Test-tubes
Tiles

Sow two or three broad bean seeds in compost in small pots so that they have at least three fully expanded leaves at the time of the investigation.

If the beans are relatively old, secondary thickening may have occurred at the base of the shoot. In the early stages of study avoid this region. Students are often mesmerized with the most detailed study of the dyed bundles in the leaf, but much of the real value of the exercise is lost unless the structure of the xylem in the whole shoot is understood.

Students should find that a major bundle runs close to each corner of the stem. At those corners where leaves are borne, part of the ascending bundle goes into the leaf and other parts 'arch' to join the bundles which run up the adjacent corners. The 3-D drawing should resemble *figure 29a. Figure 29b* gives a view facing angle 'A'.

Question and answer

a ***Interpret the final result of your observations and your sketches in terms of***

1 the supply of water to parts of plants, and

2 how water supply keeps the shoot erect.

The xylem provides the main route for the supply of water to the leaves. The movement of mineral salts into and within the plant depends on the presence of water, as do photosynthesis and other plant functions. Some mechanical force is needed to keep a herbaceous plant upright and all plants require mechanical support to display their leaves.

When the water flows into the xylem vessels of plant parts, the living cells which surround the vessels are separated from the sap by their cell membranes. Thus, water will flow by osmosis into the more

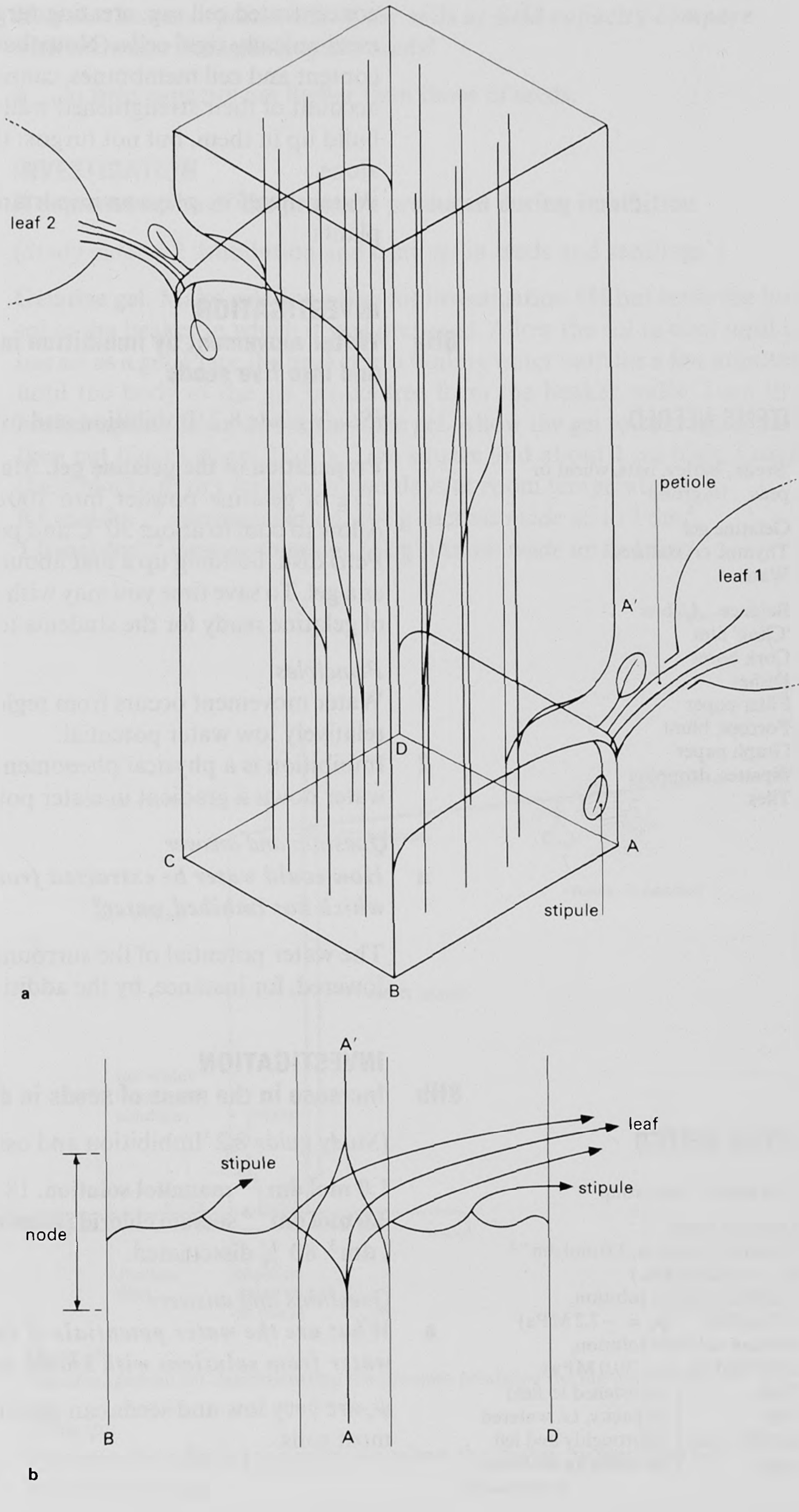

Figure 29
The arrangement of xylem in a broad bean stem.
a A three-dimensional view. The leaves are borne alternately on angles A and C.
b A facing view of the node at angle A.

concentrated cell sap, creating turgor pressure; this results in mechanically rigid cells. (Note that xylem cells, being devoid of living content and cell membranes, cannot develop turgor. They are rigid on account of their strengthened walls, and hydrostatic pressures can build up in them, but not turgor: this is a property of living plant cells alone.)
Water therefore plays an important part in the mechanical support of plants.

INVESTIGATION
8Ha Water movement by imbibition into a non-living physical system and into live seeds

ITEMS NEEDED

'Seeds', barley, oats, wheat or peas 10/group

Gelatine gel
Thymol, crystallized
Water

Balance 1/class
'Cling' film
Cork borer
Dishes
Filter paper
Forceps, blunt
Graph paper
Pipettes, dropping
Tiles

(*Study guide* 8.2 'Imbibition and osmosis in seeds and seedlings'.)

Preparation of the gelatine gel. Make a colloidal gelatine sol by mixing 25 g of gelatine powder into 100 cm^3 of boiling water while stirring. Allow to cool to about 30 °C and pour slowly onto a glass plate or into a Petri dish, building up a mat about 2–3 mm thick. Allow to cool and set as a gel. To save time you may wish to cut with a cork borer and dry discs of gelatine ready for the students to weigh.

Principles

1 Water movement occurs from regions of relatively high to regions of relatively low water potential.

2 Imbibition is a physical phenomenom involving the movement of water down a gradient in water potential.

Question and answer

a ***How could water be extracted from a system (gelatine gel or seeds) which has imbibed water?***

The water potential of the surrounding water would have to be lowered, for instance, by the addition of a solute.

INVESTIGATION
8Hb Increase in the mass of seeds in different soils and known solutions

ITEMS NEEDED

Pea seeds 140/group

Distilled water
Mannitol solution, 1.0 mol dm^{-3} ($\psi_s = -2.26$ MPa)
Sodium chloride solution, 2.0 mol dm^{-3} ($\psi_s = -7.2$ MPa)
Sodium chloride solution, saturated ($\psi_s = -30.0$ MPa)
Soils – clay, garden loam, sand } moistened to field capacity, *i.e.* watered thoroughly and left to drain for 48 hours

(continued)

(*Study guide* 8.2 'Imbibition and osmosis in seeds and seedlings'.)

1.0 mol dm^{-3} mannitol solution. 182 g mannitol *made up to* 1 dm^3.
2.0 mol dm^{-3} sodium chloride solution. 116 g sodium chloride *made up to* 1 dm^3. 80 % dissociated.

Questions and answers

a ***What are the water potentials of the seeds, as judged by the inflow of water from solutions with known water potentials?***

ψ_s are very low and seeds can therefore obtain *some* moisture from most soils.

ITEMS NEEDED (*continued*)

Balance 1/class
Dishes 7/group
Filter paper
Graph paper
Plastic bags 7/group

b ***How do the water potentials of the soils at field capacity compare with the water potentials of the seeds?***

ψ_{soil} at field capacity are higher than those of seeds.

INVESTIGATION

8I A demonstration of the pressure produced during imbibition

ITEMS NEEDED

Gelatine gel
Sucrose solution, 0.6 mol dm^{-3} ($\psi_s = -1.7$ MPa)
Sucrose solution, 1.0 mol dm^{-3} ($\psi_s = -3.4$ MPa)
Tap water

You need 3 sets per class of the arrangement shown in *figure 30*. To assemble it you need:
Dish, shallow
Graph paper
Lever system
Masses, 50 g–500 g
Retort stand and clamp
Ruler
Scale, Scalafix tape or ruler
Stopclock or stopwatch
Thread

(*Study guide* 8.2 'Imbibition and osmosis in seeds and seedlings'.)

Gelatine gel. Make gelatine gel as for investigation 8H but leave the hot sol in the beaker in which it was prepared. Allow the sol to cool until it has set as a gel. Place the beaker in a boiling water bath for a few minutes until the body of the gel breaks free from the beaker walls. Turn the beaker upside down and release the gel. Allow the gel to cool again and then cut blocks of gel 2 cm × 2 cm square and about 1 cm high. Leave these blocks to dry for one or two days at room temperature.
0.6 mol dm^{-3} sucrose solution. 204 g sucrose *made up to* 1 dm^3.
1.0 mol dm^{-3} sucrose solution. 342 g sucrose *made up to* 1 dm^3.

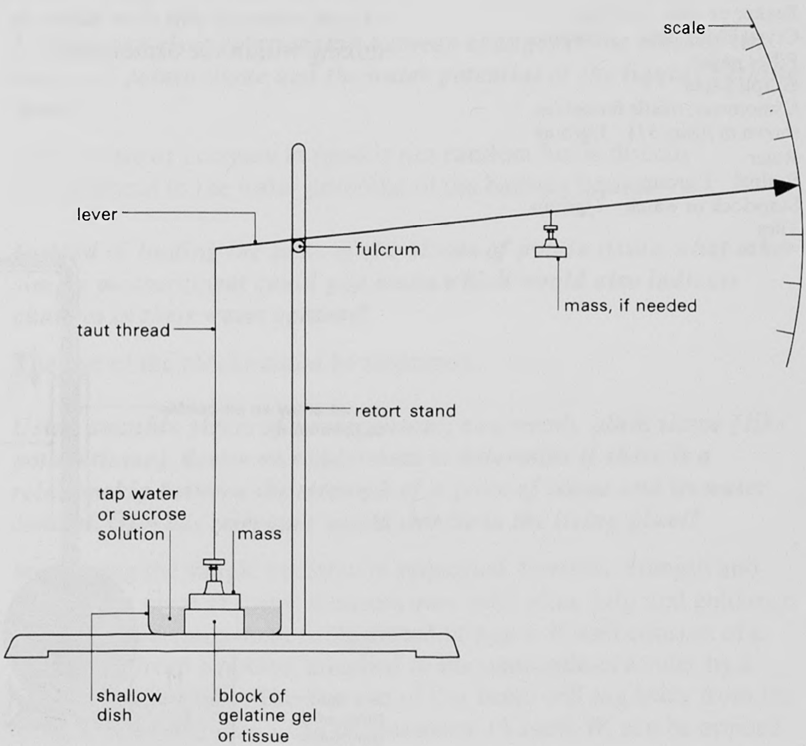

Figure 30
The arrangement for demonstrating the pressure produced during imbibition.

Principle

1 Pressure (or a force) is produced when the water moves into an imbibing system.

Question and answer

a **How does the water potential of the surrounding liquid relate to the pressure produced during imbibition?**

The higher the water potential (that is, the less negative it is), the greater the force developed.

INVESTIGATION

8J Osmotic water movement into a non-living system and into living plant tissue

ITEMS NEEDED

Potato tuber

Sucrose solutions:
1.0 mol dm^{-3};
0.1 mol dm^{-3};
0.25 mol dm^{-3}.
Water, distilled
Water, tap

Balance 1/class
Beaker or dish 3/group
Crystallizing dish 1/group
Filter paper
Graph paper
Osmometer, thistle funnel (as shown in *figure 31*) 1/group
Ruler
Scalpel 1/group
Stopclock or watch 1/group
Tiles

(*Study guide* 8.2 'Imbibition and osmosis in seeds and seedlings'.)

1.0 mol dm^{-3} sucrose solution. See investigation 8I, page 221.
0.1 mol dm^{-3} sucrose solution. 34 g sucrose *made up to* 1 dm^3 ($\psi_s = -0.26$ MPa).
0.25 mol dm^{-3} sucrose solution. 85 g sucrose *made up to* 1 dm^3 ($\psi_s = -0.67$ MPa)
Thistle funnel osmometer. This is assembled as shown in *figure 31*. The join between the wet parchment and the funnel must be leak-proof. The rate of osmosis will slow down in both because of the absence of good mixing within the osmometer.

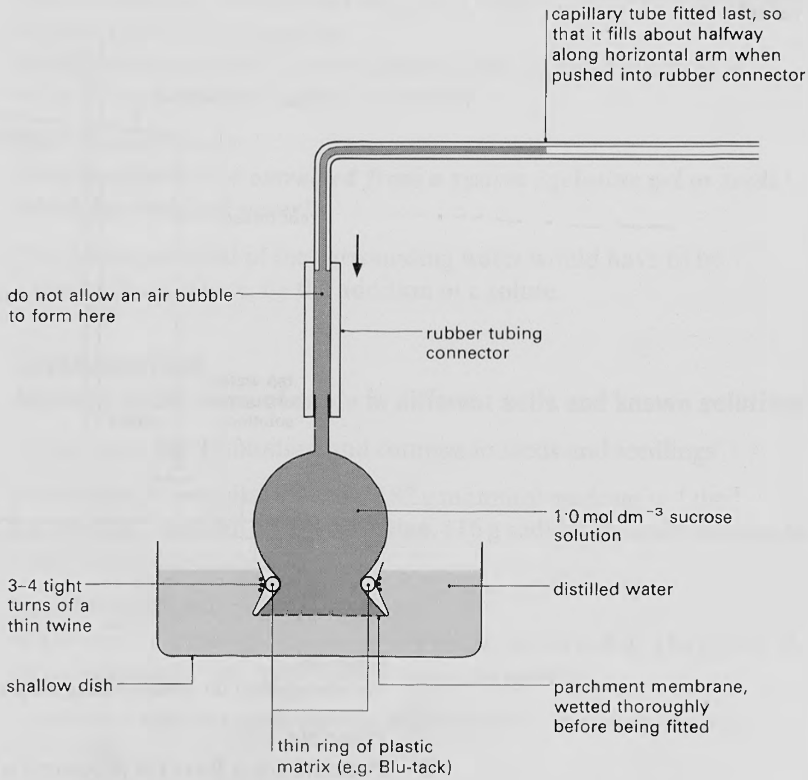

Figure 31
A thistle funnel osmometer.

Principles

1 Water moves from a region of higher water potential to one of lower water potential through a differentially permeable membrane.

2 Pieces of plant tissue of known mass are immersed in solutions of known water potential; the changes in mass are measured.

Questions and answers

a ***From the results of these experiments give a definition of osmosis in terms of water potential.***

Osmosis can be defined as the movement of water through a differentially permeable membrane, from a region of higher water potential to one of lower water potential.

b ***How is osmosis similar to and different from imbibition?***

It is similar by involving the movement of water from a region of higher water potential to one of lower water potential and different in that osmosis involves movement through a differentially permeable membrane.

c ***Is there any clear relationship between changes in the mass of the blocks of potato tissue and the water potential of the liquids bathing them?***

An increase or decrease in mass is not random but is directly proportional to the water potential of the bathing liquid.

d ***Instead of finding the mass of the blocks of potato tissue, what other simple measurement could you make which would also indicate changes in their water content?***

The size of the blocks could be measured.

e ***Using suitable pieces of homogeneous, non-woody plant tissue (like potato tissue), devise an experiment to determine if there is a relationship between the strength of a piece of tissue and its water content. Of what relevance would this be in the living plant?***

In devising the simple experiment requested, to relate strength and turgor in a piece of tissue, students may need some help and guidance. One simple arrangement is illustrated in *figure 32* and consists of a 'beam' cut from a potato, attached to the underside of a ruler by a rubber band or tape. The free end of this beam will sag away from the ruler and the distance S can be measured. (A load, W, can be applied to this end if required.) The piece of potato can be immersed in pure water or sucrose solutions without removing it from the ruler and the distance S can be measured after osmotic equilibrium has been reached in each solution. Graphical presentation of results will reveal a relationship between the flexure (sag) and the water potential of the immersing solution.

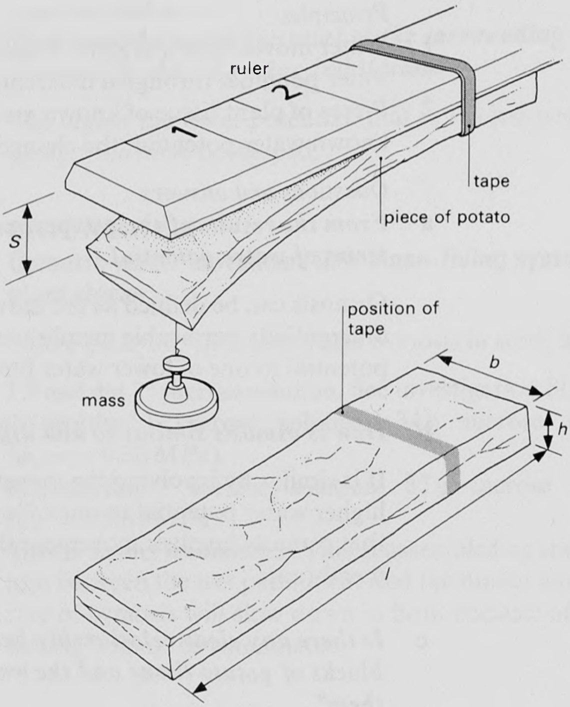

Figure 32
A technique to relate strength and turgor in plant tissue.

Though simple in principle there are many variations of this method and an opportunity is thus provided for students to plan, criticize, and assess their techniques. Some may be curious to know more of the mechanics of a loaded beam:

the flexure (sag), $S = \dfrac{4Wl^3k}{bh^3\mu}$

where
W = load applied
l = effective length of beam
h = thickness (vertical) of beam
b = breadth
μ = modulus
k = a constant dependent on the manner of support

If a rod of potato is cut, with a cork borer, and used in a similar manner, then:

$$\frac{S = 4Wl^3k}{3\pi r^2\mu}$$

An experiment of this kind should convince students that mechanical strength of tissue is greatly affected by the turgor of cells.

You can use the arrangement described in investigation 8I to demonstrate the force produced in plant cells as a result of osmotic input by using blocks of plant tissue instead of gelatine gel. The procedure is as follows:

1 Cut a piece of potato tuber or beetroot 2.0 cm × 2.0 cm square × 0.5 cm thick. (In either case, the piece should have dried a little on the bench, that is, not be fully turgid.) Place it in a dish with a known mass on it and add tap water as before. This time record the movement of the lever every 2 minutes, for 15 to 20 minutes.
2 Replace the water with 0.5 mol dm^{-3} sucrose solution if using potato tissue, or 1.0 mol dm^{-3} if using beetroot. Record the movement of the lever.
3 Then replace the sucrose solution with tap water twice, within 1 to 2 minutes. By using different masses of 50–500 g it is possible to estimate the force developed (1 kg = approximately 10 N).

Additional question

f ***What force did you estimate was developed in the plant tissue you used?***

The answer will depend on the results obtained.

INVESTIGATION
8K A demonstration of the osmotic input into seedlings, producing root pressure so that guttation droplets are extruded at special points on the leaves

ITEMS NEEDED

Barley, oats, or wheat seedlings, 8–10 cm high 10–15 group

Water

Large beaker

(*Study guide* 8.2 'Imbibition and osmosis in seeds and seedlings'.)

Seedlings. Sow the 'seeds' in pots of sand and keep well watered in natural daylight.

Principles

1. Root pressure is the result of osmotic water input.
2. Because seedlings are not tall enough for root pressure to lift the water to great heights, water is forced out of some xylem vessel ends – called hydathodes.

Question and answer

a ***Guttation occurs more frequently at night. What explanation can you give for this?***

This is presumably because transpiration is least at night so the root pressure in the xylem vessels builds up.

INVESTIGATION

8L Water movement against gravity in a physical system and in plants

ITEMS NEEDED

Leafy shoot 1/group

Methylene blue, 1 % solution
Water

Atmometer 1/group
Balance 1/group
Beaker 2/group
Capillary tubing, 70 cm long 1/group minimum
Fan 1/group
Glass tube, wide bore, with 2 one-holed rubber bungs to fit 1/group
Light source 1/group
Plastic hood, transparent 1/group
Porous pot with 1-holed rubber bung to fit 1/group
Ruler or Scalafix tape
Scalpel or razor blade
Stopclock or watch
Syringe or graduated pipette 1/group

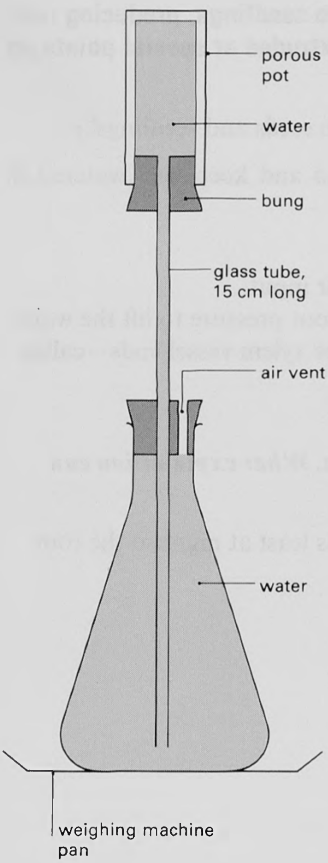

Figure 33
An atmometer.

(*Study guide* 8.3 'The cohesion theory of water movement in the plant'.)

Preparation of the porous pots for these experiments. Place a porous pot, with its opening showing upwards, in a beaker and fill the beaker with distilled water until the pot is completely submerged. Boil for at least 15 minutes and then allow to cool at room temperature while the pot remains completely submerged in the water in which it was boiled. Fit a rubber bung onto the end of the capillary tube and insert it in the mouth of the submerged porous pot, with a gentle pressure. This will fill the capillary tube with water to overflow. Place a moist finger over the end of the capillary tube and lift the assembly out of the water. Invert quickly and place the open end of the capillary tube under distilled water in a beaker.

The atmometer shown in *figure 33*. The end of the capillary tube is inserted into a bung to fit the flask, while under water. The outside of the whole assembly must be dry before taking readings.

Simplified versions of Böhm's apparatus. The leafy shoot is prepared and the arrangement assembled in the same way as for the potometer in investigation 8C. (See *figures 34a* and *b*.)

Principles

1 Physical models can be useful aids to investigations of biological systems.

2 A column of water contains some sort of cohesive force holding the molecules together.

Questions and answers

a ***How do environmental conditions affect the rate of evaporation from the atmometer?***

Dry, hot conditions with a moving air stream increase the rate of evaporation.

b ***How do the rates of evaporation compare with the rates of transpiration under similar conditions?***

Transpiration rates are similar to evaporation rates except for light/dark conditions, which have no effect on the rate of evaporation from the porous pot. Transpiration rate decreases in dark conditions and increases in the light.

c ***What do these experiments tell you about the ascent of water as a phenomenon?***

It is a phenomenon which can be explained in terms of the physical properties of water and differences in water potentials.

d ***What information do these experiments provide about the degree of control of transpiration exerted by the plant?***

Light seems to be the factor increasing the rate of transpiration and darkness reduces it.

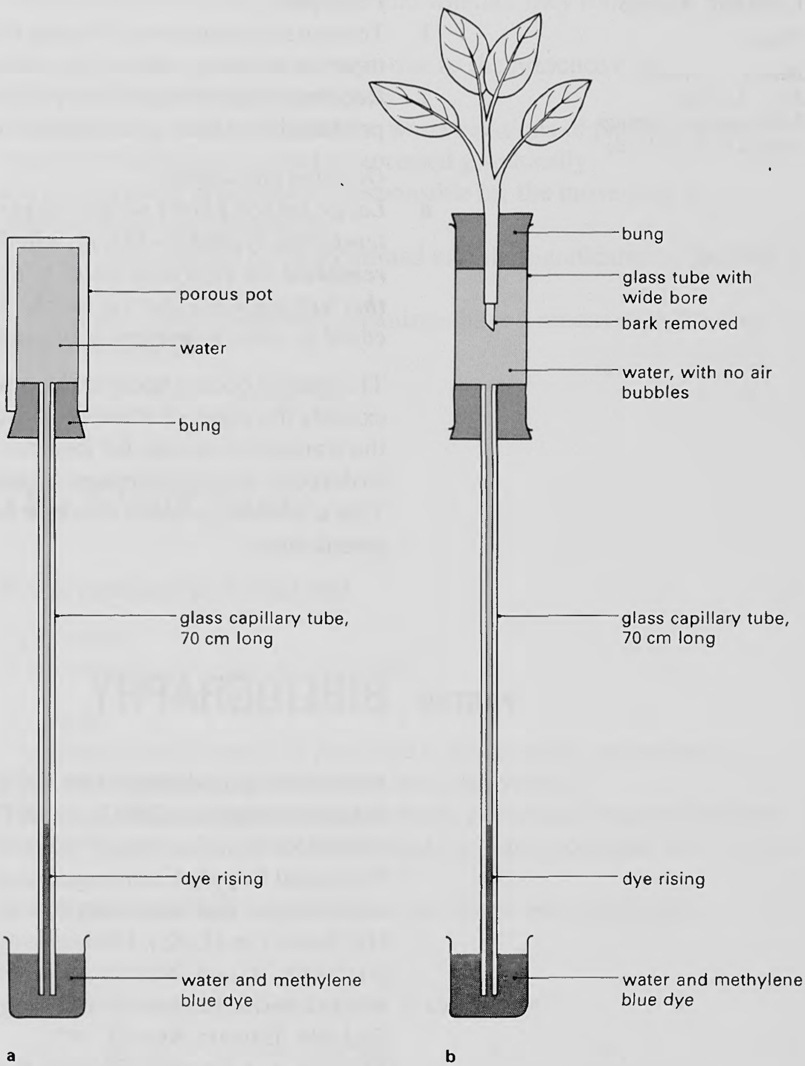

Figure 34
Simplified versions of Böhm's apparatus, using
a a porous pot
b a leafy shoot.
Note: Böhm used mercury, not methylene blue, to rise up the capillary tube.

INVESTIGATION

8M A demonstration of the mechanical support function of turgor pressure produced by water input into leaf cells

ITEMS NEEDED

Lupin leaf 1/group

Water

Beaker 1/group
Fan 1/group
Light source 1/group
Scalpel or razor blade

(*Study guide* 8.4 'What role does water play in the life of a plant?')

Principles

1 Temporary wilting occurs when the transpiration rate exceeds the input of water and the turgor pressure of the leaf cells falls.

2 Recovery from wilting takes place as a result of the turgor pressure produced by water input into leaf cells.

Question and answer

a ***Large-leaved plants such as sugar beet often wilt temporarily in temperate climates – this is called 'mid-day wilt'. It cannot be remedied by applying water to the soil. What is the explanation for this wilting when there is no shortage of water in the soil? What could be done to prevent it happening?***

The wilting occurs because the output of water vapour in transpiration exceeds the input of water to the roots. It can be prevented by reducing the transpiration rate, for instance by shading or spraying the foliage to decrease temperature and increase the relative humidity of the air. This is obviously not practicable for field crops but is done in greenhouses.

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CHAPTER 9 THE CELL AND WATER

A review of the chapter's aims and contents

1 Having considered the whole plant and water, this chapter turns to the water relations of individual cells and whether they too can be likened to physical systems.
2 Many of the properties of cells are due to the presence of a differentially permeable membrane.
3 An equation is used to express the water relations of plant cells and these relationships can also be expressed graphically.
4 Certain physical processes are responsible for the movement of substances in and out of cells.
5 A model of an animal cell is examined and the significance of the lack of a cell wall is seen.
6 Some single-celled freshwater organisms have a means of regulating their water content.

PART I The *Study guide*

9.1 Water relations of a plant cell

Assumption

1 A knowledge of the previous chapter.

Principles

1 The plasma membrane of a plant cell is differentially permeable; a plant cell therefore functions as an osmotic system.
2 There is a relationship between the water potential of the cell content and the turgor pressure of the cell, and the water potential of the cell as a whole.
3 Water molecules and mineral ions enter plant cells by physical processes.

The following equation is given to the students.

$$\psi_{\text{cell}} = \psi_{\text{p}} + \psi_{\text{s}}$$

cell water potential = turgor pressure + water potential of vacuolar sap and cytoplasm

The terminology is deliberately kept simple and the point to be emphasized is that water molecules will always move from regions of higher water potential to regions of lower water potential.

This equation is adequate for Advanced level and the treatment of the concept suggested here should avoid any confusion. Nevertheless, students can be given an indication of additional terminology. One difficulty is likely to be the handling of negative values.

STUDY ITEM

9.11 Quantitative examples of water potential in plant cells

The aim of this Study item is to help students to overcome the difficulties they have with the concept of water potential in plant cells, and to reinforce their understanding of it.

Questions and answers

a ***For each example calculate the initial water potential of the cell (ψ_{cell}), using the equation $\psi_{cell} = \psi_p + \psi_s$.***

	ψ_p		ψ_s		ψ_{cell}
1	0	+	(−0.4)	=	−0.4 MPa
2	0	+	(−0.8)	=	−0.8 MPa
3	+0.3	+	(−0.6)	=	−0.3 MPa
4	+0.5	+	(−0.5)	=	0
5	+0.4	+	(−0.8)	=	−0.4 MPa
6	+0.4	+	(−1.0)	=	−0.6 MPa

b ***What will the net direction of water flow be when the cell is placed in the bathing liquid in each case?***

Water will flow from a region of higher water potential to one where it is lower. In cells *1*, *2*, and *3* water will flow into the cell. In cell *4* there will be no net movement. In cells *5* and *6* water will flow out of the cell.

c ***Calculate the turgor pressure (ψ_p) and the water potential of the cell (ψ_{cell}) when the new equilibrium is reached in each case (changes in the water potential of the vacuolar sap will be very small and can be ignored in the calculations).***

Students may find it helpful to use the following diagrammatic way of representing this.

1
ψ_p = 0.0 MPa
$\psi_{s(internal)}$ = − 0.4 MPa
ψ_{cell} = − 0.4 MPa

$\psi_{s(external)}$ = 0.0 MPa

At equilibrium $\psi_{s(external)} = \psi_{cell}$
Therefore ψ_{cell} at equilibrium with external medium = 0.0 when ψ_p = +0.4 MPa

2 ψ_{cell} = 0.0 MPa
ψ_p = +0.8 MPa.

3 ψ_{cell} = 0.0 MPa
ψ_p = +0.6 MPa.

4 No net change: ψ_{cell} = 0.0 MPa
ψ_p = +0.5 MPa.

5 and *6* Water will leave the cell until
in *5* ψ_{cell} = −0.6 MPa
ψ_p = +0.2 MPa
and in *6* ψ_{cell} = +0.7 MPa
ψ_p = +0.3 MPa.

d ***Describe in words what would happen to the cell in example 3 if it were placed in a solution with a water potential of −1.2 MPa.***

Water will flow out of the cell and the cell will become plasmolysed. The space between the cellulose cell wall and the protoplast will be filled with the bathing liquid.

e ***Consider the two adjacent cells shown in figure* [S]187. *Which way will water flow? Give the values for the pressure potential and the water potential of the cells when equilibrium is reached.***

Cell A $\psi_{cell} = \psi_p + \psi_{s(internal)}$
$= +1.1 - 2.5$
$= -1.4\,\text{MPa}$
Cell B $\psi_{cell} = +0.4 - 2.0$
$= -1.6\,\text{MPa}$

There will therefore be a net movement of water from cell A to cell B. When equilibrium is reached the ψ_{cell} in both cells will be equal, *i.e.*, -1.5 MPa. ψ_p in cell A will then equal $+1.0$ MPa and in cell B $+0.5$ MPa, ignoring changes in $\psi_{s(internal)}$.

f ***In an experiment with onion epidermal cells, incipient plasmolysis was reached in an external bathing solution with a water potential of −0.2 MPa. What are the pressure potential and the water potential of the cell sap at this point?***

At incipient plasmolysis turgor pressure is zero. The water potential of the cell sap will be −0.2 MPa, equal to that of the bathing liquid. □

9.2 Water relations of animal cells

Principles

1 The movement of water into and out of an animal cell depends on the difference in water potential on the inside and outside of the plasma membrane, which is differentially permeable.

2 If an animal cell is to maintain its normal volume it must either be in an iso-osmotic medium or have a means of regulating its water content.

STUDY ITEM

9.21 The effect of various solutions on human red blood cells

Practical investigation. *Practical guide 3*, investigation 9D, 'The effect, on isolated animal cells, of altering the composition of the external medium'.

Principles

1 The plasma membranes of red blood cells are differentially permeable to water. If the membrane is burst by the net passage of water into the cell, the haemoglobin is released.

2 The plasma membrane of red blood cells is permeable to propane-1,2,3-triol (glycerol).

3 The water potential of a solution depends on the number of particles present in the solvent and not on their nature. Thus a dilute solution of sodium chloride, which dissociates almost completely, reduces the water potential more than a similar sugar solution.

Questions and answers

a ***Explain the results of these experiments. What conclusions can you draw from them?***

Experiment 1. The clear red solution obtained when the blood was placed in distilled water indicated that the haemoglobin had left the red cells and dissolved in the water. This is confirmed by the fact that centrifuging had no effect. The red cell membrane is normally impermeable to haemoglobin and the only way in which the haemoglobin could leave the cell would be after rupture of the cell membrane.

We presume that the membrane is differentially permeable and that it contained a solution with a low water potential. The membrane had not been able to withstand the forces built up as the water entered, and had burst. In the microscopical examination no whole red cells would have been seen, although some traces of the cell membranes ('ghosts') would be present.

Experiment 2. Table 30 in the *Study guide* shows the results of immersing red cells in a series of solutions of varying concentrations. In dilute solutions there was a net passage of water out of the cell. In the 3 % solution this was sufficient to reduce the volume of the cell, producing shrunken edges. In solutions of higher water potential, sufficient osmosis into the red blood cell took place to cause rupturing of the cells. Around 0.9 to 1.0 per cent, no apparent change occurred; this indicates that the internal water potential must be equivalent to this range of sodium chloride concentration.

Experiment 3. The result of this experiment indicates that the red cells have ruptured. Thus, osmosis into the red cells has probably occurred, although there was apparently a low water potential outside the red cells. This situation would take place if the cell membrane were permeable to propane-1,2,3-triole, thus equalizing the concentration of propane-1,2,3-triol outside and inside the cell. A water potential gradient would be set up, similar to that occurring in distilled water.

Experiment 4. The water potential of a solution depends on the number of particles present in the solution and not on their nature. The empirical formula of glucose is $C_6H_{12}O_6$ and for sucrose it is $C_{12}H_{22}O_{11}$. Thus a 3 per cent solution of glucose will contain nearly as many molecules of solute as will a 6 per cent solution of sucrose. Therefore the two solutions will be of nearly equal water potential. Because of the dissociation of sodium chloride into two particles (ions), a comparatively stronger solution of glucose is required to prevent haemolysis of red blood cells.

b ***Supposing the cells were exposed to various concentrations of***

fructose, what would be the lowest concentration you would expect not to give a clear red solution?

Fructose has the same molecular mass as glucose and therefore it would be expected that the lowest concentration which would not give a red solution would be the same strength as the glucose series of solutions, that is, about 3.0 per cent. □

A note to teachers

The term iso-osmotic is given to the students. However, the terms hyperosmotic and hypo-osmotic have been avoided, in order to lessen the chance of confusion. Because of the terminology used, hyperosmotic applies to a solution which has a *lower* water potential than another, that is, has a *more* negative water potential. Likewise, a hypo-osmotic solution is one which has a *higher* water potential than another, that is, it has a *less* negative water potential.

STUDY ITEM
9.22 The action of the contractile vacuole complex

Principles

1 The contractile vacuole complex can satisfy the osmoregulatory needs of freshwater Protozoa only in conjunction with the volume-regulatory mechanism.

2 Together, these functions constitute an effective homeostatic mechanism.

The term contractile vacuole complex has been introduced recently to cover the structures intimately associated with the contractile vacuole, but not readily visible with the light microscope. In the case of *Paramecium* the term would cover the spongiome, the collecting canals, and the pore and associated microtubules.

Questions and answers

a ***How do you think contractile vacuole fluid might be formed?***

The answer to this question is not known. One clue may exist in table (S)31 in which we can see that sodium is concentrated within the contractile vacuole. Perhaps the membranes of the spongiome can pump sodium from the cytoplasm. Such action would create local water gradients directed into the contractile vacuole complex, and thereby produce a force for fluid segregation. Such a mechanism would produce a fluid iso-osmotic with the cytoplasm or one with a lower water potential. Some reabsorption of solutes would have to occur to produce contractile vacuole fluid, which has a higher water potential.

b ***What is the cytoplasmic water potential of* Zoothamnium?**

If we assume that the treatment of *Zoothamnium* with cyanide (figure (S)195b) affects only the contractile vacuole, then the water potential of the cytoplasm equals that of the bathing medium when no swelling or shrinkage occurs. This produces an approximate estimate for the cytoplasmic water potential equal to that of 0.05 mol dm^{-3} sucrose solution. Most estimates of cytoplasmic water potential for freshwater Protozoa range from -0.12 to -0.24 MPa.

c ***Suggest why few single-celled organisms from marine or parasitic habitats have contractile vacuoles.***

These organisms live in a stable environment. If the water potential of the cytoplasm is adjusted to be iso-osmotic with the external medium the organisms would not be subjected to further osmotic stresses and so have no need for contractile vacuoles.

d ***From the information given in* figures [S]197 *and* [S]198, *describe the phases in the filling of the contractile vacuole complex and the expelling of fluid from the vacuole.***

After the vacuole has expelled its contents it is flattened and usually invisible (phase 1). The collecting canals and their distended ampullae can still be seen. The vacuole first fills (phase 2) as the ampullae empty into it, but may continue to fill for some time after that (phase 3). Filling stops with the vacuole rounding up (phase 4). Shortly after this some fluid is squeezed back to dilate the ampullae (phase 5). After a short delay (phase 6), the diaphragm sealing the pore is perforated and the fluid is expelled from the vacuole (phase 7).

e ***Why do only single-celled organisms with no cell walls, such as* Amoeba *and* Paramecium, *have contractile vacuoles?***

The most widely used mechanism of minimizing osmotically induced volume changes is a rigid cell wall which prevents cell swelling and restricts shrinkage as well. This is seen in bacteria, fungi, many algae, and plants. The cells of higher animals are protected by the extracellular fluid which bathes them. Neither of these mechanisms is seen in single-celled organisms. They cannot regulate the extracellular fluid, and a wall would impede movement and feeding. Contractile vacuole complexes are an alternative to the same problem. Differences in the ultrastructural appearance of contractile vacuole complexes of different organisms suggest that they may have evolved independently on several occasions. □

Practical investigation. ***Practical guide 3*, investigation 9E, 'The action of a contractile vacuole'.**

PART II The *Practical guide*

The investigations related to this chapter appear in *Practical guide 3, Cells, tissues, and organisms in relation to water.*

INVESTIGATION
9A Measurement of the water potential of cell sap by plasmolysis

ITEMS NEEDED

Beetroot, hand sections, or pieces of epidermis containing anthocyanin from rhubarb, pink onion, or cyclamen

Sucrose solution, 1.0 mol dm^{-3} (see investigation 8I)
Water, distilled

Burette 2/group
Forceps
Graph paper
Microscope, monocular 1/1
Microscope slides and coverslips
Pipette, dropping 1/1
Razor blade, single edged 1/1
Scalpel 1/1
Specimen tubes 4/group
Watch-glasses 5/group

(*Study guide* 9.1 'Water relations of a plant cell'; Study item 9.11 'Quantitative examples of water potential in plant cells'.)

Principles

1 Incipient plasmolysis cannot be recognized under the microscope but average incipient plasmolysis in a whole tissue occurs when half of its cells are plasmolysed and half are not. Values of ψ_s obtained by this method are valid for the cell at incipient plasmolysis, that is, zero turgor; in the turgid cell ψ_s will be higher, owing to dilution.

2 There is no need to produce this condition of incipient plasmolysis, as the concentrations of solute responsible for it can be deduced graphically.

The counting of the cells is better done under high power magnification as it is easier to discriminate between plasmolysed and not plasmolysed cells. If low power magnification is used for counting, imagine the field divided in half, as otherwise counting is too slow. An alternative method is to take the low power observation when most cells are plasmolysed, transfer the tissue to the next weaker concentration, wait 5 to 10 minutes, and see whether they have deplasmolysed.

Questions and answers

a ***From your graph, read off the molarity of sucrose which corresponds to 50 % plasmolysis. What does this represent?***

This represents incipient plasmolysis, which means that the water potential of the cell sap is equal to that of the external medium. Turgor pressure is zero.

b ***What was the water potential of the cell sap at incipient plasmolysis in the tissue you used?***

This will be read off from the graph and from table (P)1, showing the ψ_s values for sucrose solutions.

c ***When 50 % of the cells in a piece of tissue are plasmolysed what is the average turgor pressure within the cells?***

Zero.

d ***What inaccuracies are inherent in this method of determining water potential of plant cells?***

Tissues within the whole plant structure are subjected to mechanical forces which are altered when the tissue is removed. More important, during the experimental measurements solutes will leak out of the sap, thus causing the ψ_s of the sap to be underestimated.

e ***What happened to the plasmolysed cells when they were placed in tap water and what does this demonstrate about the state of the cells?***

The cells deplasmolyse, which indicates that plasmolysed cells are alive. It should be pointed out that plasmolysis is an experimental occurrence. In nature, cells of land plants are not bathed in solutions of lower water potential than that of the vacuolar sap.

INVESTIGATION
9B Diffusion of substances against gravity in a gel

ITEMS NEEDED

Copper(II) sulphate crystals
Eosin or methylene blue, aqueous
Gelatine, 3 % gel, in test-tubes 2/group
Gelatine, 3 % gel, with Universal Indicator added (in test-tubes) 1/group
Hydrochloric acid, 0.5 mol dm^{-3}
Petri dishes 3/group
Pipettes, dropping
Test-tubes with scale 3/group
Test-tube racks

(*Study guide* 9.1 'Water relations of a plant cell' – see 'Diffusion', page (S)270.)

Preparation of the tubes of gelatine. Prepare a 3 % gelatine sol, fill the tubes, and allow the sol to set by cooling. The gelatine will form a concave meniscus by shrinking. Fill the hollow to form a convex meniscus with a little more gelatine sol, and allow to solidify.
To one-third of the tubes add a few drops of Universal Indicator.

Principle

1 The movement of particles takes place against gravity by diffusion.

The advantage of demonstrating diffusion in a gel is that convection currents are absent.

The H^+ moves quickly and may reach the top of the tube within 6 to 8 hours and thus make the measurement not possible.

Questions and answers

a ***Explain what appears to have happened in each case in terms of movement of particles. Which particle moves fastest and why?***

The particles move from where they are in a higher concentration to where the concentration is lower – that is the movement is down a concentration gradient. The smallest particles (the hydrogen ions) move fastest.

b ***At what point would an equilibrium be established?***

When the particles were uniformly distributed in the system and a concentration gradient no longer existed.

INVESTIGATION
9C Movement through a differentially permeable membrane

ITEMS NEEDED

Iodine solution
Soluble starch solution, 0.2 %

Bulldog clips 2/group
Pipettes, dropping
Scissors
Measuring cylinders 2/group
Visking tubing

(*Study guide* 8.2 'Imbibition and osmosis in seeds and seedlings'; 9.1 'Water relations of a plant cell' – see 'Dialysis' p. (S)271; 10.6 'Kidney failure'.)

Iodine solution. Dilute the iodine solution normally used in the laboratory to a pale yellow colour with water.
0.2 % soluble starch solution. 0.2 g in 100 cm^3 water.
Make a paste of the soluble starch powder with a minimum of water; then add this paste to the rest of the water while it is boiling, and boil for a further 5 minutes. The solution should be clear with a slight blue haze.

This investigation is concerned only with dialysis as osmosis will have been demonstrated in an earlier investigation, 8J, 'Osmotic water movement into a non-living system and into living plant tissue'.

Principle

1 Substances dissolved in water (solutes) can be separated by differential diffusion across a differentially permeable membrane.

Questions and answers

a ***What appears to have happened in terms of movement of particles? Which kind of particle penetrates the membrane?***

Only one kind of particle appears to have moved down the concentration gradient. It is the iodine particles which penetrate the membrane.

b ***How is this process similar to and different from the diffusion which occurred in the previous experiment?***

It is similar in that there is a movement of particles down a concentration gradient. It is different from diffusion in that a differentially permeable membrane is involved, which restricts the movement of larger particles down a concentration gradient.

c ***In what way is this process, which is called dialysis, similar to and different from osmosis, which was demonstrated in investigation 8J?***

It is similar because a differentially permeable membrane is involved and different because osmosis is the movement of water only, down a concentration gradient.

ITEMS NEEDED

Blood, fresh, citrated (see investigation 4A), diluted 1 : 10 with iso-osmotic saline (5 cm^3 per group)

Saline, iso-osmotic with mammalian blood
Saline, 1.0 mol dm^{-3}
Water, distilled

Colorimeter 1/group
Microscope, monocular 1/1
Microscope slides and coverslips
Pipettes, dropping
Syringes or graduated pipettes, 1 cm^3 and 10 cm^3
Test-tubes 3/group

INVESTIGATION
9D The effect, on isolated animal cells, of altering the composition of the external medium

(*Study guide* 9.2 'Water relations of animal cells'; Study item 9.21 'The effect of various solutions on human red blood cells'.)

Saline, iso-osmotic with mammalian blood. Dissolve 6 g sodium chloride in 1 dm^3 of distilled water
1.0 mol dm^{-3} saline. Dissolve 58.5 g sodium chloride in 1 dm^3 of distilled water.

Principle

1 If blood, diluted in iso-osmotic saline, is mixed with solutions of varying osmotic concentration, the effect of the different concentrations on the cells can be observed.

Questions and answers

a ***What was the effect of each of the reagents on the red blood cells? Relate the turbidity of the liquids to the appearance of the cells as seen under the microscope.***

Distilled water – the cells burst – red blood cells were haemolysed, releasing the cell contents and producing a clear red solution.
Iso-osmotic saline – the red blood cells appear unchanged; the blood is merely diluted further.
1.0 mol dm^{-3} saline – the red blood cells change shape and appear 'spiky' or crenated.

b ***Explain the observed effects in terms of a hypothetical model of an animal cell as given in the Study guide (*figure [S]192*, page 272).***

With distilled water, the water potential outside was higher than the water potential inside the cells, so water molecules entered the cells. The cell membrane is not strong enough to prevent the inflow of water molecules, so it eventually bursts, liberating the cell's contents.
In the case of the iso-osmotic saline, the water potential inside and outside the cells is the same. There is therefore no net movement of water molecules across the membrane and the cells remain normal.
1.0 mol dm^{-3} saline has a lower water potential than the cell contents, so there is a flow of water molecules from the cells. Because there is a net outflow of water from the cells, the volume of the cells is reduced and their shape alters, giving the crenated appearance.

c ***What does this investigation suggest about the composition of the blood plasma (the natural external medium of the red blood cells)?***

As comparatively small changes in the composition of the external fluid greatly affect the cells, it would appear necessary for the natural external medium to be kept osmotically constant.

d ***In what ways do animal cells behave differently from plant cells, when immersed in solutions of different concentrations? What is the reason for the difference?***

Plant cells do not 'burst' or undergo the equivalent of haemolysis when placed in an external medium with a high water potential. Neither do they 'shrink' in the same way as animal cells, although they undergo plasmolysis when placed in an external medium with a lower water potential. The difference is because of the presence of a cell wall in plants, which is responsible for turgor pressure and which contributes an additional component to the water potential of the cell. This component is absent in animal cells.

INVESTIGATION
9E The action of a contractile vacuole

ITEMS NEEDED

Good *Discophrya* culture (see opposite) 1/class
Good *Paramecium* culture (see opposite) 1/class

Water, distilled
Sucrose solution, 0.1 mol dm^{-3} (see investigation 8J, page 222)
Methyl cellulose, 25 % (or lens tissue or cottonwool)

Burettes 2/class
Graph paper
Microscopes (see note)
Microscope slides and coverslips
Mounted needle
Paper tissues
Pipettes, dropping
Petri dishes or watch-glasses (see note)
Stopclock or stopwatch 1/group

(*Study guide* 9.2 'Water relations of animal cells'; Study item 9.22 'The action of the contractile vacuole complex'.)

Cultures. Cultures can be obtained from major biological suppliers. *Paramecium caudatum* should be selected in preference to other species. *Discophrya* or *Podophrya* can be used for the experimental part of the practical. Cultures of these organisms can be kept easily in the laboratory. For *Paramecium* boil 100 cm^3 of pond water with several barley grains. Allow to cool overnight. Inoculate with 5–10 cm^3 from a thriving *Paramecium* culture. Repeat at 3 to 4 week intervals. Keep at room temperature and in the dark. *Discophrya* is also kept in cooled boiled pond water. No barley grains are needed. The suctoria need to be fed twice a week with *Paramecium* or other ciliates (such as *Colpidium*, which are cultured in the same way as *Paramecium*). Silk threads should be added to allow the organisms to attach. For further information see Page F. C., *The culture and use of free-living Protozoa in teaching*, Institute of Terrestrial Ecology, 1981.

Microscopes. If possible, microscopes should have phase-contrast optics. If this is not possible, closing the iris in the condenser will improve the contrast so that the contractile vacuole can be seen more clearly. Remember that if the size of the contractile vacuole is to be measured, micrometer graticules will be needed for the eyepieces; these will have to be calibrated against a micrometer slide.

Small dishes or watch-glasses. The number which are needed depends on the number of experimental solutions, and in turn this will depend on the length of the practical period. If each pair of students is asked to collect data from five contractile vacuole cycles for organisms from each test solution, then at least 20 to 30 minutes should be allowed for each solution.

If time is short the experimental solutions (step 1) can be made up in advance. The students could then begin at step 5 or step 6, and ignore step 8.

The experiment which is described is similar to that which gave the results illustrated in figure (P)14. *Discophrya* and *Paramecium* will give comparable results. The advantage of using *Paramecium* is that it is a familiar organism and well documented. However, it moves actively and *Discophrya* is consequently preferred for observational work as it is immobile.

Principle

1 The contractile vacuole complex is an osmoregulatory structure found in some single-celled freshwater organisms.

Questions and answers

a ***What has happened to these cells?***

Immediately after being placed in a solution which has a lower water potential, the organisms will have a shrunken appearance. This is a consequence of water passing out of the cells. The contractile vacuole(s) should be relatively inactive, and in the case of *Paramecium* locomotion is severely impaired. By the end of the practical the cells should have recovered their normal shape, or at least be tending towards it. The contractile vacuole should be active again. This illustrates the fact that the cells have increased the levels of cytoplasmic solutes so that there is a water potential gradient drawing water into the cells. This is brought about by the volume regulation mechanism, and is an important point to be stressed. This recovery of cell volume cannot be brought about by the contractile vacuole complex alone.

b ***Do both contractile vacuoles behave similarly?***
Is there any evidence that these vacuoles really are contractile?

The behaviour of both contractile vacuoles is similar and is illustrated by figure (P)13a and b, but the two vacuoles do not behave synchronously. Whether contractile vacuoles are indeed contractile is a contentious question. However, the filling of the vacuole terminates with the vacuole actively adopting a rounded profile. This can only be brought about by the action of some contractile elements, either in the membrane of the vacuole or intimately associated with it. The term contractile vacuole therefore seems quite appropriate.

c ***Does measuring the frequency of contractions of the contractile vacuole provide a satisfactory measure of the osmoregulatory activity of this organelle?***

The relationship between output and frequency of contractions is not linear. Measuring the frequency of contractions does not provide an accurate measurement of the osmoregulatory activity of this organelle. However, the responses of frequency to osmotic stress resemble those of total output, and so measuring frequency alone can provide an approximate estimate of the osmoregulatory activity of this organelle.

d ***Why is the relationship between vacuolar activity and external water potential not linear?***

The response of the contractile vacuole to osmotic stress does not form a linear relationship with the water potential of the surrounding medium. A linear relationship would only be expected if the cell behaved as a passive sac surrounded by a differentially permeable membrane. However, in the event of osmotic stress, the volume regulatory mechanism actively changes the water potential inside the cell. This action causes the relationship to deviate from linearity.

e *Draw some typical cells. Has their appearance changed in any way? Why?*

See the response to question **a**.

PART III BIBLIOGRAPHY

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CHAPTER 10 CONTROL BY THE ORGANISM

A review of the chapter's aims and contents

1 The concept of homeostasis has already been introduced and the theme is continued in this chapter.
2 The emphasis is on the functioning whole organism. This is illustrated by the way in which animals, and to a lesser degree plants, are adapted structurally and physiologically to maintain a viable internal environment in the widely different environmental conditions of their habitats.
3 The kidney is introduced as a major organ responsible for maintaining the constancy of the internal environment of a vertebrate, and the relation between function and structure is considered in some depth.
4 In order to provide some understanding of the way in which biological knowledge has developed, the historical background to our present day understanding of kidney structure and function is given.
5 Throughout the chapter, biological data are provided for analysis and to support certain principles.
6 The application of biology is illustrated by consideration of existing medical solutions to the problems resulting from kidney failure.

PART I The *Study guide*

10.1 Water control in animals

Assumptions

1 A knowledge of the previous two chapters is assumed, in particular, an understanding of the concept of water potential, ψ ('psi').
2 Some knowledge of nitrogen metabolism (Chapter 6) is helpful to an appreciation of this section.
3 Certain aspects of kidney function and structure (glomeruli), covered later in the chapter, are referred to. (If preferred, the kidney can be studied before this section.)

Principles

1 Many animals have the ability to osmoregulate, that is, to maintain the osmotic concentration of their body fluids at a level different from that of the medium. The term homeostasis is used to describe the maintenance of near constant internal conditions despite substantial variation in the external environment.
2 Marine and freshwater environments pose contrasting problems. Teleosts and marine elasmobranchs are used to illustrate how these problems may be solved. Problems of living in the brackish water and changing salinities of estuaries may be pursued further, if wished. Eels and salmon are a reminder that there are still some biological

problems to which we do not yet have the answer.

As a group, mammals are largely terrestrial but they occupy widely differing habitats, so the problems of water control vary. Desert-living mammals illustrate the adaptations to hot, dry conditions.

STUDY ITEM

10.11 (A matching pairs exercise) (*J.M.B.*)

The water potentials of fresh water and sea water, and the body fluids of various animals are given in the table.

		Water potential (MPa)
	Fresh water	−0.01
	Sea water	−1.85
A	Freshwater crayfish	−0.82
B	Freshwater mussel	−0.08
C	Marine invertebrate	−1.85
D	Marine teleost	−0.65

Using this evidence only, indicate which animals would be MOST likely to

1 lose most salt

2 gain most salt

3 gain most water

4 show least apparent change

☐ **Key:** 1–A, 2–D, 3–A, 4–C.

STUDY ITEM

10.12 Animals living in deserts

Principles

1 The survival of animals in hot, dry conditions depends on specialized adaptations for the control of water and salt content as well as temperature control.

2 The mechanism depends largely on whether the animal is large or small.

Questions and answers on the camel (Camelus dromedarius)

a ***Summarize the reasons for rejecting the idea that camels store water. What assumption was made about camels to make the idea tenable in the first place?***

No anatomical site for water storage has been found. The capacity of the 'water sacs' does not constitute a significant water store. Analysis of the liquid showed it to have a salt content resembling that of blood. Oxidation of fat is physiologically uneconomical and fat cannot be considered to be a water store. The assumption made was that camels drink water in anticipation of their needs. Camels do not drink in excess of their requirements.

b *What point does this illustrate about the acceptance of long-established explanations for biological phenomena?*

An explanation must not be accepted without factual evidence to support it.

c *When a camel and a man are subjected to the same conditions of dehydration, from where does the greater percentage water loss take place in each case?*

Camel–body fluids.
Man–blood plasma.

d *When water is lacking the water potential of the blood decreases. What happens in the camel which does not appear to happen in Man?*

Water is withdrawn by osmosis from the body fluids. One should perhaps ask the question 'Why doesn't this happen in man as well?'. In fact, water is withdrawn from the cells into the blood by osmosis in both animals. But there is a difference in the proportional loss of water from the tissues of Man and of the camel because of the different concentrations of osmolytes in the blood.

e *What is the physical effect of this situation on the blood of Man and how does it explain why the camel survives when Man does not?*

The blood becomes more viscous, putting a strain on the heart pumping the blood. This means the blood does not circulate so efficiently and carry metabolic heat to the skin where it can be lost. The body temperature therefore quickly increases and death follows.

f *By how many degrees does the body temperature of the camel fluctuate when it is deprived of water?*

6.0 °C.

g *How does the rise of body temperature affect the absorption of heat from the environment during the day, when the air temperature may be above 40 °C?*

This reduces the gradient between the body temperature and air temperature, so less heat is transferred from the environment to the camel.

h *By what physical processes will the camel lose heat during the night?*

Radiation and convection.

i *What advantage is there in allowing the body temperature to fall below normal during the night?*

If the temperature falls to 34 °C at night, much of the day will pass before the body temperature reaches over 40 °C and sweating begins.

j *Summarize how the fluctuating body temperature helps the camel to conserve water.*

The fact that the body temperature of the camel rises during the day means that less water is lost by evaporation. The loss of heat by radiation and convection at night does not require water.

k *What additional means of water conservation are illustrated in the photograph of the camels in* figure [S]203? *How are they different from the methods discussed previously?*

Camels will lie still, with the legs tucked under the body. This reduces the amount of metabolic heat produced and reduces the surface area over which evaporation can take place.
Thick fur (and the fat in the hump) acts as an insulating layer, thus reducing the transfer of heat from the environment.
The first example is a behavioural adaptation which conserves water; the second conserves water by a physical means. The other adaptations have been largely physiological.

Questions and answers on the kangaroo rat (Dipodomys)

l *How much is the water intake in the kangaroo rat? How does it compare with the amount of water lost?*

The water intake is 53.7 g. This is 6.2 g less than the amount lost.

m *What is the source of the water taken in?*

It is derived from the oxidation of food (metabolic water).

n *What does the graph* (figure [S]205) *suggest is a means of making good a water deficit?*

The relative humidity of the atmosphere. If the food eaten was in equilibrium with the relative humidity of the air the kangaroo rat will have gained the water taken in with the food. The graph indicates the additional amount of water available if the food grains have been allowed to absorb water until they reach equilibrium with air at different relative humidities.

o *At what point on the graph is the kangaroo rat just in water balance?*

Where the lines for total water gain and total water loss intersect.

p *Where in the nasal passage is the temperature lowest?*

The lowest temperature is at the tip of the nose and at low relative humidity of the air this is below the air temperature.

q ***How does this help the kangaroo rat to conserve the water in the expired air?***

The air coming from the lungs will be at body temperature and will be saturated with water vapour. When it reaches the lower temperatures at the tip of the nose this water will condense on the walls of the nasal passages and be conserved.

r ***Summarize the ways in which the kangaroo rat conserves water and is thus enabled to survive the arid conditions of the desert environment.***

Being nocturnal and burrow-dwelling, the kangaroo rat avoids the high daytime temperatures. It does not sweat. It produces very dry faeces and has efficient kidneys able to produce very concentrated urine. Its water needs can therefore be met from metabolic water, so reducing the need to drink water. By lowering the temperature in the nasal passages below that of the air temperature at low relative humidities, the kangaroo rat reduces the amount of water lost by exhalation from the lungs. □

10.2 Plants and the availability of water

STUDY ITEM
10.21 Plants and water

Assumptions

1 Some knowledge of plant anatomy.
2 A knowledge of the movement of water.
3 An understanding of the function of stomata.

Principle

1 Plants exhibit structural and physiological adaptations to the availability of water in their environment.

Questions and answers

a ***Make a list of the structural features of the plants shown in* figure [S]207 *and explain how each could be an adaptation to the availability of water in the environment.***

Structural feature	*Adaptation to lack of water*
A thick cuticle and waxy deposits on leaf surface Sunken stomata Small sub-stomatal spaces Densely packed mesophyll cells Ability to roll leaf	reduce transpiration
Succulent stems, no leaves Succulent leaves	store water
Hairs	maintain an 'unstirred layer'
Deep and widely spread root system	taps water supplies deep underground and over large area

Structural feature	*Adaptation to lack of water*
Shallow, widely spread root system near surface	takes advantage of any dew or minor rain shower
Leaves sunk deeply in soil Leaves shed Leaves reduced to spines	reduces transpiring surface

b ***What physiological adaptations would you expect to find in plants growing in hot, dry conditions?***

Restricted opening of stomata, for instance, being open at night and closed during the day. Use of the CAM pathway (see Chapter 7) for carbon fixation. As a result of the opening of stomata at night some xerophytes take in carbon dioxide in the dark. From this they make organic acids which are the source of carbon dioxide for photosynthesis during the day.
Slow growth. This is likely to be the result of the restricted influx of carbon dioxide as a result of restricted stomatal opening.
Low water potential of root cells. This enables water to flow more readily from soil into roots.
Ability of the tissues to withstand desiccation without injury.

c ***What mechanism is common in the regulation of the water balance in plants and in the animals you have studied?***

It depends fundamentally on osmotic relations, and input must ultimately equal metabolic use plus output.

d ***What physiological adaptation will allow water to flow into halophytes from the soil?***

A low water potential in their cells will enable water to flow in from the environment (such as a salt marsh).

e ***What explanation might there be for the succulent nature of many of these plants?***

Water flows readily into many halophytes, partly on account of high transpiration rates. Succulence is more likely to be a metabolic response to salinity than to 'physiological drought', as was once thought. □

See the Bibliography for fuller accounts of xerophytic and halophytic adaptations.

10.3 The internal environment

The words written by Claude Bernard in the nineteenth century –

'... *la fixité du milieu intérieur est la condition de la vie libre* ....'

are perhaps the most famous ever written by a physiologist. Certainly their significance should be recognized by present day students. Modern physiologists have since established the role of the kidney in the maintenance of a stable internal environment.

Instructions for the dissection of the urinary system (and the reproductive system) of a mammal and the relation of this system to other organs of the body are given in Practical investigation 10A, which also includes the macroanatomy of the kidney.

Practical investigation. *Practical guide 3*, investigation 10A, 'The relation of the urinary system of a mammal to other systems of the body'.

10.4 The functioning of the kidney

Assumption

1 Some understanding and knowledge of nitrogen metabolism.

Principles

1 Regulation of the internal environment (homeostasis) by the kidney, in conjunction with receptor and humoral systems, is achieved in a number of ways (regulation of the amount of water in the body, maintenance of a proper balance of salts, excretion of nitrogenous waste, and regulation of pH).

2 A study of the histology of the kidney enables deductions about function to be made.

3 The historical development of the knowledge of kidney structure and function illustrates how scientific knowledge is accumulated.

4 Appropriate techniques are needed to acquire a full understanding of the structure and function of the kidney.

STUDY ITEM

10.41 The composition of human urine and blood plasma

Practical investigations. *Practical guide 3*, investigation 10B, 'Injection of the arterial blood system in the kidney', and investigation 10C, 'The histological structure of the nephron'.

This Study item can be used to form a hypothesis about the formation of urine – for example, that urine is formed from the blood plasma by a selective process.

Principles

1 The kidney changes the composition of the blood by removing metabolic wastes, but not substances useful to the body.

2 A comparison of the concentration of materials in the plasma and urine indicates the selective role of the kidney (nephron) in excretion.

Questions and answers

a ***Which substances have a urine/plasma concentration ratio greater than 20, thus showing a high degree of concentration?***

Urea, creatinine, uric acid, sulphate, phosphate, ammonia.

b ***Which substances present in the plasma are absent from the urine? Why?***

Proteins are large molecules which in normal kidneys do not pass the filter of Bowman's capsule.
Glucose is a small molecule which is filtered but which is normally completely reabsorbed by the proximal convoluted tubule. Only if the level of glucose in blood is too high does it appear in the urine.

c ***Which substances appear in the urine in concentrations which are higher than in the plasma and*** **vice versa*****?***

Substances in urine in concentration higher than in plasma are water, urea, creatinine, uric acid, sodium, potassium, magnesium, chloride, sulphate, phosphate, and ammonia. Substances in plasma in concentration higher than in urine are proteins and glucose. The concentrations of substances in the urine vary; these answers apply only to the particular circumstances given in the table.

d ***Which substances on the list are metabolic waste products?***

☐ Urea, creatinine, uric acid, and ammonia.

For firsthand investigation of kidney structure see Practical investigations 10B and 10C.

STUDY ITEM

10.42 Filtration and other processes in the nephron

Principle

1 Materials pass into the nephron by two main processes, filtration under pressure and secretion.

Questions and answers

a ***What is the major barrier to the filtration of fluid into the Bowman's capsule?***

The basement membrane.

b ***What process must occur in the nephron to explain this evidence?***

The tubular part of the nephron must actively secrete dye into the
☐ lumen.

Counter-current flow

The concept of a counter-current flow is a complex one to appreciate and you may find that you have to spend some time over this section in order that the students understand the function of the loop of Henle.

STUDY ITEM

10.43 Habitat, kidney structure, and excretory product, related to early development

Table (S)37 includes generalized information derived from a number of investigations of different species.

Question and answer

a ***Using these data and any other information, write a critical account of, or discuss, the following statement: 'In studying excretion we learn that a full understanding of living organisms only comes from considering structure, physiology, and environment as inter-related parts of a complex system'.***

The following points should emerge:

1 Ammonia is the major nitrogenous waste product only in aquatic organisms, where there is a plentiful supply of water to remove this highly toxic, soluble substance efficiently from the body.

2 Urea is the nitrogenous waste product when water is in shorter supply. (Ammonia is excreted by amphibian tadpoles but at metamorphosis it is replaced by urea.) Urea is soluble but less toxic and therefore seems to be an adaptation to water shortage.

In elasmobranchs the accumulation of urea in the blood decreases the water potential until it is below that of the medium. This means that water is not withdrawn from these animals' bodies.

3 Trimethylamine oxide, which is soluble and non-toxic, is also excreted by marine teleosts, which face the same problem of desiccation as marine elasmobranchs. Trimethylamine oxide is a significant excretory product in marine fish, by contrast with freshwater fish; it is also excreted by some crustaceans. Ammonia, urea, trimethylamine oxide, creatine, and creatinine are all produced to varying degrees by different species of marine teleost. Ammonia (NH_4^+) is not invariably the principal waste material, as it is in freshwater forms. Nevertheless, even in marine teleosts, ammonia constitutes up to 90 % of total nitrogen loss.

4 The major nitrogenous waste product of the terrestrial groups (reptiles, birds, and mammals) might be expected to be urea. Although mammals do excrete urea, birds and terrestrial reptiles excrete uric acid, which is insoluble. This difference appears to be related to the method of reproduction. Urea is associated with viviparity (mammals) and uric acid with embryonic development which takes place inside an egg protected by a shell. The urea produced by mammalian embryos is removed in the maternal blood. Uric acid produced by the embryos of reptiles and birds is deposited in the allantois. Some reptiles, tortoises, and turtles which lay their eggs in damp places are in an intermediate position and excrete urea and uric acid. □

STUDY ITEM

10.44 (A matching pairs exercise) *(J.M.B.)*

Table [S]38 shows the percentage of total nitrogen in ammonia, urea, and uric acid excreted by certain tortoises and turtles.

Percentage of total nitrogen excretion

Species	*Ammonia*	*Urea*	*Uric acid*
A	5	5–10	50–60
B	6	20–30	50
C	6	50–60	5
D	20–25	20–25	5

Relate these data to the most likely habitat of each of the following species.

1 aquatic

2 amphibious

3 terrestrial (not desert)

4 desert-living

☐ Key: 1–D, 2–C, 3–B, 4–A.

10.5 The regulation of the salt and water balance

Principles

1 Posterior pituitary gland and adrenal cortex hormones influence the ability of cell membranes to control the entry and exit of water and salts.

2 The mechanisms involved are examples of homeostatic feedback mechanisms.

STUDY ITEM

10.51 (Essay) *(J.M.B.)*

1 How does the structure of a mammalian red blood cell (Chapter 4) differ from that of a proximal tubule cell of the kidney and how can these differences be related to their respective functions?

2 How do you account for the fact that there is a correlation between the relative thickness of the kidney medulla in different species of mammals and their ability to produce concentrated urine?

Part **1** of the essay should include a detailed account of the structure and the correctly related function of the two types of cell; for example, it should show how the size and flexibility of the red blood cell enable it to squeeze through capillaries and how the microvilli of the brush border of the proximal tubule cell increase the surface area for absorption.

Part **2** should include a clear explanation of the counter-current multiplier system in the loop of Henle, the role of ADH, and the impermeability of the ascending limb of the loop of Henle to water. ☐

10.6 Kidney failure

This section provides some background on kidney failure and its treatment. The alternatives of dialysis or transplants are discussed, including the social and moral issues involved.

PART II The *Practical guide*

The practical investigations related to this chapter appear in *Practical guide 3, Cells, tissues, and organisms in relation to water.*

INVESTIGATION
10A The relation of the urinary system of a mammal to other systems of the body

ITEMS NEEDED

Freshly killed small mammal (equal numbers of both sexes) 1/2
Fresh lamb's or pig's kidneys from butcher 1/4

Ethanol

Cotton thread
Cottonwool
Dissecting board/dish 1/2
Dissecting instruments 1/2
Hand lens 1/1
Pins, dissecting

(*Study guide* 10.3 'The internal environment.')

We recommend that teachers refer to the joint statement of the Association for Science Education, the Institute of Biology, and the Universities' Federation for Animal Welfare, entitled 'The use of animals and plants in school science'; this is reproduced in the Appendix.

This investigation is best carried out by individual students but for reasons of economy one mammal between two students is suggested although even this may not be possible. If possible arrange the practical so that the dissected mammal is used to the greatest possible advantage. For example, the alimentary canal could be dissected or at least revised, and the reproductive system should certainly be dissected at the same time as the urinary system.

Any small mammal such as rat or mouse is suitable. As the kidneys of these are rather small, fresh lambs' or pigs' kidneys from the butcher are better, although not essential, for an examination of the macro-structure of the kidney.

Principles

1 Dissection allows firsthand experience of systems and organs and their relation to each other.

2 The mammalian reproductive system illustrates the relationship between structure and function. (The viviparous method of reproduction is an adaptation to the colonization of land.)

Questions and answers on the urinary system

a ***From what part of the kidney does the ureter leave and where does it join on to the bladder?***

b ***To what is the artery supplying the kidney with blood connected, and to what is the vein carrying the blood from the kidney connected?***

The answers to these questions will emerge from the dissection.

c ***From the relationships of the arterial blood supply, what hypothesis can you make about the pressure of blood entering the kidney?***

The renal artery is a short thick vessel. As the blood in the dorsal aorta is under high pressure, from the anatomical arrangement it would be expected that arterial blood will reach the kidney under high pressure.

Questions and answers on the male reproductive system

a ***What is the relationship between the ureters (which transmit urine from the kidneys), the vasa deferentia (which transmit sperms from the testes), the urethra, and the penis?***

The point to note is that the urethra in the male transmits both sperms and urine.

b ***What functions can you suggest for the prostate gland?***

This question is intended to raise the problem of providing the fluid medium required by sperms. The bulk of this, in fact, is provided by the seminal vesicles whose function is glandular, not the storage of sperms. The prostate produces an alkaline secretion which helps to neutralize any acid urine remaining in the urethra and also some of the acid secretions of the female.

c ***Is there any evidence to show that the testes were originally located in the abdominal cavity but, as the animal developed, descended into the scrotal sacs?***

Yes, the spermatic cords. Each cord is composed of the spermatic artery and vein connecting the testis with the dorsal aorta and inferior vena cava respectively.

d ***What indication, if any, did you find of a blindly-ending sac (resembling a uterus) extending forwards into the abdominal cavity and ending at a point where the vasa deferentia and urethra join?***

The uterus masculinus is sometimes well developed, particularly in guinea pigs.

e ***A rudimentary uterus (known as the uterus masculinus) is sometimes well developed in certain male mammals, notably the guinea pig. What conclusion can you draw from the existence of an element of femaleness in a normal male animal?***

The potential for femaleness is present in the male but is inhibited in its expression by those factors resulting in maleness.

Questions and answers on the female reproductive system

a ***In the light of your findings, where do you think it is most likely that fertilization and the development of the embryo take place?***

The wall of the Fallopian tube (oviduct), although slightly muscular, is quite thin and the lumen is narrow. Fertilization takes place in the Fallopian tube, but it is hardly likely that an embryo could develop here. In humans, implantation in the oviduct occasionally occurs. This is a serious condition involving the surgical removal of the embryo. By contrast the lumen of the uterus is much wider and the wall thick and muscular.

b ***How does the function of the urethra in the female differ from that in the male?***

The urethra in the female transmits urine only.

c ***What indication, if any, is there of a male portion among the female reproductive organs? How do you reconcile your findings with question d above?***

There is none. Evidently in mammals the secretion of female sex hormone precedes that of the male. Moreover, secretion may begin before the genetic sex-determining mechanism has exerted its full effect in inhibiting the development of ovarian tissue, if the animal is a genetic male.

INVESTIGATION
10B Injection of the arterial blood system in the kidney

ITEMS NEEDED

Fresh lamb's kidney (enclosed in fat) 1/2 or 3
Corrosion preparation of mammalian kidney (not essential)

Hydrochloric acid, 2.0 mol dm^{-3}
Latex, red
Latex, blue (optional)
Pepsin, 2 %
Ringer's solution, mammal

Beakers
Cotton
Forceps
Glass tubing, narrow
Gloves and goggles 1/1
Microscope, monocular
Microscope, binocular, stereo
Microscope slides and coverslips
Rubber tubing, narrow
Scalpels
Seekers
Specimen tubes
Syringe, 5 cm^3, disposable 1/2 or 3

(*Study guide* 10.4 'The functioning of the kidney'.)

2 per cent pepsin. 2 g in 100 cm^3 water.
Ringer's solution, mammal
Calcium chloride (anhydrous), 0.3 g
Potassium chloride, 0.25 g
Sodium chloride, 8.5 g
Distilled water, 1 dm^3

Principle

1 The path of the arterial blood supply is marked by the injection of rubber latex, which has been warmed in a water bath, into the arterial blood vessel. The latex solidifies and the external tissues are digested away by enzyme action.

The kidneys used in this investigation must be fresh, undamaged, and with reasonable lengths of blood vessels still attached. If the fat is still around the kidneys you are more likely to achieve this.

It is best to remove this fat with the fingers, by just pulling it gently away.

The vein is often overlooked. It is thin-walled and much wider than the artery or ureter. The artery is distinguished from the ureter in the lamb's kidney as it branches just before it enters the kidney. The glass tube should go into one of these branches, if possible.

The time needed for the digestion of tissues varies and depends on the temperature and the freshness of the enzyme.

This can be a very rewarding exercise and gives a much more vivid picture of kidney structure and its blood supply than a microscope section. Corrosion preparations are obtainable from suppliers and these, too, demonstrate the blood supply to the kidney.

Questions and answers

a ***What parts of the kidney structure can you identify? Make a sketch of these.***

Students should be able to identify the afferent arteriole, Bowman's capsule, parts of the tubule and, possibly, parts of the afferent blood vessels.

b ***Compare the distribution of the branches of the renal artery, supplying the glomerulus, with a conventional diagram of the structures. In what way does the distribution in your preparation differ from the diagram?***

The distribution will be less regular and less complete when compared with the conventional diagram.

c ***What can be inferred from the preparation, about the resistance to flow in the glomerulus blood vessels?***

As little or none of the injection material will have passed beyond the glomerular blood vessels it can be assumed that resistance to flow is high.

d ***Does the preparation give any indication of the nature of the barrier between the blood vessels of the glomerulus and the lumen of Bowman's capsule?***

Unless extreme pressure has been used during the latex injection, the barrier will not have been penetrated by the latex. Thus it can be assumed that a physical barrier is present.

INVESTIGATION
10C The histological structure of the nephron

ITEMS NEEDED

Microscope, monocular 1/1
Projector, 35 mm slide 1/class
Slides and/or 35-mm transparencies of kidney section

(*Study guide* 10.4 'The functioning of the kidney'.)

Principle

1 Observation and interpretation of histological structure of the nephron can be used to make some deductions about the function of the various parts.

The data that follows can be assembled from firsthand examination of slides of kidney sections and from the information given in the photomicrographs. They should be written beside the students' drawings of the various parts of the nephron.

Glomerular blood capillaries	Red blood cells may be seen in lumen. Capillary wall, thin, with pores. Podocytes envelop glomerular capillaries. Prominent basement membranes.
Bowman's capsule	Squamous epithelium with flattened nuclei.
Proximal convoluted tubule	Cuboidal epithelium. Prominent brush border of microvilli almost fills the lumen. Presence of numerous mitochondria.
Distal convoluted tubule	Clearly defined lumen, no brush border.
Loop of Henle	Rounded shape of tubes, no brush border, no red blood cells in lumen;
–descending loop	flattened epithelium;
–ascending loop	cuboidal epithelium.
Collecting duct	Columnar epithelium, well defined cell outlines, no brush border.

Questions and answers

a ***From the examination of sections of kidney and photomicrographs, what can you deduce about the function of various parts of the nephron?***

Very few deductions can be made just from the examination of slides, under a light microscope. But the photomicrograph showing the brush border of the microvilli indicates an increased surface area where absorption can take place, and numerous mitochondria suggest an energy-requiring process.

b ***How can more precise evidence about the function of the various parts of the nephron be obtained?***

By removing liquid from the various parts of the tubule and analysing it.

STUDY ITEM

10C1 (Multiple choice exercise) *(J.M.B.)*

The following drawings, which are not to scale, represent transverse sections of tubes found in the kidney. Which one of the rows A to D correctly identifies the tubes?

	Figure (P)29	*Figure (P)30*	*Figure (P)31*
A	distal convoluted tubule	capillary	proximal convoluted tubule
B	collecting duct	base of loop of Henle	distal convoluted tubule
C	proximal convoluted tubule	base of loop of Henle	collecting duct
D	collecting duct	capillary	proximal convoluted tubule

☐ **Key:** C.

INVESTIGATION

10D Determination of chloride content of urine collected in different circumstances of salt intake

ITEMS NEEDED

Specimens of urine, collected after different intakes of salt

Iron(III) nitrate, saturated solution
Methylbenzene (toluene)
Potassium thiocyanate, $0.1\ mol\ dm^{-3}$
Silver nitrate, $0.1\ mol\ dm^{-3}$

Boiling-tubes with bungs
Burette 1/group
Conical flasks 1/group
Glass rods
Labels
Pipettes, dropping
Syringe, disposable, $2\ cm^3$ 1/group
Syringe, disposable, $10\ cm^3$ 1/group

(*Study guide* 10.5 'The regulation of the salt and water balance'.)

Potassium thiocyanate, $0.1\ mol\ dm^{-3}$. 9.7 g in $1\ dm^3$ water.
Silver nitrate, $0.1\ mol\ dm^{-3}$. 17 g in $1\ dm^3$ water.

Principle

1 The fact that chloride absorption is regulated by the kidney can be demonstrated by estimating the amount of chloride (sodium chloride) in urine after different intakes of salt.

Students should be told only to handle their own urine samples, to avoid the risk of cross-infection.

The following results have been obtained and are meant to show a difference in the amount of chloride excreted rather than to give an accurate answer.

Specimen 1 $3.91\ g\ dm^{-3}$
Specimen 2 $1.06\ g\ dm^{-3}$

The method does have one drawback, which is that since silver thiocyanate is less soluble than silver nitrate, the thiocyanate tends to react with some of the silver nitrate. This means that more thiocyanate ion is run out of the burette than is necessary, leading to a lower than normal calculation for chloride ion concentration.

The following are the results obtained by a class.

Subject	*Specimen 1* (cm^3 of potassium thiocyanate)	*Specimen 2* (cm^3 of thiocyanate)
1	3.55	2.66
2	2.31	0.88
3	2.13	1.24
4	0.88	0.18
5	5.5	4.26
6	3.64	2.25
7	1.78	1.95
8	2.84	1.33
9	4.44	2.13
10	4.52	2.04
11	3.99	1.78
12	1.75	0.90

Questions and answers

a ***Explain the chloride content of the two specimens of urine in terms of the regulation, by the kidney, of the salt content of the body.***

The results should show a difference in the chloride, and therefore in the sodium, content of the urine, which can be related to the intake of salt. This shows that the kidney is selective in the amount of sodium and chloride ions removed in the urine.

b ***If you carried out a determination of chloride ions on blood collected at the same times as the urine specimens, what results would you expect?***

You would expect to find little difference in the chloride ion content of the two specimens, showing that the kidney is acting as a regulator of the salt content of the body.

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CHAPTER 11 CO-ORDINATION AND COMMUNICATION

A review of the chapter's aims and contents

1 The need for co-ordinated responses in the animal is discussed within the general framework of negative feedback.

2 The details of the monosynaptic reflex are examined as an example of a negative feedback system using a nervous mechanism.

3 The main features of the function of the neurone are developed with particular emphasis on the resting membrane potential and the changes which occur during the passage of an action potential.

4 The idea of chemical signals as a generalized mechanism of signalling between cells is developed. The essential similarity between the actions of neurotransmitters and hormones is stressed.

5 The contraction of skeletal muscle is used as an example of a response initiated by a neurotransmitter. The idea that changes in the intracellular calcium ion concentration might mediate the response is presented, and the mechanism by which this might occur is indicated in terms of what is known about the molecular processes which lead to contraction.

6 Drug action is illustrated by showing how specific compounds might affect the generation of an action potential or the transmission of a signal at a synapse or neuromuscular junction.

7 It is demonstrated how a study of drug action can help the understanding of the mechanisms involved in chemical signalling.

PART I The *Study guide*

11.1 Control systems

Principles

1 Homeostasis requires a monitoring of both the internal and external environments and the ability to respond to changes which are detected.

2 A response occurs after the synthesis of more than one piece of afferent information and responses are frequently complex, incorporating more than one effect.

3 Communication between the site where the change is detected and the site where the response is given may involve both chemical (hormonal) and electrical (neural) signalling.

Assumptions

1 A knowledge of the meaning of the terms 'reflex action', 'reflex arc', 'neurone', and 'synapse'.

2 A general understanding of the concept of homeostasis.

STUDY ITEM

11.11 Identifying the neural and hormonal pathways in a control system

This exercise allows the student to check the model given in figure (S)228 against information previously given in another context.

Questions and answers

a ***Identify the receptor and effector in the control of the water potential and concentration of ions in the plasma.***

b ***Do the afferent and efferent pathways involve neural or hormonal signalling?***

Figure (S)219

a The receptor is the osmoreceptor in the brain (hypothalamus).
The effector is the kidney, or more specifically the collecting duct wall.

b The afferent pathway transmits a signal from the hypothalamus to the posterior pituitary. This pathway is neural.
The efferent pathway is hormonal: ADH in the blood stream.

Figure (S)220

a The only receptor shown is the adrenal cortex.
The effector is the distal tubule of the kidney nephron.

b No afferent pathway is shown which matches figure (S)228 although ACTH (adrenocorticotrophic hormone) does provide an afferent pathway to the cortex of the adrenal gland. This pathway is hormonal.
□ The efferent pathway is also hormonal: aldosterone.

11.2 The monosynaptic reflex

Practical investigation. *Practical guide 4*, investigation 11A, 'The spinal cord and spinal roots'.

Principles

1 The monosynaptic reflex is used to illustrate the components of the model in figure (S)228. This apparently simple reflex involves a number of pathways and the final effect is a co-ordinated response from a number of muscle groups.
2 Such a co-ordinated response would normally be part of the process of producing a controlled movement, such as walking and correcting for changes in terrain.
3 The role of the cell body of the motor neurone as an integrating centre is stressed. The efferent information conveyed to the muscle is controlled by a combination of commands coming from the brain and reflex responses from muscle spindles, as well as other sensory information.

STUDY ITEM

11.21 The knee jerk

Questions and answers

a ***What are the possible explanations for the observed response (the powerful jerk)?***

A full answer would be very complex. However, an important factor is the time lag between the stimulus and the response (approximately 20 milliseconds for a knee jerk). The duration of the tap is very short, and the response will occur after the removal of the stimulus. Moreover, the muscle contraction will last for a further, relatively long period (perhaps 100 to 200 milliseconds). The contraction is thus unopposed by the stimulus.

b ***What would you expect to happen if the tap were replaced by a sustained pressure on the patellar tendon?***

In this situation there is a sustained stimulus producing a matching and sustained response. Tension would develop in the quadriceps femoris muscle to oppose the stretch in the patellar tendon.
Stretch receptors respond both to the amount of stretch and to the rate of stretch. The 'tap' causes an extremely rapid stretch, that is, a very large stimulus produces a large response. On the other hand, sustained pressure on the tendon only constitutes a small stimulus because the total change in length is small. Application of pressure to the tendon with a finger or a blunt object would probably stretch the muscle spindle much more slowly than the tap, and so the 'rate of change' stimulus would again be small.

c ***What natural situations might elicit the monosynaptic reflex in the quadriceps femoris (extensor muscle).***

None. The stretch reflex only occurs as part of a co-ordinated movement under normal circumstances.
Clearly an intended stretch of the quadriceps femoris should not be opposed by a stretch reflex. However, the sensitivity of the stretch receptor can be reduced by the contraction of the intrafusal muscle fibres, which have their own motor neurones (the γ-efferent fibres). Thus a co-ordinated movement involves changes in nervous signalling both to the main (extrafusal) muscle fibres and to the intrafusal fibres. □

STUDY ITEM

11.22 The pupillary reflexes

These reflexes can readily be demonstrated in a dimly lit room. If the room is dark enough, the pupil will at first be dilated. Constriction of both pupils should be observed when a light is switched on, even when one eye is shielded from the light. Then ask the subject to focus on an object which is brought towards the eye. Again constriction should be observed.

Questions and answers

a ***What would you expect to occur to the radial muscle during pupillary constriction?***

By analogy with the knee jerk, one would expect its tension to be reduced. However, it should be noted that the size of the pupil is controlled by a balance between the tone of the radial muscle and that of the circular muscle, and a simple analogy with the knee jerk is not really appropriate.

b ***What type of information initiates the three pupillary reflexes?***

Two distinct types of information: general information about the overall level of illumination, and more specific visual information which probably relates to obtaining a focused image on the retina.

c ***What does this suggest about the site of integration?***

The complex input suggests that the site of integration is likely to be within the brain.
Much visual information is processed in the retina. The afferent pathway then runs via primary neurones in the optic nerve. Most of these neurones have synaptic connections with second order neurones, which carry information to the visual cortex, where pattern recognition and other aspects of visual processing occur. The accommodation reflex must involve this pathway.
However, some of the primary neurones in the optic nerve pass directly to the mid-brain, where pupillary constriction is initiated. There must also be a pathway to this region from the visual cortex. The pathway back to the pupil also involves two neurones with a relay station in a ganglion. The reflexes are thus multisynaptic.

d ***Suggest some possible causes for the loss of both pupillary light reflexes.***

1 Damage to the eye (for example, total blindness), so that the afferent signal is not initiated.
2 Damage to the afferent pathway at any stage before integration.
3 Damage to the integrating centres.

e ***What might cause loss of the consensual reflex alone?***

Damage to the efferent pathway to the opposite eye.

f ***Why is loss of the pupillary light reflex a very serious sign in an unconscious patient?***

Loss of the reflex is most likely to indicate a failure in the function of the brain, particularly the mid-brain. This could be caused by physical damage or might be the result of an inadequate oxygen supply. Either way the condition is likely to be very serious.

g *Suggest one other situation in which a reflex response of the pupil might be initiated.*

☐ A sudden shock or emotional stress causes the pupil to dilate.

11.3 The neurone

Principles

1 Individual nerve cells, neurones, are shown to be discrete but able to communicate with each other at synapses.

2 The historical development of this concept is mentioned, and the importance of technical breakthroughs in the development of ideas is emphasized.

3 The function of a neurone is often suggested by its shape: it is governed by the specific connections it makes with other cells.

4 Neurones are surrounded by companion cells which in some cases may have a nutritive function, or may develop to form a myelin sheath.

Practical investigation. *Practical guide 4*, investigation 11B, 'The histology of nerve and muscle tissue'.

Questions and answers

a ***If the neurone theory is correct, and each cell is independent and physically separate, what evidence is there that each neurone is not also a functionally separate unit?***

The knowledge available to the student will be limited at this stage. However, the monosynaptic reflex (section 11.2) shows that neurones are able to communicate with one another. The question is intended to be open-ended and the answer will depend on the experience and imagination of the student.

b ***If neurones function in networks, how could information be transmitted from one cell to the next?***

The simplest answer, that an electrical signal in one cell directly initiates an electrical signal in the next, is rarely the case. Normally information is transmitted chemically: the presynaptic cell releases a chemical (neurotransmitter) which initiates a response in the post-synaptic cell.

11.4 The nerve impulse

Principles

1 Information is conveyed along the neurone in the form of an electrical impulse, the action potential, which can be studied indirectly by measurements made from the surface of the nerve, or directly by inserting a microelectrode into the cytoplasm of the nerve axon (axoplasm).

2 The resting membrane potential is governed by the uneven distribution of potassium and sodium ions and the high permeability

of the membrane to potassium ions. The uneven distribution of ions is maintained at the expense of metabolic energy through the action of the sodium pump.

3 During the action potential, there is a massive, but transient, increase in the permeability to sodium ions which momentarily reverses the resting potential. During recovery, the sodium permeability returns to its resting level and there is a transient rise in the potassium permeability. The entire process lasts for only a few milliseconds and the number of ions crossing the membrane in each action potential is very small.

4 The changes in permeability of the cell membrane depend on the presence of specific voltage-dependent channels which are selectively permeable to either sodium or potassium ions.

Questions and answers

a ***What does this suggest about the nature of nervous transmission?***

Not very much. The early neurophysiologists were careful to point out that this did not prove that nervous transmission was itself electrical, but only that it could be stimulated by electricity.

b ***What conclusion can be drawn from this observation?***

The early workers were puzzled by the finding that compressing a nerve impeded transmission. They had been thinking in terms of the nerve acting like an electrical cable, which should be unaffected by compression.

Practical investigation. *Practical guide 4,* investigation 11C, 'The physiology of a nerve–muscle preparation'.

c ***How could you determine the conduction velocity of a motor nerve, using a kymograph?***

Prepare a muscle with as long a piece of intact motor nerve as possible. Set up the kymograph so that the moment of stimulation is marked, and record the contraction produced after stimulating the nerve at two different points. The separation of the two responses denotes the time taken for the impulse to travel between the two points of stimulation.

d ***Does this experiment prove that information is transmitted along the neurone in the form of an electrical signal?***

No. All it shows is that an electrical stimulus will initiate the transfer of information.

STUDY ITEM

11.41 Recording action potentials from a nerve

Questions and answers

a ***What p.d. would you expect to record during the resting state?***

Since all points on the surface of the axon are at the same potential, no potential difference would be recorded.

b ***What changes in p.d. would you expect to see during the passage of an action potential?***

When the action potential first reaches the nearer of the two recording electrodes the first electrode will become negative with respect to the second one. When the action potential is midway between the two electrodes, the potential differences will again return to zero. When the action potential reaches the second electrode there will again be a potential difference between the recording electrodes. The nearer electrode will now be positive with respect to the other one. When recorded with an oscilloscope the action potential has an upward and downward peak and is accordingly described as diphasic (*figure 35*). The action potential in figure (S)237 has only one peak and is described as monophasic.

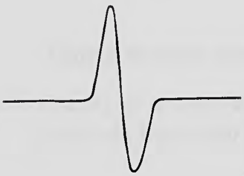

Figure 35
A diphasic action potential.

c ***What would be the effect of increasing the distance between the electrodes in this experiment?***

The two oppositely directed potential differences would be further separated in time. The time interval between the peaks, and the distance between the electrodes, provide another means of determining conduction velocity. If the electrodes are sufficiently far apart, the two phases of the response will be separated by a plateau whilst the whole of the action potential is within the space between the electrodes (*figure 36*). □

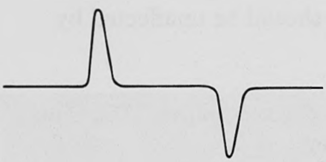

Figure 36
A diphasic action potential with the electrodes widely separated.

The resting potential

Practical investigation. *Practical guide 4*, investigation 11D, 'The nerve impulses in the earthworm's ventral nerve cord'.

Question and answer

a ***What does the existence of a p.d. across the membrane of a cell imply about the distribution of anions and cations on either side of the membrane?***

It implies that there is a separation of charge, suggesting that the anions and cations must be unevenly distributed between the two sides of the membrane.

STUDY ITEM

11.42 The ionic composition of nerve

Questions and answers

a ***Calculate the intracellular concentrations of these three principal ions. Assume that for each kilogram of nerve tissue, 200 cm³ comprise extracellular fluid with the same ionic composition as plasma, 600 cm³ are intracellular water, and the remainder (200 g) is dry material.***

In nerve, each kg of tissue contains 200 cm^3 of extracellular fluid of the same ionic composition as plasma. This fluid therefore contains 28 mmol of Na^+ $\left(\text{that is, } \frac{200}{1000} \times 140 \text{ mmol dm}^{-3}\right)$, 1.0 mmol of K^+, and 20 mmol of Cl^-.

Therefore, in the intracellular fluid there are 9 mmol of Na^+ (that is, 37 − 28), 90 mmol of K^+, and 5 mmol of Cl^- all in 600 cm^3 of intracellular fluid. This equates to intracellular concentrations of 15 mmol dm^{-3} of Na^+ $\left(\text{that is, } \frac{1000}{600} \times 9 = 15\right)$, 150 mmol dm^{-3} of K^+, and 8.33 mmol dm^{-3} of Cl^-.

b ***What do your results show about the intracellular composition of the nerve?***

They show that the concentrations of Na^+ and Cl^- are much lower, and the concentration of K^+ much higher, inside the nerve than outside.

c ***Recalculate the data, assuming that extracellular fluid = 250 cm³ kg⁻¹ and intracellular water = 550 cm³ kg⁻¹.***

Using the figure of 250 cm^3 kg^{-1} for the volume of the extracellular fluid means that the extracellular fluid contains 35 mmol of Na^+, 1.25 mmol of K^+, and 25 mmol of Cl^-. This would leave 2 mmol of Na^+, 89.75 mmol of K^+, and no Cl^- at all in 550 cm^3 of intracellular fluid, that is, the intracellular concentrations would be computed as 3.64 mmol dm^{-3} of Na^+, 163.2 mmol dm^{-3} of K^+, and no Cl^-.

d ***What would be the effect of a small error in the estimate of the volume of extracellular fluid on the calculated values for the intracellular ion composition?***

The calculation in question **c** shows that quite a small error in estimating the volumes of the intracellular and extracellular fluids makes a considerable difference in the apparent intracellular concentrations. Since it is difficult to make precise measurements of these volumes, early estimates of the composition of the interior of the axon were rather inaccurate. ☐

The ionic basis of the resting potential

Questions and answers

a ***What processes in living cells could lead to the ions being distributed so that there are more anions than cations inside the cell, and vice versa outside?***

In non-biological systems, the forces acting on ions to cause movement are those associated with the kinetic energy of the ions and the electrical forces which they experience. In biological systems, ions can also be subjected to specific 'pumping' mechanisms in which metabolic energy is used to perform work.

b ***What is the consequence of this in terms of the separation of charge?***

There will be more anions than cations in the 'high concentration' compartment, and *vice versa* in the 'low concentration' compartment.

c ***What will be the effect on the potential difference across the barrier?***

The 'high concentration' compartment will become negative in relation to the 'low concentration' compartment: that is, a potential difference will be established.

STUDY ITEM

11.43 Some properties of nerve conduction

Questions and answers

a ***What other code system is available to the nervous system?***

The intensity of a signal can only be encoded by the frequency of the action potentials. The nature of the signal is encoded by the specificity of nerve pathways, that is, by which particular neurone is firing.

b ***What would be the effect of the longitudinal potential gradients on the mobile ions along the axon?***

Ions will move down the potential gradients: for example, outside the axon Na^+ will move from positive towards negative, and Cl^- will move in the opposite direction. This will reduce the potential difference across the membrane.

c ***What effect would this have on the adjacent segment of membrane?***

An action potential in one segment of a neurone will depolarize the adjacent segment and initiate an action potential, provided that the membrane is not recovering from a previous action potential. Thus the local flow of ions ('local currents') along the axon will allow the action potential to be propagated from one end of the axon to the other.

d ***What ensures that the action potential passes along the axon in one direction only?***

The action potential passes in one direction only because the region which the action potential has just left is still in the process of recovering and is insensitive to depolarization. ☐

11.5 Chemical messages

Principles

1 Direct electrical coupling between some types of cell does occur. However the transfer of information between nerves is nearly always by means of a chemical transmitter substance.

2 Chemical transmission occurs at synapses, and in this respect there are certain differences between the voluntary and autonomic systems.

3 There is a close similarity between the process of synaptic transmission and other types of chemical signalling, that is, in endocrine and neuroendocrine systems.

STUDY ITEM
11.51 Evidence for chemical transmission at nerve terminals

Questions and answers

a ***What is the most probable hypothesis to explain this observation?***

Stimulation of the vagus must release a substance which can slow the heart. This substance, as well as slowing the innervated heart, is present in sufficient quantities in the perfusion fluid to affect the second heart as well. The most likely source of the substance is the nerve itself.

b ***How could further evidence to support this hypothesis be obtained?***

By isolating the chemical agent involved and demonstrating that it could slow the heart. Since the actual amounts of acetylcholine released are extremely small, this would be technically very difficult. If the substance could be isolated, the ultimate aim would be to identify the chemical. This would not have been possible in 1921 and would be difficult even with modern techniques. Ideally, it should also be demonstrated that the same substance is present in the nerve terminals of the motor neurones.

c ***Is the fact that acetylcholine can slow the heart sufficient evidence to identify it as the agent which does so naturally?***

No. Other agents apart from acetylcholine might also be able to slow the heart.

d ***What are the advantages of destroying acetylcholine almost as soon as it is released?***

Firstly, to limit the duration of the response. Secondly, to help to localize the response to the site at which the acetylcholine is released.

e *What conditions would have to be fulfilled for Loewi's experiment to be successful?*

Sufficient acetylcholine must enter the perfusion fluid for the second heart to be affected. This suggests that:
1 large amounts of acetylcholine are released from the vagus;
2 cholinesterase does not break down all the acetylcholine at the site of its release;
☐ **3** the frog's heart is sensitive to very small quantities of acetylcholine.

STUDY ITEM
11.52 Adrenaline and noradrenaline

Questions and answers

a ***If this were not the case, could any distinction be drawn between a neurotransmitter and a hormone?***

No. Hormones can be defined as chemical substances that are secreted into the blood and carried by it to other cells, on which they act.
In mammals adrenaline is a hormone released from the adrenal medulla and involved in the regulation of metabolism. However, it has also been identified as a neurotransmitter in the mammalian brain. There is increasing evidence to suggest that some of the small peptide hormones of the gastrointestinal tract are also present in the brain, where a role as neurotransmitters is suspected. Thus the same chemical substance may act as both neurotransmitter and hormone in the same organism.

b ***How could noradrenaline be rapidly removed from the synaptic region other than by a local extracellular enzyme?***

Since the synaptic cleft is very narrow, the noradrenaline will tend to be confined to its site of release. It can thus be removed by being taken up into the cells which border the synaptic cleft.
Quantitatively, uptake by the presynaptic terminal is the most important mechanism for removal of noradrenaline. This noradrenaline is then available for re-use. The remainder of the noradrenaline is destroyed by enzymes which are more remote from the synapse than is cholinesterase. One of these enzymes, monoamine oxidase (MAO) is found in mitochondrial membranes, both in the presynaptic nerve terminal and also distributed widely throughout the body. Another enzyme, catechol O-methyl transferase (COMT) is found in the cytoplasm of many different cell types.

c ***Since these three compounds are structurally so similar and yet have quite distinct actions, what does this suggest about the way in which chemical messengers are recognized?***

The recognition site (receptor) must be highly specific and able to
☐ discriminate small structural differences.

Questions and answers

a **What does this suggest about the target cells?**

A single target cell must possess at least two types of receptor. In a cardiac cell, or in a neurone which acts as an integrating centre, the two types of receptor must lead to opposite responses.
A similar multiplicity of receptors can occur for a hormonal response. The fat cell responds to insulin by synthesizing more triglyceride. The opposite response, breakdown (hydrolysis) of the triglyceride, will occur in response to a number of hormones. Each of these has its own receptor on the fat cell's membrane, although all ultimately act through the adenylate cyclase system (see *Study guide II*, Chapter 24).

Practical investigation. *Practical guide 4*, investigation 11C, 'The physiology of a nerve–muscle preparation'.

b **What are the consequences of the narrowness of the synaptic cleft?**

This ensures that it is difficult for the transmitter to diffuse away from the synaptic region. The concentration of transmitter at the post-synaptic membrane will therefore be raised.

c **How could depolarization, initiated in channels sensitive to acetylcholine at the neuromuscular junction, start up an action potential in the muscle membrane?**

Depolarization in the end-plate region will depolarize the adjacent region of muscle membrane. If this depolarization exceeds the threshold, an action potential will be initiated.

d **What would have to happen for an action potential to be initiated in a post-synaptic cell in this situation?**

Again, for an action potential to be initiated the post-synaptic cell needs to be depolarized to its threshold. For this to occur the overall effect of all the incoming synaptic inputs must be to depolarize beyond its threshold.
The adding together of the sensory inputs is termed summation. Each depolarization induced at a synapse is transitory and localized. For the threshold to be reached, several individual excitatory inputs must occur sufficiently close together in both space and time. These requirements are described as spatial and temporal summation.

e **What are the common features shared by synaptic transmitters and hormones?**

In both the chemical agent is usually stored in vesicles. Release of the vesicles is initiated by a rise in intracellular concentration of calcium ions. Vesicles fuse with the cell membranes and release their contents.

f ***What possible mechanisms for such an influence can you suggest?***

One possibility is the activation of a membrane-bound enzyme which can modify the concentration of a key intracellular molecule. For example, the activation of adenylate cyclase raises the concentration of intracellular cyclic AMP (see *Study guide II*, Chapter 24). As well as acting on membrane receptors, hormones may enter their target cells and act on cytoplasmic receptors.
Steroid hormones, which are chemically related to cholesterol, are lipid-soluble and can cross the cell membrane. All steroid receptors appear to be in the cytoplasm, and not on the membrane.
Recent evidence suggests that, for some other hormones, combination with a membrane receptor induces a process termed endocytosis. This is the reverse of the process by which transmitters and hormones are released, and it allows the hormone to enter the target cell even though it has a very low lipid solubility. The full significance of such a process in hormone action is still under debate.

11.6 Skeletal muscle

Principles

1 The arrival of an action potential at the axon terminal of a motor neurone can lead to the contraction of a muscle fibre.

2 The interaction of acetylcholine with the post-synaptic receptors of the motor end-plate causes a local depolarization; this initiates an action potential in adjacent regions of the muscle plasma membrane.

3 The spread of the action potential down the transverse tubules induces the release of calcium ions from the sarcoplasmic reticulum into the interior of the muscle cell. The release of calcium ions is the molecular signal which initiates contraction; the re-uptake of calcium ions by the sarcoplasmic reticulum terminates contraction.

4 The force of contraction is generated by the relative movement of two molecules, actin and myosin, a concept known as the sliding filament theory.

5 Two other molecules, troponin and tropomyosin, provide the mechanism whereby changes in the intracellular concentration of calcium ions can affect the actin and myosin.

6 A rapid series of action potentials can lead to a progressive rise in intracellular concentration of calcium ions, thereby raising the force of contraction. This effect is enhanced by the series elastic component of the muscle.

Practical investigation. *Practical guide 4*, investigation 11B, 'The histology of nerve and muscle tissue'.

Question and answer

a ***Under what circumstances would it be appropriate for motor units to be very large or very small?***

A large motor unit means the simultaneous contraction of many motor fibres. Large motor units are appropriate for situations in which powerful forces need to be generated, for example, in the large postural muscles of the back. Small motor units allow very delicate movements to be made, for example, in the muscles of the fingers.

Small muscles associated with fine movements often have a second characteristic. As well as being organized into small motor units they are frequently composed of fibres having short twitch durations.

STUDY ITEM

11.61 The contraction of muscle

Questions and answers

a *At the bottom of* figure [S]245b *the myofibrils can be seen to consist of alternating bundles of thick and thin filaments which overlap with each other. What does this suggest about the way the muscle might shorten?*

The filaments may slide past each other. This idea is supported by comparing electronmicrographs of myofibrils in stretched and contracted states.

b *Numerous mitochondria lie close to the myofibrils. What is their function?*

To provide energy for some aspect of the contraction process.

c Figure [S]246a *shows a cross-section of a myofibril. Through what level of* figure [S]245b *would the section pass? Explain your answer.*

The section would pass through a region where the thick and thin filaments are overlapping. This must be so because both thick and thin filaments are present in the cross-section.

d *What might be the function of the myosin cross-bridges?*

To hold the thick and thin filaments together, and possibly to pull them towards each other when the muscle contracts.

e *From* figures [S]245 *and* [S]246, *and from the above text, suggest a hypothesis outlining the sequence of events leading to muscle contraction.*

An action potential is carried into the muscle fibre by the transverse tubules. Calcium ions are released from the sarcoplasmic reticulum and bind with the troponin molecules. Binding sites for myosin cross-bridges are then exposed on the actin chain. ATP supplies the energy for the myosin cross-bridges to bind with the actin chain. The whole cycle of events leading to contraction is complex, but the main element is the sequential formation and breaking of links between actin and myosin through the myosin cross-bridges. Activation of the cross-bridge by ATP causes it to change its shape and to move through an arc whilst attached to an actin molecule. The actin and myosin separate and the myosin recovers its original shape ready for the next cycle of movement. At any given moment, about half the cross-bridges are forming links whilst the other half are recovering. It is actually the binding of an ATP molecule to the myosin which enables it to break the link with actin. In the absence of ATP, the bond is fixed and the muscle becomes very stiff and rigid. This is the cause of the stiffness seen shortly after death (*rigor mortis*). Normally, however, ATP is plentifully available and one might expect the muscle to maintain a continual state of active contraction.

It is prevented from doing so by the tropomyosin and troponin (see figure (S)246). Tropomyosin is a linear molecule which binds to actin, covering the binding sites, and preventing the attachment of the myosin cross-bridges. In order to uncover the binding sites, the position of the tropomyosin is altered as a consequence of calcium ions binding to a specific site on a troponin molecule, which in turn is attached to the tropomyosin. The binding of the calcium ions leads to a change in the shape of the troponin molecule, which pulls the tropomyosin molecule to one side and exposes the binding sites on the actin. Thus, the release of calcium ions from the sarcoplasmic reticulum 'switches on' the contractile process by exposing the actin binding sites. The removal of the calcium ions returns the troponin molecule to its original shape and contraction can no longer occur. ☐

Practical investigation. *Practical guide 4*, investigation 11C, 'The physiology of a nerve–muscle preparation'.

11.7 Neuroactive drugs

Principles

1 The use of pharmacological agents can extend our knowledge of physiological processes, such as the transmission of action potentials and synaptic transmission in the autonomic nervous system.

2 Local anaesthetics act by selectively blocking the voltage-dependent Na^+ channels and preventing the transmission of action potentials in pain fibres.

3 Organophosphates and nerve gases inhibit the action of cholinesterase, the enzyme which breaks down acetylcholine, whilst botulinum toxin prevents acetylcholine from being released.

4 Acetylcholine is shown to be the transmitter not only at neuromuscular junctions but also at parasympathetic endings and in ganglia in the autonomic nervous system.

5 The specific effects of certain drugs suggest that three different types of receptor are involved. A similar division is suggested by the use of other drugs, which specifically block ganglionic transmission.

6 The similarities and differences between the effects of adrenaline and noradrenaline indicate the existence of at least two kinds of adrenergic receptor.

STUDY ITEM

11.71 The site of action of neuroactive drugs

Questions and answers

a ***What does this suggest about the action of curare on the muscle?***

Curare prevents the action potential in the motor neurone from eliciting an action potential in the muscle, even though the muscle is still excitable. Curare must either prevent transmission of the action potential by the nerve, or block neuromuscular transmission.

b ***What does this suggest about the action of curare on the nerve?***

Curare does not prevent nervous transmission.

c ***What is the most probable site of action of curare?***

The neuromuscular junction.

d ***Suggest possible ways in which curare might act.***

1 By blocking the voltage-dependent Ca^{2+} channels in the pre-synaptic nerve ending, thus preventing the rise in intracellular Ca^{2+} concentration and the release of synaptic vesicular contents.
2 By preventing the release of acetylcholine from synaptic vesicles in some other way.
3 By preventing the combination of acetylcholine with the post-synaptic receptor.
□ **4** By modifying the action of cholinesterase.

Questions and answers

a ***What electrical changes would this produce in the muscle end-plate?***

Since fewer receptor sites are occupied, the electrical change induced will also be smaller. Since an action potential is not initiated, the electrical response must be a sub-threshold depolarization.

b ***What electrical changes in the muscle end-plate would an action potential in the motor nerve initiate under these circumstances?***

Since the acetylcholine is not being broken down, one would expect its concentration to become higher, and to remain high for longer. The prolonged action of acetylcholine would produce a sustained depolarization of the motor end-plate, and this is what is observed.

c ***What effect would you expect such changes to have on neuromuscular transmission?***

Two possibilities suggest themselves. The sustained depolarization of the end-plate might mean that multiple action potentials were produced. Equally, once the end-plate was depolarized, further action potentials in the motor neurone might be unable to initiate an action potential in the muscle, thus blocking neuromuscular transmission. It is the latter process which actually occurs.

d ***What would you expect to be the effect of botulinum toxin on neuromuscular transmission?***

To block it completely.

e ***What do these observations suggest about the acetylcholine receptors?***

They suggest that, although acetylcholine is the common transmitter at the three sites, the receptors are not all the same. The different effects of agonists and antagonists tell us that there are at least three types of cholinergic receptor.

f ***How could the similarities and differences between the actions of adrenaline and noradrenaline be explained?***

More than one type of adrenergic receptor must exist. At least one of these receptors must respond to both adrenaline and noradrenaline. Other receptors must respond differently to each substance. Receptors in the heart must respond to both compounds. One would expect there to be two types of receptor associated with the arterioles in different locations.

STUDY ITEM

11.72 The effect of drugs on the eye's pupil

Questions and answers

a ***Determine whether each of the following drugs would cause dilatation or constriction of the pupil:***
1 phenylephrine (which mimics the sympathetic nervous system),
2 eserine (which inhibits cholinesterase),
3 cocaine (which inhibits an enzyme which breaks down noradrenaline),
4 atropine (which blocks the acetylcholine receptor).

1 Since phenylephrine mimics the sympathetic system it would be expected to cause dilatation.

2 Since eserine inhibits cholinesterase, the inactivator of acetylcholine, it would be expected to enhance the effect of acetylcholine and lead to constriction.

3 Since cocaine inhibits the breakdown of noradrenaline, it would be expected to enhance the effect of the sympathetic system and lead to dilatation.

4 Since atropine blocks the acetylcholine receptor, it would be expected to inhibit the parasympathetic system, allowing the sympathetic system to act unopposed. This will tend to produce dilatation.

b ***What would be the effect of fear (which activates the sympathetic nervous system)?***

Fear stimulates the release of adrenaline from the adrenal medulla and raises the level of activity of the sympathetic nervous system, leading to dilation of the pupil. □

PART II The *Practical guide*

The investigations related to this chapter appear in *Practical guide 4, Co-ordination, response, and behaviour.*

INVESTIGATION 11A The spinal cord and spinal roots

ITEMS NEEDED

Spinal cord, mammalian, T.S. nerve entry, permanent preparation 1/1

Ammonium iron(III) sulphate (iron alum), aqueous, 1 %
Mulligan's fluid
Tannic acid (aqueous), 2 %

Microscope, monocular 1/1

(*Study guide* 11.2 'The monosynaptic reflex'.)

Microscopical preparations are used to study the anatomy and histology of the spinal cord and the roots of spinal nerves.

Mulligan's fluid
Copper(II) sulphate (hydrated), 5 g
Hydrochloric acid, concentrated, 1.5 cm^3
Phenol, 40 g
Water, distilled, 1 dm^3
(*TAKE CARE:* hydrochloric acid and phenol are toxic and corrosive.)

Assumption

1 An appreciation of the gross structure of the spinal cord in three dimensions and the ability to relate this to the appearance of the cord in transverse section.

Principles

1 The grey matter of the spinal cord contains the cell bodies of the motor neurones; the dorsal root ganglion contains the cell bodies of the sensory neurones.

2 The functioning of the spinal cord depends on the arrangement of synapses in the grey matter. Electron microscopy has revealed much information about synapse structure and function.

If slides of the spinal cord including the two roots are not available, then slides showing the roots separately should be examined. Transparencies are an alternative or additional resource.

If a preserved spinal cord or brain material is available, it could be examined, dissected, and stained as follows:

1 Slice the tissue, 5 mm thick, and wash well in running water.
2 Place slices in Mulligan's fluid for 2 minutes and wash for 1 minute.

3 Place in 2 % tannic acid (aqueous) for 1 minute and wash for 3 minutes.
4 Place in 1 % ammonium iron(III) sulphate (iron alum) (aqueous) until grey matter turns black (30 seconds).
5 Wash well.

Myelinated nerve fibres remain unstained as the stain cannot penetrate the fatty sheath.

Questions and answers

a ***How can the dorsal root be distinguished from the ventral root by its gross appearance?***

The dorsal root has a swelling or ganglion but the ventral root does not.

b ***Where in the spinal cord and spinal roots are cell bodies found?***

In the grey matter and in the dorsal root ganglion.

c ***How can the cell bodies of motor neurones be distinguished from those of the sensory neurones?***

The shape is of irregular triangles or polygons, owing to the multipolar nature of the cell body, which has several dendrites extending from it. The cell bodies of sensory neurones are roughly spherical, as they have only one process extending from them.

d ***In which region of the spinal cord would you expect synapses to be found?***

In the grey matter.

e ***How do the arrangement of synapses and the anatomy of the cell bodies of the motor neurones relate to the function of the spinal cord in co-ordinating nervous activity?***

Figure (P)4 shows numerous synapses arranged along the surface of a cell body. Dendrites will provide an even greater surface for synaptic bulbs. Each motor neurone can therefore be stimulated in a very large number of ways.

f ***What features of the synapse's function can be inferred from* figure *[P]5?***

The distinct gap or synaptic cleft illustrates the discrete nature of neurones. This poses the problem of transmission across the gap. Chemical transmission is a possibility since the gap is small. The synaptic bulbs are full of vesicles, while the dendrite has none. Mitochondria could provide the energy for manufacturing the transmitter or for moving and rupturing the vesicles, or both. The round vesicles are stimulatory and the oval ones inhibitory, but this cannot be inferred from the figure.

INVESTIGATION
11B The histology of nerve and muscle tissue

ITEMS NEEDED

Permanent preparations of:
Nerve, T.S. and L.S. 1/1
Nerve, T.S. osmium(VIII) oxide (osmic acid) 1/1
Striated muscle, T.S. and L.S. 1/1
Striated muscle, teased, motor end-plates 1/1

Microscope, monocular 1/1

(*Study guide* 11.3 'The neurone' and 11.6 'Skeletal muscle'.)

Microscopical preparations are used to study the histology of nerve and muscle tissue.

Assumption

1 The ability to relate the appearance of tissues in transverse and longitudinal section to their three-dimensional structure.

Principles

1 Nerve fibres (axons) are surrounded by a fatty myelin sheath derived from Schwann cells. The sheath is interrupted at the nodes of Ranvier.
2 The striated appearance of muscle fibres is due to the arrangement of actin and myosin filaments within each sarcomere of the myofibrils.
3 Impulses are passed from nerve fibres to muscle fibres at the motor end-plates.

35 mm transparencies are a suitable alternative to microscope slides. Other material could be examined to illustrate the range of different types of neurone (for example, in the cerebellum) or muscle fibre (that is, smooth and cardiac).

Questions and answers

a ***What can you infer about the nature of the myelin sheath?***

The myelin sheath is stained black with osmium(VII) oxide and must contain fatty material. Electron microscopy reveals that it is composed of many layers of membrane tightly coiled around the axon. The membrane is formed from the surrounding Schwann cell.

b ***What happens to the myelin sheath at the nodes of Ranvier?***

The sheath is interrupted.

c ***What processes could occur at the nodes of Ranvier which might be difficult or impossible at other parts of the axon?***

The exchange of materials, for example, ions between the axoplasm and the extracellular fluid.

d ***What is responsible for giving muscle fibres their striated appearance?***

The arrangement of thin actin and thick myosin filaments within each sarcomere of the myofibrils.

e ***Can you suggest a possible mechanism that could bring about the shortening of a muscle fibre?***

The actin filaments slide into the spaces between the myosin filaments, shortening each sarcomere and therefore the total length of the myofibrils. In a fully contracted muscle the I bands, which contain actin filaments only, disappear. Cross-bridges can be seen between the actin and myosin filaments. These are believed to produce the force which causes the sliding action during myofibril contraction.

f ***What is the relationship between a motor axon and the muscle fibres which it innervates?***

Each axon branches and innervates a number of muscle fibres. Each muscle fibre has one motor end-plate on its surface.

g ***What features of the motor end-plate's function can you infer from figure [P]14?***

There are obvious similarities with synapses studied in investigation 11A. Vesicles and mitochondria can be seen within the axoplasm, and chemical transmission across the synaptic cleft seems likely.

INVESTIGATION
11C The physiology of a nerve–muscle preparation

ITEMS NEEDED

Frog, freshly killed 1/group

Ringer's solution, frog

Cocktail sticks, wooden or plastic
Cotton thread
Forceps, blunt 1/group
Kymograph (or electronic arm and chart recorder) with paper, pen, and ink 1/group
Muscle bath with lever 1/group
Pin 1/group
Pipette 1/group
Scissors 1/group
Seeker 1/group
Stimulating electrodes, 1 pair, with stand 1/group
Stimulator 1/group

(*Study guide* 11.4 'The nerve impulse', 11.5 'Chemical messages', and 11.6 'Skeletal muscle'.)

An investigation into the physiology of nerves and muscles by stimulating the nerve and recording the contraction of the muscle on a kymograph.

Ringer's solution, frog
Calcium chloride, anhydrous, 30 g   0.3
Potassium chloride, 25 g   0.25
Sodium chloride, 6.5 g
Water, distilled, 1 dm^3

Assumptions
1 A knowledge of the histology of nerve and muscle tissue.
2 The ability to convert units of distance on the recording paper into time intervals.

Principles
1 Raising the stimulus voltage increases the amount of muscle contraction until a point is reached when all the axons are stimulated and all the muscle fibres contract.
2 Two stimuli given at a suitable interval can produce summation of the response. Repetitive stimuli increase the amount of contraction until tetany is reached, when each muscle fibre is fully contracted. Continuous stimulation leads to fatigue of the muscle.
3 Nerve and muscle fibres exhibit a refractory period; immediately after stimulation they are unresponsive to further stimuli.

Only one frog is necessary, as a skilful demonstration by the teacher could be the most appropriate way of organizing this work. There is alternative practical work that does not involve the use of vertebrates;

for example, locust thorax preparations can be used where the metathoracic ganglion is stimulated and responses of the hind limb recorded, or the hind leg can be dissected and stimulated via the crural nerve. Work on human physiology could also be tried, for example, recording contractions of the flexor muscles of a finger.

Electronic arms could be used which convert the movement of the muscle into electrical voltages, either for display on a chart recorder or storage in a data memory unit. (More information can be found in the equipment booklet *Harris data memory system*, Philip Harris Limited, 1980.)

Questions and answers

a ***Why are wooden or plastic cocktail sticks used to separate the nerve from the muscle tissue?***

This reduces the possibility of mechanical damage to the nerve and also avoids unnecessary stimulation by local currents produced by the presence of metal in the Ringer's solution.

b ***During the course of the dissection, did the muscle show any signs of being stimulated? If so, what do you think caused the stimulation?***

The muscle will probably twitch during dissection, particularly when the sciatic nerve is cut. This is probably due to the nerve fibres being damaged and stimulated by the local currents produced.

c ***How do you account for the responses of the muscle to stimuli of different voltages?***

Below a certain threshold no axons and therefore no muscle fibres are stimulated. As the stimulus voltage increases, more axons fire; therefore more muscle fibres contract. Eventually all the axons are stimulated and all the muscle fibres contract, so that further increases in stimulus voltage produce no effect.

d ***What is the period of delay between a stimulus being delivered to the nerve and the muscle responding? What mechanical and physiological factors could contribute to this?***

The delay is likely to be of the order of 0.02 s. This could be due to a delay in initiating the nerve impulse, the velocity of the nerve impulse, a delay in crossing a synapse, a delay in initiating muscular contraction, the rate of muscular contraction, or mechanical effects in the lever system.

e ***What is the period of delay when the muscle is stimulated directly? What can be deduced from this about the velocity of the nerve impulse?***

There is likely to be little measurable difference in the period of delay when the muscle is stimulated directly; this suggests that the impulse velocity is very fast. It should be pointed out that this technique eliminates the first three factors given above, though it is still possible that nerve fibres within the muscle may be stimulated, rather than the muscle itself.

f ***How could the nerve–muscle preparation be used to measure the velocity of the nerve impulse? State what measurements you would have to make.***

Place two sets of stimulating electrodes under as long a portion of sciatic nerve as possible. Repeat the procedures described in stages 10 and 11, stimulating first with one set of electrodes and then the other. Measure the distances between the stimulus marks and the beginning of the responses and convert these to time intervals. Measure the distance between the electrodes. Divide the difference in time intervals by the distance to calculate the velocity. This could be attempted as part of the practical work.

g ***What effects do two successive stimuli have on the muscle?***

With a long time interval between the two stimuli two separate muscle twitches are recorded. As the time interval is decreased, there comes a point when the second stimulus reaches the muscle fibres before the first twitch has had time to die away completely. The second twitch is then superimposed on the first, giving an effect called summation. When the time interval is further decreased, only one normally sized twitch will be seen. This is because after stimulation both nerve and muscle fibres exhibit a refractory period, during which they are unresponsive to further stimuli.

h ***What effects do repetitive stimuli have on the muscle?***

Increased frequency of stimulation increases the amount of contraction up to a maximum, known as tetany. This effect depends on the amount that each muscle fibre contracts and should not be confused with the effects of stimulus voltage described in the answer to question **c**.

i ***From this investigation, what determines the amount that a muscle contracts?***

The frequency of stimulation affects how much a muscle contracts, providing a graded response.

j ***How do you account for the effect of continuous stimulation on the muscle?***

The muscle will exhibit fatigue and stop contracting. Shortage of ATP is the immediate cause. This could lead to a discussion of muscle biochemistry.

INVESTIGATION

11D The nerve impulses in the earthworm's ventral nerve cord

ITEMS NEEDED

Earthworm, large, healthy, in dish of Ringer's solution 1/group

MS 222 solution, 1.0 g in 1 dm^3 diluted Ringer's solution

Ringer's solution, earthworm (frog Ringer's diluted × 6; see page 280)

Cathode-ray oscilloscope, single beam, sensitive to 10 mV cm^{-1} (ideally) or 50 mV cm^{-1} (minimum) 1/group

Dissecting dish, metal, wax-bottomed 1/group

Dissecting instruments – entomological scissors and forceps (recommended), scalpel, and wooden handled seeker 1/group

Dividers 1/group

Electrodes, platinum, 1 pair suitably mounted: one connected to the core and the other to the screen 1/group

Leads with crocodile clips

Length of coaxial cable, connected to electrodes

Pins (entomological)

Pipette 1/group

Pre-amplifier, if oscilloscope is not sensitive enough 1/group

(*Study guide* 11.4 'The nerve impulse'.)

A simple reflex arc is investigated by using a cathode-ray oscilloscope to record the nerve impulse.

Assumptions

1 An understanding of the pathway and the mechanisms involved in the earthworm's escape reflex.

2 An understanding of the terms 'potential difference' and 'action potential'.

3 The ability to use an oscilloscope properly and interpret results in terms of the gain and time base settings.

4 The ability to carry out dissection carefully and accurately.

Principles

1 Nerve impulses can be recorded as changes in potential on the screen of a cathode-ray oscilloscope.

2 These action potentials are of fixed size, but their number depends on the strength of stimulation.

3 Repetitive stimulation causes some kind of failure within the reflex arc. Action potentials are not produced indefinitely.

It is vital that a clean, undamaged preparation is produced. Extra worms should be provided and they must be large. All the anaesthetic must be removed and the cord frequently moistened with Ringer's solution.

The number of oscilloscopes needed can be reduced by several preparations being tried on one machine in turn. A pre-amplifier could be used between the electrodes and the oscilloscope input.

Electrodes can be home-made, for example, from stainless steel pins pushed through a bung and connected as shown in *figure 37*. One electrode must be soldered to the screen of the coaxial cable, which is connected to the earth input of the oscilloscope.

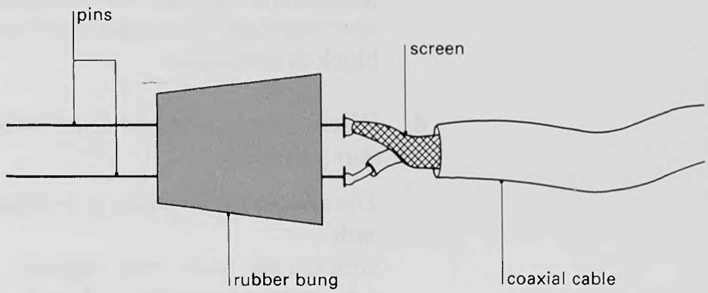

Figure 37
Home-made electrodes.

The earthing of the dish (stage 7) has proved satisfactory in producing a clean straight line on the screen. Mains interference will appear as waves of 50 cycles s^{-1} frequency and must be eliminated. It may be necessary to experiment with different earthing arrangements to achieve the best results.

Questions and answers

a ***How do the potentials produced by stimulating the two ends of the worm compare? Refer to magnitude (in mV) and duration (in ms) in your answer. Make scale drawings of the potentials from your measurements.***

The potentials should be of the order of 20 mV in size and of 1 ms duration. Those produced by anterior stimulation should be smaller.

b ***What is the effect on the potentials of varying the strength of the stimulus? Can you give an explanation for this result?***

Very gentle stimulation should elicit one or two action potentials, while stronger stimulation produces a burst of five to ten. Each potential should however be of a standard size. The burst could be due either to a particular tactile receptor firing at a higher frequency, or to more receptors being stimulated.

c ***What is the effect of repetitive stimulation on the potentials? What possible explanations are there for this result? What experiments could be performed to test these ideas?***

The reflex arc will fail and after several stimuli no more action potentials will be seen. This can be related to the waning of the escape response that takes place under these conditions, that is, the muscles that bring about the response cease to be stimulated. In this case there must be some failure on the afferent side of the reflex, for example, sensory adaptation, synaptic failure at the junctions between the sensory neurones and the giant fibres, or possibly failure of the nerve fibres themselves. By using stimulating electrodes, stimuli could be delivered to one side of a suspected block, and recordings made from the other side. The cessation of action potentials would confirm a block at that point.

d ***Discuss the results and implications of any further investigations that you made.***

The middle of the worm is, as might be expected, less sensitive than the ends.

Stimulating both ends together can produce both types of action potential (see question **a** above); this suggests that they are transmitted independently. The smaller potentials produced by anterior stimulation are in fact transmitted by the median giant axon and the larger ones by the lateral giant axons.

Strong acids and vibration may also elicit action potentials.

PART III BIBLIOGRAPHY

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KATZ, B. *Nerve, muscle and synapse.* McGraw-Hill Book Company, 1966. (This classic book has something to offer at all levels. The opening chapters on the electrical aspects are tough-going but unnecessary for understanding the rest of the book.)

CHAPTER 12 THE RESPONSE TO STIMULI

A review of the chapter's aims and contents

1 Animals and plants normally respond to stimuli in such a way as to increase their chances of survival.

2 In studying responses to stimuli one must ask what sort of stimuli initiate the responses, and how the responses are executed.

3 To answer the second of the above questions one must understand how stimuli are perceived at the cellular level. This question is easier to answer for animals, where specialized receptor cells can be identified, than for plants.

4 In analysing an animal's response to a stimulus the properties not just of the receptors but of the entire reflex mechanism must be taken into account.

5 The analysis of responses to stimuli provides admirable examples of the use of the scientific method, particularly formulating and testing hypotheses.

PART I The *Study guide*

12.2 Animal responses

Principles

1 Animals respond to stimuli by moving the whole, or part, of the body. At the behavioural level the movement may be random (kinesis) or in a particular direction, which is determined by the source of the stimulus (taxis).

2 In responding to stimuli specialized receptor cells are involved. They are connected to nerves, and thus to muscles, in reflex arcs whose properties determine the overall features of the response.

The first part of the section deals with the behavioural aspects of responses. There then follows an account of the structure and functioning of receptor cells. The part played by different facets of the reflex system in determining the overall response is covered by a Study item on the earthworm's escape response.

Orientation

Practical investigations. *Practical guide 4*, investigation 12A, 'The response of *Tribolium* to humidity' and investigation 12B, 'The response of *Calliphora* larvae to light'.

STUDY ITEM

12.21 Taxic and kinetic responses

(Modified from a J.M.B. paper)

Although sharp divisions are artificial, this exercise should enable students to appreciate the difference between taxic and kinetic responses, and also the adaptive significance of both.

Questions and answers

a ***Which of the following responses are examples of taxis and which of kinesis? Explain your choices.***

1 When exposed to daylight the mollusc **Lepidochitona cinereus** *starts moving randomly within a few minutes.*
2 The brine shrimp swims on its dorsal surface if illuminated from above, but on its ventral surface if the light is placed below its tank.
3 The average rate of change of direction of a planarian is greater in a high than in a low intensity of light.
4 A woodlouse moves more rapidly as the humidity decreases.
5 A centipede stops moving when more than 50 per cent of its dorsal surface is in contact with an object.
6 A species of insect larva which feeds on vine roots moves towards a source of CO_2 when there is a difference of 0.1 per cent by volume over a distance of 10 cm, but away from a source of CO_2 when the concentration gradient is steeper.

Taxis: 2 and 6. Both involve movement in relation to the source of a directional stimulus.
Kinesis: 1, 3, 4, and 5. All involve a change in the rate of movement or turning but not a change in direction.

b ***Suggest a reason why each of the above responses is important in the animal's life.***

1 The *Lepidochitona's* response enables it to reach dark places, for example, under stones and rocks.
2 The brine shrimp's response enables it to maintain a correct dorso-ventral orientation when swimming.
3 The planarian's response enables it to find dark places, for example, under stones.
4 The woodlouse's response enables it to find damp places, for example, under stones and logs.
5 The centipede's response keeps it in protected places, such as the soil and leaf litter.
6 This insect larva's response guides it towards the vine roots, which it uses as food, but away from potentially noxious CO_2 levels. □

Sensory cells and neurones

Principles

1 An animal's sensory system provides information about the type, location, and intensity of a stimulus.

2 The specificity of this information lies in the properties of the receptor and the pathway from the receptor to the sensory cortex of the brain or other central destination.
3 The intensity of a stimulus is signalled by the number of action potentials which it initiates in each sensory neurone and the number of sensory units which are activated.
4 The initiation of action potentials depends on the stimulus first causing a change in the membrane potential in the receptor cell.
5 The magnitude of this potential is graded according to the strength of the stimulus.

Assumption

1 That the student is familiar with the physiology of the nerve impulse described in Chapter 11 (this section will make little sense otherwise).

Questions and answers

a ***Since only one type of signal is used in a nerve cell, how can the* type *of stimulus be distinguished?***

By the specificity of the receptor. Each receptor responds to only one type of stimulus.

b ***What changes in the membrane's properties could produce:***
1 depolarization (as in most receptors) and
2 hyperpolarization in the vertebrate photoreceptor?

1(i) A fall in permeability to K^+. (ii) A rise in permeability to Na^+. (iii) A general rise in the membrane permeability, which allows the permeability to Na^+ to increase more than the permeability to K^+ (this appears to be the actual mechanism).

2(i) A rise in permeability to K^+. (ii) A fall in permeability to Na^+ (which is what actually occurs).

c ***What effect would you expect tetrodotoxin to have on the receptor region of the nerve ending?***

You would expect it to block any voltage-sensitive Na^+ channels present (see page 349 in the *Study guide*).

d ***What hypothesis was being tested by this experiment?***

That the failure of the receptor region of the nerve ending to produce action potentials is due to the absence of voltage-sensitive Na^+ channels in this region.

e ***What conclusions can be drawn about the ion channels in the cell membrane of the receptor region?***

They are not voltage-dependent. They are affected by the specific stimulus to which the receptor responds (the specific stimulus causes an increase in the general cation permeability of the receptor region). The magnitude of the increase in permeability is related to the strength of the stimulus.

f ***What will happen when the action potentials generated in the terminal branches reach the common axon?***

They will be propagated along the axon provided it is not still in a refractory period following the passage of a previous action potential. Thus, most, but not all, of the action potentials initiated in the peripheral receptors will be propagated. The axon will transmit with a frequency that represents the collected information from all the receptors which it serves.

g ***In what situations would you expect the sensory units to be (1) large, and (2) small?***

The size of a sensory unit will be greater in those situations where fine discrimination is unnecessary, such as the cutaneous receptors of the back, whilst sensory units in, for example, the finger tips serve much smaller areas.

h ***How could you compare the sizes of the tactile (touch) sensory units in different areas of the skin?***

This is easily achieved by touching the skin *lightly* with a pair of dividers. Two separate points of contact will be detected if there is an unexcited sensory unit between them. Determine the smallest distance between the points which will allow the stimulus to be felt as two separate pin pricks rather than one. Repeat this in different places on the body surface. Such two-point discrimination is much better in the finger tips than on the back, or even on an arm.

Sensory adaptation

STUDY ITEM
12.22 Dark adaptation

No prior knowledge is assumed, but many students will already be aware that the retina possesses two types of photoreceptor, rods and cones. This knowledge may make some of the questions rather easy.

Questions and answers

a ***Describe in words the changes shown graphically in* figure [S] 253.**

The sensitivity increases in *two* stages. In each stage the improvement is initially rapid and becomes progressively slower (it appears to be roughly exponential).

b ***How much does the sensitivity increase***
1 in the first 5 minutes, and
2 overall?

1 Nearly forty-fold. (This is an exercise in interpreting a logarithmic scale.)
2 More than ten thousandfold.

c ***What possible explanation can you offer for the shape of the curve?***

Two stages of adaptation imply two types of receptor. The initial rapid adaptation represents adaptation of the cones, and the larger but slower change corresponds to adaptation of the rods.

d ***What is the mechanism for the increase in sensitivity [of the retina] during dark adaptation?***

Resynthesis of 'unchanged' light-trapping pigment (unbleached photopigment).

e ***Consider the changes in the capabilities of the visual system when illumination is low (loss of colour vision and impaired visual acuity). What do they suggest about the properties of the visual system?***

The visual system appears to operate in two ways. Under bright illumination colour vision with high visual acuity is possible: under low level illumination both are lost. Two modes of function suggest two sets of receptors with different properties.

f ***What does this suggest about the sensitivity of the different parts of the eye?***

Under bright illumination the centre of the visual field is most sensitive and possesses high acuity. Under very dim illumination, maximum sensitivity shifts to the periphery.

g ***Collect together all the information about the properties of vision given in this Study item. What conclusions can be drawn about the properties of visual receptors in humans?***

There are two types of receptor. One type (cones) receives information from the centre of the visual field, has high acuity, and allows colour vision, but only works under high levels of illumination. The second type (rods) receives information from the periphery of the visual field, has low acuity, and does not allow colour vision, but works under conditions of very poor illumination. This is the basis of the *duplicity theory of vision.*

There are three different types of cone in the normal subject. Each has a different spectral sensitivity. Analysis of the *relative* response of the three types of receptor makes colour vision possible.

There is only one kind of rod but this is extremely sensitive. Under optimal conditions, the rods may detect a single photon, the smallest possible unit of light energy. □

Practical investigation. *Practical guide 4*, investigation 12C, 'Short-term changes in responsiveness'.

STUDY ITEM
12.23 Analysis of the earthworm's giant fibre response

Principles

1 Studies of lower organisms may help us to interpret the functions of higher organisms.
2 Earthworms exhibit a rapid escape response mechanism, involving reflex activity and the rapidly transmitting giant nerve fibres.
3 This system exhibits fatigue. To analyse the cause of this, it is necessary to make a careful comparison, by experiment, of several hypotheses.
4 Fatigue is due to failure at the junctions of the sensory nerve to giant fibre and the giant fibre to motor nerve.

This investigation is concerned as much with cultivating an understanding of various aspects of testing hypotheses and experimental design as presenting a description of the working of the giant fibre reflex arc.

Questions and answers

a ***Of these explanations, which do you think is, or are, most likely to be correct, and why?***

The fact that failure is rapid suggests that it occurs at inter-neural synapses (that is, the hypotheses A and D are correct), rather than at the neuromuscular junctions or in the muscle fibres.

b ***Can you think of any additional hypothesis which might explain the observation?***

Failure *could* be due to adaptation of the tactile sensory endings in the skin, failure at synapses in the course of the sensory and/or motor neurone pathways, or failure at the septa which divide up the giant fibre. (Students will perhaps not appreciate the last point and will need extra reference on the nature of giant fibre synapses.)

c ***Is this technique suitable for testing hypothesis A?***

and **d** ***Can you think of another technique that could be used for testing hypothesis A? In what way is yours different?***

There really is no alternative. Ideally, the stimulating electrodes should be as close as possible to the nerve cord and the recording electrodes as close as possible to the segmental nerve, in order to avoid the possibility of including synapses other than those situated directly between the sensory neurones and the giant fibre. Some students may suggest stimulating an individual sensory neurone, and recording from the median giant fibre: a good suggestion, but impossible to carry out!

In fact, it is not necessary anyway – a good point for discussion.

e ***What conclusions can you draw from these results concerning the transmission of nerve impulses on the sensory side of the giant fibre reflex?***

Giant fibre impulses (action potentials) are given to the first three stimuli but to none thereafter. The conclusion is that the sensory-to-giant synapses fail, in this case after they have transmitted three impulses at this particular frequency.

f ***If hypothesis A were correct, what result would you have expected?***

Exactly what we have got, or something very like it. For example, impulses might cease to be given after fewer, or more, stimuli than in the example shown in figure (S)256.

Alternatively, questions **e** and **f** could be approached in the following way.

If hypothesis A is true, then some predictions about the outcome of experiment *1* can be made. For example:

1 If there is a failure at the synapse between the sensory neurone and the giant fibre, then stimulation of the sensory neurone will not be followed by electrical activity in the giant fibres.
2 If there is *not* a failure at the synapse, then stimulation of the sensory neurone will be followed by electrical activity in the giant fibres.

The record expected in each of these cases might be of the kind shown in *figure 38*.

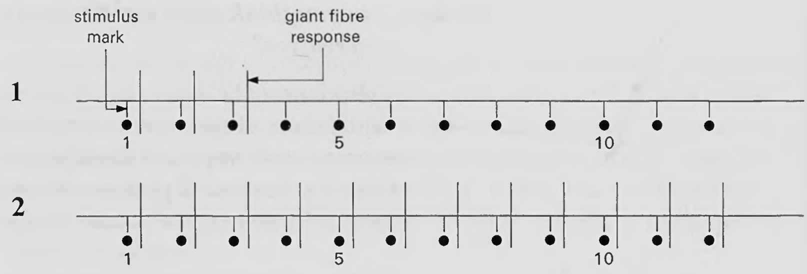

Figure 38
The giant fibre response.

Now examine figure (S)256, a drawing of an actual record obtained using the arrangement shown in figure (S)255. (Do the results show hypothesis A to be false, or can the hypothesis be accepted as correct?)

g ***Examine hypothesis A really critically. Is there any possible alternative explanation of the results obtained in experiment (1)? If so, how might hypothesis A be reworded?***

As stated in answer to questions **c** and **d**, it is possible that the failing synapse is located in the course of the sensory neurone pathway or within the giant fibre itself. This may be unlikely but it cannot be ruled out and hypothesis A might be reworded to include this possibility.

(At this stage the pupils can either continue straight on or, if it is felt they should explore the significance of experiment *1* further, they could be asked to devise experiments which could be carried out in order to eliminate the possibility mentioned above and prove beyond doubt that failure occurs at synapses between the sensory neurones and the giant fibres.

Here are three experiments which could be done:

1 Stimulate one end of the giant fibre repeatedly and record impulses from the other end. If there is no failure, this eliminates the possibility of failure occurring at junctions within the giant fibre itself.

2 Stimulate the distal (peripheral) end of the segmental nerve and record impulses from the median (central) end. If there is no failure, this eliminates the possibility of failure occurring in the course of the sensory neurone pathway.

3 Stimulate the segmental nerve as close as possible to the nerve cord and record from the nerve cord as close as possible to the segmental nerve, so as to include *only* the sensory-to-giant synapses.

A further question could also be put to students, namely:

Now that we have located a site in the giant fibre reflex where failure in transmission occurs, is it necessary to test the remaining three hypotheses, and if so, why?

A suitable answer would be:

What we have done so far is to show that failure does indeed occur on the sensory side of the giant fibre reflex, but we have not ruled out the possibility that it *also* occurs on the motor side. Hypotheses B, C, and D must therefore be tested by experiments, even though we have found that A is correct.)

h ***In the results shown in* figure [S]258 *how many shocks are sent into the nerve cord before the muscle fails to give any further contractions?***

Five.

i ***Do you consider that these results provide sufficient evidence that failure occurs* somewhere *on the motor side of the giant fibre reflex?***

Yes.

(At this stage two additional questions might be asked:

1 Does this experiment confirm any of the individual hypotheses B, C, or D, or does it fail to distinguish between these three possibilities?

The answer is that it fails to distinguish between hypotheses B, C, and D.

2 If your answer to question 1 is that the experiment fails to distinguish between the three hypotheses, then what was the point of doing this experiment? Think about this carefully before going on to the next stage in the argument.

The answer would be that this is merely a preliminary experiment to find out if failure occurs *somewhere* on the motor side of the reflex. The results show that it does, but provide no information about the exact site where failure might be occurring. So we know we must go on to test the individual hypotheses B, C, and D. If, however, this experiment showed that failure does *not* occur on the motor side of the reflex, then we would not need to go on.)

j ***What conclusions do you draw from the results shown in* figure [S]259*?***

At the frequency used in this experiment, the muscle contracts every time it is stimulated. There is no rapid failure.

k ***Is hypothesis B confirmed or refuted?***

Hypothesis B is refuted.

l ***What conclusions do you draw from the results shown in* figure [S]261*?***

The conclusions are that rapid failure does not occur at the neuromuscular junctions, *nor* at synapses in the course of the motor neurone pathways. The latter possibility is eliminated because the stimulating electrodes are placed at the extreme central end of the segmental nerve, so that impulses have to traverse the full length of the motor neurone pathways before they reach the muscle.

m ***Assuming hypothesis C was correct, what record would you have expected the oscilloscope to give?***

The disappearance of muscular response on repetitive stimuli.

(The results show that hypothesis C is disproved. An additional question to be introduced here might be:

Can we now abandon hypothesis C as incorrect with absolute certainty? If not, what further hypothesis would have to be put forward, and how might it be tested?

Yes; hypothesis C can now be abandoned. The only slight reservation is that, although the neuromuscular junction itself does not fail rapidly, it is conceivable that failure occurs in the 'coupling' between the electrical response of the muscle and the actual mechanical contraction. It is unlikely but possible. To test it, stimulate the segmental nerve repeatedly and either record or watch the muscle fibres contracting.)

n *The experiment from which the results shown in* figure [S]262 *were obtained was set up to test the validity of hypothesis D. Explain, with the help of a diagram, the technique you consider suitable for this experiment.*

To test hypothesis D it is necessary to stimulate and record on either side of the synapses which lie between the giant fibre and the motor neurones. The simplest way of doing this is to stimulate the giant fibre through a pair of stimulating electrodes placed in contact with the nerve cord, and record the electrical responses of the muscle through a pair of recording electrodes placed in contact with the surface of the muscle. A second pair of electrodes placed in contact with the other end of the nerve cord will give a record of giant fibre impulses elicited whenever the nerve cord is stimulated. The set-up is shown in *figure 39*.

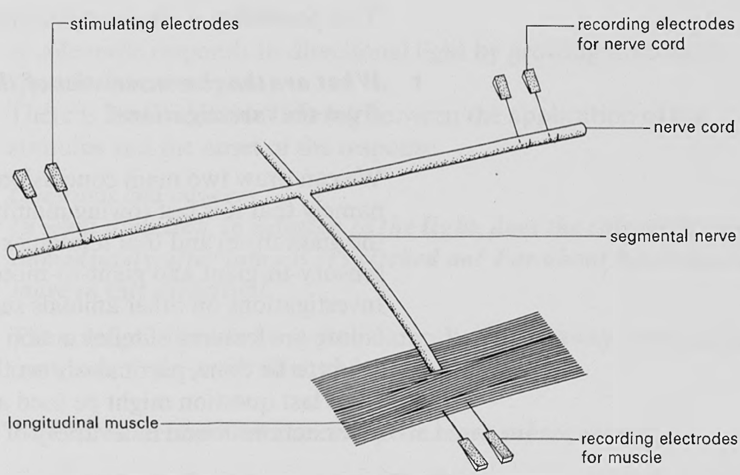

Figure 39
An arrangement for an experiment to test the validity of hypothesis D.

An alternative (more difficult) experiment would be to stimulate the giant fibre and record impulses from the motor neurones in the segmental nerve. But this is not necessary; it is satisfactory to record the muscle potentials, for we have already shown that failure does not occur at the neuromuscular junctions or at synapses in the motor neurone pathways.

o *What conclusions can you draw from these results concerning the transmission of impulses on the motor side of the giant fibre reflex?*

The giant-to-motor junctions fail rapidly on repetition. They transmit regularly to begin with, then irregularly, and finally they stop.

p *Assuming hypothesis D to be correct, what tracing would you expect to find?*

The results confirm hypothesis D. The tracings are those that we would expect.

q **Can the results be explained in any way other than by hypothesis D?**

No. The possibility that failure occurs *within* the giant fibre is, in this case, eliminated by having a second pair of recording electrodes recording giant fibre impulses from the other end of the nerve cord.

r **Does experiment (5) eliminate the possibility that failure occurs, not at synapses between the giant fibre and motor nerve fibres, but at synapses between successive motor nerve fibres within the segmental nerves?**

No.

s **If the answer to question r is 'no', then how would you eliminate this possibility experimentally? Does one of the other experiments eliminate it and if so, which one?**

This possibility is eliminated by experiment *4*.

t **What are the characteristics of this reflex action which can be drawn from the investigations?**

We can draw two main conclusions about *this* particular reflex, namely that it is fast (owing mainly to the high transmission speed of the giant fibre) and that it fatigues quickly (owing to failure at the sensory-to-giant and giant-to-motor junctions).
Investigations on other animals suggest that rapid action and synaptic failure are features of reflex action in general, but much more work needs to be done, particularly on the latter.
(This last question might be used as a lead into further reading on reflex actions found in a variety of species.) □

12.3 A plant's response to light

Principles

1 Plants respond to directional stimuli such as light by differential growth responses called tropisms. The response to light is called phototropism.

2 The influence of the stimuli is transmitted by a chemical agency to the area of response.

The series of classic experiments described in this section cannot readily be reproduced without special techniques and skills. However, they provide secondhand evidence about the nature of the phototropic response. The material is presented in a historical sequence. By working through the text, students can be led to formulate a hypothesis dealing with the mechanism of the phototropic response of coleoptiles. The section will also serve as a vehicle for considering aspects of experimental design. The inconclusive state of our knowledge of phototropic responses should be stressed.

Only responses to directional stimuli are discussed. Responses to non-directional stimuli, for example, seed germination, flowering, and stomatal opening and closing, are discussed in other parts of the *Study guide.*

Practical investigation. *Practical guide 4*, investigation 12D, 'The tropic responses of coleoptiles and radicles'; procedure A, 'The effect of unidirectional light on a coleoptile'.

STUDY ITEM

12.31 The response of a coleoptile to unidirectional light (*J.M.B.*)

This exercise will introduce students to the phototropic response and will enable them to speculate on the underlying mechanism. The phenomenon can also be investigated practically: see *Practical guide 4*, investigation 12D, procedure A.

Principles

1 A coleoptile responds to directional light by growing towards it (positive phototropism).

2 There is a considerable time lag between the application of the stimulus and the onset of the response.

Questions and answers

a ***In what direction, in relation to the light, does the coleoptile move immediately after lamp B is switched on? For about how long does it move in this direction?***

The coleoptile moves away from lamp B, that is, away from the light. It continues to do so for about 15 minutes.

b ***In what direction does the coleoptile move subsequently?***

Towards lamp B, that is, towards the light.

c ***Explain this response in terms of the growth of the coleoptile.***

After a time lag, growth occurs faster on the side of the coleoptile away from the light than on the side towards the light. In consequence the coleoptile bends towards the light.

d ***What does the time lag suggest about the mechanism by which the coleoptile responds to light?***

The long time lag between the application of the stimulus and the onset of the response (in this case 15 minutes) suggests that the mechanism by which the stimulus is perceived and translated into action is slow, probably involving a chemical messenger rather than nerves. ☐

Practical investigation. *Practical guide 4*, investigation 12E, 'The nature and detection of the stimulus involved in phototropism'.

Questions and answers based on figure (S)264

a ***What does the lack of response in B suggest about the site of perception of the light stimulus?***

That it is perceived by the tip – which is ineffective if covered with opaque foil.

b ***The response in treatment A suggested that the actual bending occurred in a region below that which was covered by the opaque cap in B. What would you infer from this?***

That something must pass from the tip, which receives the stimulus, to the elongating zone a few millimetres below.

c ***What do you conclude from C? Does it reinforce your answer to question b?***

If the tip is excised, the base has no means of perceiving the stimulus, nor could any signal pass from tip to base, thus reinforcing the idea in question **b**.

Question and answer based on figure (S)265

d ***What can you conclude about the nature of the 'signal' passing between the site of perception in the tip and the bending zone in the base? Could it be the equivalent of a nervous impulse, as in animals? If it is a chemical, what can you say about its solubility?***

A nervous impulse requires a nerve to conduct it; if a nerve is cut, it no longer works. Therefore A, which would sever a conducting nerve, should not work – but it does, so the signal is *not* equivalent to a nervous impulse. If it is a chemical, then it must be virtually insoluble in fat (C), but fairly readily soluble in water (gelatin and agar are effectively 'stiff' water).

Question and answer based on figure (S)266

e ***Why does this suggest that the substance diffusing from the tip is a growth promoter? What measurements of the cells in the base might confirm this?***

The side receiving the diffusate from the eccentric tip grows more than the other side – hence the coleoptile bends away. One would need to measure a few epidermal cells, from about 2 to 3 mm behind the excised tip, to see their relative sizes – and, ideally, to compare them with cells from the same region of a coleoptile grown in the dark.

STUDY ITEM

12.32 The mechanism underlying the phototropic response of coleoptiles

The experiments considered so far strongly suggest that the phototropic response is brought about by the differential distribution of a substance which is produced by the tip of the coleoptile and promotes growth further back. The experiments featured in this Study item lend further support to this idea.

Principle

1 If an impenetrable barrier is placed on one side of a coleoptile between the tip and the elongating region, the growth response to light is affected.

Questions and answers

a ***Explain these results in terms of the hypothesis outlined above.***

The growth pattern of coleoptiles in uniform light indicates that auxin passes down from the tips of the responding region only on that side which is not blocked by the mica plates. This indicates that there is little or no lateral movement of auxin during its pathway down the coleoptile. In the second group the unidirectional light affects the distribution of auxin. This results in the middle coleoptile showing no response, since the signal concentration will be low on the darkened side of the coleoptile which is the blocked side. In the last group, the response will be normal because the block is on the illuminated side.

b ***What should the next stage of the research be?***

Many possible experiments could be performed, but one series which might suggest itself to students more readily than others is to test the idea of lateral translocation by placing mica plates longitudinally in the coleoptiles. If the coleoptile tips are then placed on divided agar blocks and subjected to unilateral illumination, auxins moving downwards will accumulate in the agar. Bioassay techniques will determine the relative concentrations of auxins in each half block and hence each side of the coleoptile. These techniques are discussed in a
□ subsequent part of the chapter.

Question and answer based on figure (S)269

a ***Which of the three options does this favour?***

Option 3.

Question and answer based on figure (S)270

b ***Explain why these results show unequivocally that light causes a redistribution of growth substance. Do they give any support to the idea of destruction by light?***

In A there is less than usual (16° compared with 23°) auxin on the illuminated side coupled with more than normal (31° compared with 22°) on the darkened side, suggesting that about 7° to 8° equivalents moved from the light to the dark side. If the drop to 16° on the lighted side in A was due to photodestruction, then it should also have occurred on the illuminated side in B. But it did not; there was no significant difference between the light versus dark halves in B. The glass barrier would stop lateral movement, but not photodestruction.

12.4 A plant's response to gravity

Principles

1 Plants respond to the force of gravity by growing towards or away from it (gravitropism).

2 As with phototropism, the gravitropic response is believed to be brought about by a chemical agency.

Assumption

1 Students should be familiar with simple experiments involving the use of a clinostat, and the conclusions that can be drawn from them.

Practical investigation. *Practical guide 4*, investigation 12D, 'The tropic responses of coleoptiles and radicles'; procedure B, 'The effect of gravity on a radicle'.

STUDY ITEM

12.41 The response of a radicle to gravity (*J.M.B.*)

This exercise will introduce students to the gravitropic response and will enable them to speculate on the underlying mechanism. The phenomenon can also be investigated practically: see *Practical guide 4*, investigation 12D, procedure B.

Principles

1 A radicle responds to the force of gravity by growing towards it (positive gravitropism).

2 As with phototropism, there is a considerable time lag between the application of the stimulus and the onset of the response.

Questions and answers

a ***In what direction, in relation to the force of gravity, does the radicle move immediately after being rotated through 180°? For approximately how long does it move in this direction?***

The radicle moves away from the force of gravity. It continues to do so for about 9 minutes.

b ***In what direction does the radicle move subsequently?***

Towards the force of gravity.

c ***Explain this response in terms of the growth of the radicle.***

After a time lag, growth occurs faster on the upper side of the radicle than on the lower side. In consequence the radicle bends downwards towards the force of gravity.

d ***What does the time lag suggest about the mechanism by which the radicle responds to the force of gravity?***

The time lag between the application of the stimulus and the onset of the response suggests that the mechanism by which the stimulus is perceived and translated into action is a slow one, probably involving a chemical messenger. In this respect the mechanism may be similar to that for phototropism. ☐

Questions and answers

a ***How do you think the falling velocity of the statoliths is measured?***

Measurement of the velocity of falling statoliths is achieved by taking sections from tissue in which the orientation has been changed, for example, inverted, after varying periods of time following inversion. By measuring the average distance of numerous statoliths in many cells from a standard reference point, for example, the upper cell wall, then the average distance travelled in known time intervals can be calculated.

b ***Suggest a reason why changing the temperature alters the falling velocity of the statoliths.***

An obvious possibility is that a change in temperature affects the viscosity of the cytoplasm through which the statoliths fall. However, the velocity increased around threefold when the temperature was raised from 10 °C to 20 °C; this suggests that the change is not entirely due to the changing viscosity of the cytoplasm, since this is only slightly affected by a 10 °C rise in temperature. Also the rise from 30 °C to 40 °C would be expected to reduce viscosity even further – yet the velocity of the statoliths declined between these temperatures.

c ***Smaller organelles such as mitochondria are not competent to create this sense of gravity; nor are soluble molecules such as auxin. Why is this?***

Small organelles are ineffective presumably because their mass is insufficient. Soluble molecules such as auxin will not redistribute simply by falling, any more than the dye in a bottle of ink will settle out. Brownian movement will counteract this.

d ***It seems that the size of the gravitropic response is proportional to the amount of root cap present. Suggest an explanation.***

The greater the amount of root cap present, the larger the number of statoliths. A large number of statoliths may initiate a larger gravitropic response. However, it is possible that the root cap has the additional function of producing a chemical messenger. This possibility is explored in the subsequent part of the chapter.

STUDY ITEM

12.42 Further experiments on the role of the root cap in gravitropism

Like the classic experiments of Went and others, this is an admirable example of experimental design. The experiments test the hypothesis that the gravitropic response of the root is caused by a substance which is produced by the root cap and inhibits growth on the lower side of the root.

Questions and answers

a ***Do the results support the hypothesis that the gravitropic response is brought about by a growth inhibitor produced by the root cap?***

Yes.

b ***Explain the results of treatments G to N in terms of this hypothesis.***

When placed horizontally a normal root (G) shows a very strong response and deviates about 60° from the horizontal, growing downwards. If mica barriers are inserted on the upper surface (H), they have little effect, but if implanted on the lower side (J) they completely prevent the response (even suggesting a tiny upwards response). This is because the mica barrier blocks the growth inhibitor on the lower side. M and N confirm that the root cap is involved in producing the response and that barriers inserted behind the elongating zone are ineffective. Removal of half the root cap has a similar effect to inserting a barrier on the opposite side of the root (K and J; L and H).

c ***Why do you think treatment L produced a smaller downward angle of curvature than the control (G)?***

Only half of the root cap was present, and so only half of the normal amount of growth inhibitor was produced.

d ***What hypothesis was being tested by treatment Y?***

That the growth inhibitor moves from the top side to the bottom side of a horizontal root.

e ***What was the purpose of the metal foil?***

It was intended to prevent the inhibitor substance from moving across the root.

f ***Why do you think treatment Z produced a smaller downward angle of curvature than the control (X)?***

Injury could have accounted for the difference between X and Z, and would also have contributed towards some of the difference between X and Y. □

Recent reviews have highlighted the inadequacy of the traditional explanations for gravitropism and phototropism based solely on auxin.

While there is ample room for criticism, there is a real danger that the baby will be thrown out with the bathwater. Some of the evidence for modifying the traditional explanation of gravitropism is discussed in the *Study guide*, particularly the evidence for the involvement of growth substances other than IAA. However, the case against the role of auxin in the phototropic response of coleoptiles is negligible, and at present there is no reason to reject the early work, which has been extended and confirmed in recent years, suggesting that auxin redistribution is the main cause of the curvature of coleoptiles in response to low light dosages.

Whilst long exposures to quite bright light also cause strong, positive phototropic responses, the mechanism becomes more complicated. It is very probable that light-stimulated auxin destruction on the illuminated side complements any auxin redistribution within the apex. There is also evidence to suggest that a cell's sensitivity to light, that is, its decrease in elongation rate, is so rapid that it seems doubtful whether there has been time to destroy or redistribute hormones. In other words, there may be a direct effect of light on cells which almost instantaneously reduces their growth rate by an unknown mechanism. Supporting evidence comes more from dicotyledons than from cereals, but, if the effect exists, it may well function in both. Again this evidence comes from experiments using much brighter light than is needed to induce a good positive phototropic response in a cereal coleoptile.

Although it may be too complicated for most students at this stage, it has been known since the mid-1930s that the coleoptile response is not so simple as the text suggests. In fact, at certain rather restricted light dosages the coleoptile will show *negative* phototropism.

If auxin redistribution is responsible for this it is predicted that there should be more auxin on the illuminated side when curvature is away from the light. Experiments by Asana (1938) showed this to be the case. He collected the diffusate separately from both the illuminated and the dark halves of the illuminated coleoptiles. On average, the illuminated side did contain more activity, and this would be expected to produce greater growth and thus curvature away from the light.

Work with externally applied, radioactively-labelled IAA, to determine whether it too can be redistributed under the influence of unilateral illumination of the coleoptile, has produced variable results. The technique has two main snags. Early experimenters had to use concentrations of IAA which were far too high, physiologically, in order to be able to detect the weak radioactivity. Thus there is a likelihood that they completely swamped the natural transport system. Secondly, it is known that the apical 0.5 mm of the tip is the critically sensitive region to light and also for redistribution of auxin. If the externally applied IAA is not confined to entering this very small region of the coleoptile – but gets in lower down the tip – then it is unlikely to be redistributed. These snags were duly overcome, and good experimental support was obtained for the redistribution of radioactivity in externally applied IAA in accordance with expectations. On the other hand some other careful workers were unable to produce similar results – so the discrepancy needs explaining.

The reason for maintaining the traditional explanations is that they still illustrate a very attractive and logical scientific idea – chemical messengers for internal communication, that is, hormones. The scientific evidence is still valid within the constraints given. It should not be extrapolated to cover all other cases of phototropic response. The complications and arguments which then arise seem to be more suitable topics for academic study in further education. (See also *Study guide II*, Chapter 24.)

PART II The *Practical guide*

The investigations related to this chapter appear in *Practical guide 4, Co-ordination, response, and behaviour.*

INVESTIGATION
12A The response of *Tribolium* to humidity

ITEMS NEEDED

Culture of *Tribolium* beetles (20 beetles/group)

Calcium chloride or silica gel (anhydrous)
Cobalt chloride or cobalt thiocyanate paper (anhydrous)
Water

Chalk 1/group
Choice chamber 1/group
Elastic band 1/group
Muslin or fine mesh material
Paper, plain and graph
Petri dishes 2/group
Polythene, black, or other lightproof material
Pooter and/or small paint brush 1/group
Sponge for choice chamber (optional) 1/group
Stopclock or stopwatch with seconds indicator 1/group
Wax pencil or felt pen 1/group

(*Study guide* 12.2 'Animal responses'.)

A choice chamber is used to investigate this response.

Assumption

1 An understanding of the terms 'null hypothesis' and '5 per cent significance level'.

Principles

1 *Tribolium* beetles exhibit a non-random distribution in a humidity gradient.
2 The preference for a particular environment can be explained in terms of the variations in velocity and rate of turning of the beetles.
3 Suitable control procedures, replication, and statistical analysis are necessary if firm conclusions are to be drawn from experimental work.

Tribolium confusum or *T. castaneum* is available from major biological suppliers and is easily reared in a jam jar of wholemeal flour. Woodlice can be used instead, and other species of terrestrial arthropod could be used, if available.

The beetles, sieved from the flour before the practical investigation starts, can be distributed in Petri dishes or specimen tubes. They can be collected and transferred using a pooter or small artist's brush.

If more than one count is to be taken from a single chamber, it is essential that the counts are independent of each other. This can be achieved by:

1 Rotating the upper part of the chamber through 180°, allowing the humidity gradient to be re-established and then taking the count.
2 Leaving at least one hour between counts.

3 Disturbing each beetle with a small artist's brush.
4 Removing the beetles and adding a fresh sample.

The χ^2 test should be used for the analysis of data from procedure A. The control results can be used to work out the expected distribution of the beetles assuming that they are not influenced by humidity. If the total number of beetles in the two sets of results (that is, the control set and the experimental set) are not the same, the figures should be scaled accordingly (see *Mathematics for biologists*).

In procedure B, movements could be observed in uniform environments, that is, either humid or dry, though this will double the number of observations required. Movements could also be traced directly on to paper, if a 2 cm × 2 cm grid is drawn on the top of the chamber and a similar plan drawn on the paper.

Questions and answers (Procedure A)

a ***State the null hypothesis used in the analysis of the data.***

The null hypothesis is that humidity does not influence the distribution of the beetles and so the probability of finding any one beetle on (say) the lefthand side of the chamber is the same as in the control.

b ***What was the purpose of the first series of observations on the beetles (stages 6 and 7)?***

These act as the control. There should be no significant difference between the results obtained and a 1:1 distribution.

c ***Why were the upper parts of the chambers rotated through 180° (stages 7 and 11)?***

This ensures that the counts are independent of each other and acts as an additional control for factors such as the slope of the bench.

d ***Comment fully on the results of the analysis (stage 12).***

In a humidity gradient there should be a significant difference between the results obtained and the null hypothesis. The beetles should show a preference for the dry environment. However, the previous environment may affect the physiological state of the animals in such a way as to influence the response made in a choice chamber. For example, beetles which have been desiccated will show a preference for the higher humidity. This could be the subject of further investigation.

Questions and answers (Procedure B)

e ***Does the velocity of the beetles vary with humidity? If so, explain how this could bring about the observed orientation in a humidity gradient.***

The beetles should move faster in the more humid environment. This will increase their chances of entering a less humid area, where they will slow down and therefore tend to remain.

f *Does the rate of turning of the beetles vary with humidity? How could this bring about the orientation in a humidity gradient?*

The results are likely to be inconclusive, partly because the beetles' movement is restricted by the sides of the chamber so that turns are forced. An increased rate of turning on entering a humid environment would increase the animals' chances of re-entering a drier area.

g *What do the results suggest about the direction of the beetles' movement?*

The beetles should move in random directions.

INVESTIGATION
12B The response of *Calliphora* larvae to light

ITEMS NEEDED

Calliphora larvae, fully grown (just before pupation), 1 stock container

Adhesive tape
Black card, about 30 cm × 30 cm 1/group
Electric lamps, to give parallel beams of light, if possible, or ray boxes 2/group
Light meter 1/class
Paper, plain
Petri dishes 2/group
Retort stands and clamps 2/group
Ruler and protractor 1/group
Sheet of glass, about 30 cm × 30 cm 1/group
Wax pencil or felt pen 1/group

(*Study guide* 12.2 'Animal responses'.)

The type of response which an animal exhibits towards light is investigated.

Assumption

1 An appreciation of the possible influence of other variables on the responses of organisms.

Principles

1 *Calliphora* larvae exhibit a negative, directional response to light.
2 The larvae are capable of orientating themselves with respect to different intensities of directed light.
3 Such responses are likely to be of survival value in the wild.

The genus *Calliphora* includes the bluebottles, whose maggots are available in fishing tackle shops as 'gentles'. Other species with maggot-like larvae could be tried, for example, *Musca domestica*, the housefly and *Lucila sericata*, the greenbottle.

It is necessary to use a black-out for this investigation. However, the responses are usually so positive that it is not necessary to exclude the light completely.

Ray boxes are the most suitable source of light, though normal bench lamps will do as an alternative.

To avoid cuts it is best to bind the edges of the sheets of glass with suitable material.

Questions and answers

a ***Is the larva's response to light directional or random?***

The response should be clearly directional, in marked contrast to the previous investigation (12A).

b ***If there was directional movement, was it towards the light (photopositive) or away from the light (photonegative)?***

Away from the light (photonegative).

c *What other factors could have affected the response and how could you try to eliminate their effects?*

Heat from the lamps would be an obvious factor. This could be eliminated by using a heat filter such as sheets of glass or a rectangular trough of water placed in the light beams.

d *How can you account for the fact that different individuals may not all respond in the same way?*

The directed response to light by *Calliphora* larvae is only fully developed in the final larval stage (third instar). Larvae in earlier stages are not capable of orientating so accurately in a light beam.

e *How do the animals respond to the second light beam when the first is switched off?*

The larvae should change direction, through 90°, to move away from the source of the second beam.

f *How do the animals respond to the second beam when the first is left on?*

The larvae should change direction and move on a line somewhere between the two beams. If the lamps are of equal intensity then the path should be at about 45° to the direction of the first beam.

g *What effect does an increase in intensity of the second beam have on the orientation of the larvae?*

The larvae should move away from the source of the second beam to a greater extent. The angle made with the direction of the first beam should be between 0° and 45°, depending on the difference in the intensity of the two beams.

h *What is the importance of these responses to light in the normal life of the larvae?*

In the last larval stage the animal burrows into the ground before pupation. Thus the photonegative response would have survival value in increasing the likelihood of the larva reaching a suitable environment. It will also be an advantage at the earlier stages, since the larvae will be more likely to burrow into their food (for example, a carcass) and so avoid the dangers of desiccation and predation at the surface.

INVESTIGATION
12C Short-term changes in responsiveness

ITEMS NEEDED

Earthworm, large, healthy 1/group

Water

Dish, crystallizing, diameter 12 cm 1/group
Electric lamp (optional) 1/group
Light meter (optional) 1/group
Pipette 1/group
Seeker 1/group
Stopclock or stopwatch with seconds indicator 1/group

(*Study guide* 12.2 'Animal responses'.)

The waning of an earthworm's responsiveness to the stimulus of touch is studied.

Assumption

1 A knowledge of the reflex arc involved in the earthworm's escape response is desirable though not essential.

Part I

Principles

1 The response to a particular stimulus can wane as a consequence of the presentation of the stimulus.
2 A waning of responsiveness usually has some degree of specificity to the stimulus used and is therefore unlikely to be due to muscular fatigue.
3 A recovery of responsiveness may indicate that more than one process underlies waning.

Earthworms will need to be collected in advance and may be kept for several days in a bucket of damp soil, in a cool place. Any excessive handling will rapidly render the worms unresponsive and they must be allowed time to recover before the start of the experiment.

Questions and answers

a ***1 How does the earthworm respond to a single stimulus of touch?***
2 How does the response change with repeated stimulation?
3 How many stimuli were given before the worm became unresponsive?

1 With a single, rapid withdrawal of the part stimulated. The term 'escape response' reflects the likely survival value of the reflex.
2 The response should diminish in magnitude and eventually disappear.
3 It is likely that individual worms will show wide variations in the rate of waning, particularly when the stimulus strength is difficult to control.

b ***1 What is the effect of stimulating an unresponsive worm in a different place?***
2 What does this indicate about the possible mechanisms underlying the waning of the response?

1 The response should reappear, provided that the new point of stimulation is sufficiently far apart from the first. Neighbouring segments, however, may also be affected by the waning and may not produce a response when stimulated.
2 The recovery of responsiveness immediately after stimulation in a new place shows that waning is not due to muscular fatigue. If waning

is shown to affect neighbouring segments, then sensory adaptation can also be ruled out. Waning would therefore appear to be a property of the nervous system.

c *How does the rate of waning to the new stimulus compare with the rate of waning to the first stimulus?*

It is likely that waning will be faster and the responses of smaller magnitude. This suggests that at least part of the waning process is generalized or response-specific in contrast to the stimulus-specific aspects mentioned above.

d *1 Does recovery of the response occur with time, and if so, is this recovery (i) rapid, and (ii) complete?*
2 If recovery takes place in distinct phases, what might this indicate about the initial waning?

1 Recovery should be rapid but may not be complete.
2 Many organisms show a period of rapid recovery followed by a period of slow, or even no further, recovery. This suggests that more than one process may be responsible for waning. The term 'habituation' is often used to refer to both short-term and long-term changes in responsiveness, though W. H. Thorpe restricts its use to more permanent effects. Students should be warned that such a term embraces behavioural phenomena in which an apparent similarity may be the result of diverse underlying processes.

Part II

Principle

1 Responsiveness to one stimulus may be affected by a concurrent source of stimulation.

Questions and answers

a *Does the degree of contact between the worm and the rough surface affect the response of the worm to touch?*

The response should be greater, the greater the degree of contact. Earthworms maintain a high degree of contact, that is, positive thigmotaxis, with the walls of their burrows and this can be a powerful stimulus affecting responsiveness to other stimuli.

b *In what way does light intensity affect the earthworm's responsiveness?*

Bright light will probably increase responsiveness and decrease the rate of waning.

c ***Review your results and suggest how short-term changes in responsiveness, such as the ones you have investigated, might be to the advantage of the organism.***

Rapid waning to a stimulus that proves to be innocuous will save time and energy that would otherwise be spent making unnecessary responses. Changes due to the degree of contact and the intensity of light can be related to the most likely function of the response, that is, to draw a worm back into its burrow from the surface.

INVESTIGATION
12D The tropic responses of coleoptiles and radicles

ITEMS NEEDED

Germinating legume seeds, with radicles 1.0 to 2.0 cm long
Germinating wheat grains, with coleoptiles 0.5 to 1.0 cm long

Barrel and plunger, cut from 5 or 10 cm^3 disposable syringe · 1/group
Graph paper
Lamp 1/group
Micrometer graticule, eyepiece 1/group
Rubber bands or Blu-tack
Stopclock or stopwatch 1/group
Wire, 4 cm long 1/group

(*Study guide* 12.3 'A plant's response to light' and 12.4 'A plant's response to gravity'.)

Phototropic and gravitropic responses are investigated in coleoptiles and radicles viewed down the microscope.

Assumptions

1 The ability to cope with the inversion of image when using a microscope.
2 An understanding that rotation of the plant organ through 180° effectively reverses the direction of the stimulus.

Principles

1 Cereal coleoptiles exhibit positive phototropism; radicles exhibit positive gravitropism.
2 The delay between the reversal of the stimulus and the reversal of the response suggests that chemical processes underlie such tropisms.

Preparation of germinating grains and seeds. Wheat grains should be used (other cereals are less satisfactory). They need to be germinated in the dark, several days before they are required. Mung beans or peas provide suitable radicles, but these must be pinned to a board after soaking and placed vertically in a moist atmosphere to ensure that the radicles grow straight. Any method that fixes the plant organs firmly to the microscope stages can be used.

Students may need to be reminded that the image is inverted under the microscope and will appear to move in the opposite direction to the actual movement.

It is not necessary for students to complete both investigations. Half the class could use coleoptiles and the other half radicles, and the results could be compared afterwards. It would be profitable to use the basic technique to study other aspects of tropic responses, for example:

1 The effect of decapitation of the tip, or covering it with an opaque cap.
2 The effects of different intensities or wavelengths of light.
3 The effect of different durations of light, for example, 5 minutes compared with continuous exposure.

Questions and answers (Procedures A and B)

a ***How do the coleoptile and radicle respond to the stimuli of light and gravity respectively?***

The coleoptile will move towards the light source (positive phototropism) and the radicle will move downwards (positive gravitropism).

b ***When the plant organs are rotated through 180°, the direction of the stimulus was, in effect, reversed. Was the movement of the organ immediately reversed? If not, what was the period of delay?***

No; there is a delay, usually of 15 to 20 minutes.

c ***Did the whole plant organ bend, or was there a definite region which responded?***

There is a definite region of bending, several millimetres behind the tip, corresponding to the regions of maximum elongation.

d ***What advantages would these responses bring to a plant growing in its natural habitat?***

The coleoptile will grow towards the light, so that photosynthesis can occur when the first leaves emerge. The radicle will grow into the soil, providing anchorage and allowing absorption of water and minerals as the root system develops.

e ***Suggest a hypothesis to account for your experimental results.***

The relative slowness of the response and the delay period mentioned in question **b** suggests that these tropisms depend on the transmission of a chemical signal to the region of bending.

INVESTIGATION
12E The nature and detection of the stimulus involved in phototropism

ITEMS NEEDED

Pots containing 10 to 20 germinating wheat coleoptiles, growing in soil or sterile growing medium 4/group

Aluminium foil
Black polythene
Boxes for white light, blue light, red light, and complete darkness 4/class
Cocktail sticks
Filters: Cinemoid: No. 14 ruby and No. 20 deep primary blue
Protractor 1/group
Scissors 1/group

(*Study guide* 12.3 ‘A plant’s response to light’.)

The particular wavelengths of light which are important in a phototropic response are investigated.

Assumption

1 An understanding of the nature of white light and the relationship between wavelength and colour.

Principles

1 Phototropism is a response to blue light. This contrasts with the action spectrum for phytochrome-mediated responses, suggesting that a different mechanism is involved.

2 The tip of the coleoptile is sensitive to light, but the response takes place behind the tip. This implies the transmission of a ‘message’.

Wheat grains should be soaked in water for 24 hours and then germinated in complete darkness. This could be done in the boxes with the slits covered with black polythene or foil. They can also be grown in cottonwool in Petri dishes. The experiment should be started as soon as the coleoptiles appear.

Cocktail sticks are useful for shaping the foil caps and sleeves. The coleoptiles should only be exposed to the minimum amount of light (preferably red light) necessary to allow the caps and sleeves to be put on.

The boxes should be painted matt black on the inside, with a horizontal slit about 1 cm high cut in one side. The boxes made for A4 paper are suitable; they should measure about 22 cm × 30 cm × 24 cm.

Make sure that the coleoptile tips are aligned in the beam of light emanating from the slit. The responses can be recorded by measuring the angles of curvature of the coleoptiles and then working out the average.

Questions and answers

a **What controls did you use for this experiment?**

Coleoptiles exposed to complete darkness and to white light act as controls.

b **To which colours of light was a response shown?**

Blue light should elicit a response, but not red light.

c **Elsewhere in the course you will read about the pigment known as phytochrome (Study guide II, Chapter 25). Do these results indicate that phytochrome is involved in the phototropic response of coleoptiles? Give your reasons.**

The results should suggest that phytochrome is not involved since it absorbs red or far-red light.

d **Which part of the coleoptile is sensitive to light?**

The tip, since covering it should eliminate the response. Covering the region behind the tip should have no effect.

e **Which part of the coleoptile responds, that is, which part actually bends?**

The region of maximum bending is behind the tip.

f **Suggest hypotheses to explain your answers to questions d and e.**

A 'message' of some sort must be transmitted downwards from the tip. The relative slowness of the response rules out mechanisms involving electrical impulses and suggests that the message may be hormonal.

g **What further experiments could be done to test your hypotheses?**

1 Remove the coleoptile tips and replace them after inserting water and fat soluble barriers. Compare the responses with the controls.
2 Extract the chemical and test its effect on decapitated coleoptiles. Usually this is done by placing tips on blocks of agar.

h *Why do you think that matt black surfaces are needed inside the box rather than white or silvered surfaces?*

To prevent reflection and scattering of the light, which should come from one direction only. Any light is absorbed by the matt black surface.

i *Explain why red light can be used in a darkened room before the experiment.*

The results of the experiment should show that red light is ineffective in producing a phototropic response.

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CHAPTER 13 BEHAVIOUR

A review of the chapter's aims and contents

1 In the introduction, the question 'why do animals do what they do?' is posed. Four different answers are given, in terms of *causation*, *ontogeny* (development), *function* (survival value), and *phylogeny*. This is followed by discussions of each of these four aspects of behavioural investigation. The examples have been chosen with a view to illustrating something of the history of ethology, and to providing a source of ideas for possible independent experiments or project work.

2 The concepts and methods peculiar to ethology (the study of animal behaviour) are set out. Ethology is not simply an exercise in telling nice stories about natural history but can be a good vehicle for teaching scientific method.

3 Behaviour does not occur in isolation, but must be understood in relation to the external environment on the one hand, and to physiological processes on the other.

PART I The *Study guide*

13.1 Introduction

Assumption

1 A basic understanding of the scientific method.

Principles

1 The distinction between causal and functional explanations in biology. A confusion of the different types of questions and answers can lead to great difficulties.

2 The concept of repeatability. This is fundamental to scientific experimentation and presents special problems in the study of behaviour. It is easy to demonstrate how difficult it is to achieve consistency between observers, by using natural history films, or some aspect of human behaviour such as a play, school assembly, or bizarre behaviour on the part of a teacher.

13.2 Studying the causation of behaviour

Assumptions

1 Some understanding of the nature of receptors, sense organs, and reflex arcs.

2 The ability to treat data statistically, including use of the chi-squared test. (See *Mathematics for biologists*.)

Principles

1 Behaviour is involved in many aspects of homeostasis.

2 Internal changes can give rise to variations in response to stimuli. These are described as motivational changes.

Practical investigation. *Practical guide 4*, investigation 13A, 'Turning behaviour in woodlice'.

Practical investigation. *Practical guide 4*, investigation 13B, 'The reproductive behaviour of the three-spined stickleback'.

Questions and answers

a ***What hypotheses might be advanced to explain the behaviour of the male towards***

1 another male, and

2 a gravid female (one full of eggs)?

Although the obvious answers are that

1 having a red belly elicits attack, and

2 having a non-red, swollen belly elicits courtship

it is worth considering other possibilities. For example, these answers assume that the stimuli are visual, but it would be reasonable to suggest that other sensory modalities, including tactile and chemical stimuli, might be involved.

Students might well propose answers of a functional nature, such as: 'courtship is important for successful reproduction' or 'territoriality is important in spacing out males so that they each have enough food to rear their brood'. These explanations are not appropriate here, but are valuable in that they serve to emphasize the dangers of confusing causal and functional answers.

b ***How would you set about testing your hypotheses, and what controls would you have to introduce?***

The following possibilities should be considered:

1 Colour. Many possible models of various shapes and colours could be used. Controls should be included for the influence of the *wavelength* of light as opposed to the *intensity* of light, by using black, white, and various grey models.

2 Shape. Obviously the volume of the models should be kept constant to separate the effects of shape and absolute size. This could be tested in a variety of ways; perhaps Archimedes' principle is the simplest.

3 Chemical cues. The results of the model experiments suggest that these cannot be important. However, live sticklebacks could be presented to one another, with one isolated in a glass container, so that visual cues but not chemical ones would be transmitted.

STUDY ITEM
13.21 Newt courtship

Questions and answers

a *Suggest two different ways of testing this hypothesis.*

As suggested in the text, the obvious way is to prevent the tail-touch being given. Another would be to give the tail-touch at the appropriate point in the courtship by means of a rod, after one has first moved the female away. The expected results would be that spermatophores *would* be deposited if a tail-touch is given, regardless of the presence or absence of the female, but *not* when the touch is withheld.

b ***What assumption is the experiment making in using***
1 an anaesthetized female, and
2 a harness as illustrated in* figure [S]285*?
Are these assumptions justified in your opinion? How could you demonstrate whether or not the experimental technique is itself likely to influence the outcome of the tests?

The principle behind this question is an important one: the Principle of Interference. Whenever one observes or manipulates, one interferes, and this is a fundamental problem of scientific method. However, one of the special features of ethology is that animals should be interfered with as little as possible.

1 It is being assumed that the anaesthetic has no effect on the male's behaviour. It could be that the anaesthetic (MS 222) adheres to the female's skin and has an inhibitory effect on the male.

2 It is being assumed that the behaviour of a harnessed female, controlled by the experimenter, is identical to that of a free female. Whether this is true depends on the skill of the experimenter.

One probably could not easily demonstrate whether or not the experimental technique had influenced the outcome of the tests. This judgment must finally be left to the skill and experience of the scientist concerned.

c ***What statistical test would be appropriate to determine the probability that these results could have occurred by chance? Calculate the p value (see* Mathematics for biologists*).***

The chi-squared test.

The null hypothesis here is that spermatophore deposition is not influenced by whether or not the female touches the male's tail. This is tested by comparing the behaviour of the two groups of males (those that do and those that do not receive a tail-touch), using a χ^2 test on the 2×2 contingency table (table (S)48). This involves working out the expected values for each result; these are enclosed in brackets in the following table.

$$\text{The expected value (E)} = \frac{\text{row total} \times \text{column total}}{\text{total number of observations}}$$

$$\text{Thus, for the top-left result, E} = \frac{32 \times 35}{67} = 16.72$$

	Spermatophore deposited	*Spermatophore not deposited*	*Totals*
Tail-touch given	31 (16.72)	1 (15.28)	32
Tail-touch withheld	4 (18.28)	31 (16.72)	35
Totals	35	32	67

The formula for χ^2 is: $\chi^2 = \sum \frac{(0 - E)^2}{E}$

Thus, taking the values from the table:

$$\chi^2 = \frac{(31 - 16.72)^2}{16.72} + \frac{(1 - 15.28)^2}{15.28} + \frac{(4 - 18.28)^2}{18.28} + \frac{(31 - 16.72)^2}{16.72}$$
$$= 12.196 + 13.345 + 11.155 + 12.196$$
$$= 48.892$$

For a 2×2 table, degrees of freedom = 1. From a χ^2 table, we find that the probability of obtaining a value of 48.90, when df = 1, is less than 0.001. Therefore, the null hypothesis is rejected.

d ***Do these data support the hypothesis that the female's tail-touch is the stimulus that releases the deposition of a spermatophore?***

The test does indeed show that the results are very unlikely to be due to chance, and therefore they support the hypothesis that the female's tail-touch is the stimulus that releases spermatophore deposition.

e ***What explanation can you think of for these observations?***

Motivational changes within the male, as well as external stimuli, play a part in causing spermatophore deposition. The observations described suggest that a male's sperm supply may be the basis of these motivational changes. Each time a male deposits a spermatophore, he reduces the supply of sperm immediately available to him. It appears that, early in an encounter, when he has a lot of sperm, a male 'responds' without receiving a stimulus, but that later, when he has little sperm left, he fails to respond even when he receives a stimulus. This is a motivational effect. Males with a lot of sperm are strongly motivated to mate, and require relatively little stimulation from the female. Those with little sperm are weakly motivated and require much more stimulation. In fact, males often require two, three, or even four tail-touches before they will deposit their last spermatophore. ☐

Practical investigation. *Practical guide 4*, investigation 13C, 'Sex pheromones in the Mediterranean flour moth'.

Learned responses to stimuli

Practical investigation. *Practical guide 4*, investigation 13D, 'Associative learning in animals'.

STUDY ITEM
13.22 Classical conditioning

Principles

1 Conditioning is a simple form of learning involving associations of stimuli and responses.

2 Classical conditioning involves the substitution of one stimulus (an unconditioned stimulus) with another (a conditioned stimulus) to produce a similar response (initially an unconditioned response which becomes a conditioned response when linked to the conditioned stimulus).

3 Certain aspects of human behaviour are explicable in terms of classical conditioning.

This and the next investigation establish important links between biology and psychology. Much valuable discussion could emerge and students can be encouraged to read beyond the strict confines of biology *per se*. The way in which the experiments described help us to understand human behaviour should be emphasized; questions **i** and **j** give an opportunity for discussion.

Questions and answers

a ***We also salivate under certain conditions merely at the sight of food. From your own experience of this, when would you expect a dog* not *to salivate at the sight of food?***

When it was not hungry.

b ***What other stimuli will cause us to salivate?***

The smell or thought of food, especially if we are hungry. (Incidentally, these answers illustrate the way in which an organism's physiological state influences behaviour. Students might be asked if they know of any other circumstances in which behaviour is modified by physiological factors.)

c ***Food in the mouth causes gastric secretion. In what ways might the information 'food in the mouth' be conveyed to the stomach?***

Probably nervous co-ordination, possibly hormonal. (Students might point out that the sight or smell of food also causes gastric secretion. This is, of course, a conditioned response.)

d ***How could you prove that salivation at the sight of food is a learned response?***

Never let the animal *see* the food before it is presented. There remains the problem of smell, sounds made by the experimenter, *etc.*, which could easily become associated with the food. To exclude these is difficult.

e ***What can be concluded from these observations?***

The more often the sound of the tuning fork was presented with the meat powder, the more the dog salivated to the sound alone.

f ***What conclusions can you draw from the above observations?***

A neutral stimulus (vanillin) is not associated with the noxious stimulus when presented shortly afterwards. However, a neutral stimulus (amyl ethanoate) is very rapidly associated with a noxious stimulus when presented shortly before it. This assumes that vanillin and amyl ethanoate are equally neutral. The data do not provide evidence for this.

g ***What do you conclude from these results?***

Stimulation in a new place has no effect, so the conditioned response is the same. This is known as stimulus generalization. There appears to be no diminution of the response, but more refined studies have shown that there is a gradient of stimulus generalization, that is, the strength of the conditioned response decreases as the test stimulus becomes more distant from the original conditioned stimulus.

h ***What additional pieces of information would help you to interpret the above data?***

Where the new part of the skin stimulated was located. How this affected the secretion of saliva and the motor defence reflex. (See question **g**.)

i ***Can you list any examples of possible conditioned reflexes that you have observed in yourself or your companions?***

and **j** ***How far do you think the habits of humans can be explained effectively in terms of conditioning?***

These questions are intended for general discussion but the following observations may help.

1 Phobias are often the result of conditioning processes in childhood. Neutral stimuli become associated with unpleasant stimuli. Henceforth the neutral stimuli elicit a conditioned response.

2 Stimulus generalization is the basis of phobias and other reactions similar to the original stimuli. For instance do daughters choose as husbands men who are similar to their fathers? Does it depend on a daughter's relationship with her father? How much influence have other stimuli such as anxiety-avoidance responses (for example, incest)?

3 Harsh punishment by parents may result in a conditioned fear of them by their children and, through stimulus generalization, of similar people. Further, it is suggested that the hostility aroused in the children when they cannot overcome the fear becomes *displaced* and directed towards other targets, resulting in vandalism, prejudice against groups dissimilar to their parents, for example, of different colour or religion. Displacement activity is thus a result of conflicting responses, one of which is usually conditioned, for example, fear of parents.

4 Is the following a plausible hypothesis? A child does something and is punished, and the act is verbally called 'bad'. The response is an unpleasant feeling because of the punishment. In time the word 'bad' alone, *without* punishment, produces the now conditioned response of feeling unpleasant. Later the child is told 'stealing is bad' and the concept of 'stealing' assumes some of the unpleasant feelings associated with 'bad'.

☐ **5** Advertisements often associate a product with sexual success.

STUDY ITEM
13.23 Operant conditioning

Principles

1 Some behaviour patterns of animals can be firmly established by a process called positive reinforcement. Negative reinforcement may eliminate the behaviour pattern.

2 Reinforcement plays a role in human learning.

Some students will be able to talk from personal experience of the application of operant conditioning in programmed learning situations. In wider terms, a follow-up discussion might centre round a review of the basic ideas of the various 'schools' of psychology.

Questions and answers

a ***Parents sometimes try to stop a baby crying by giving it a sweet. In terms of operant conditioning, what effect is this likely to have?***

It will reinforce the behaviour to which the response was the gift of a sweet. That is, the baby will cry to obtain another sweet.

b ***Which type of reinforcement would provide the more efficient learning?***

and c ***What reasons can you give for your choice?***

From figure (S)289 you can see that the number of responses made by those animals subjected to complete reinforcement rapidly fell off (extinction). Those animals subjected to partial reinforcement were much more persistent and responses continued over a longer post-reinforcement period. Partial reinforcement is much more efficient in promoting long-term learning.

d ***How could you apply this theory if you were required to train young children to tidy up their playthings before bedtime?***

It should be noted that with complete reinforcement initial training is much more rapid. With partial reinforcement, training is more prolonged but its effects last longer if reinforcement ceases. (Which would be the best method to use with a young child and what would be the implications?)

e ***Why is such an achievement rewarding?***

In human learning situations 'correctness' is rewarding. It affords individual success and, here, satisfaction. It also receives social approval. □

13.3 Studying the development of behaviour

The nature–nurture issue

Question and answer

a ***In what ways do you think the instinct–learning issue is relevant to human behaviour?***

Clearly, this question could stimulate very wide-ranging discussion. One interesting area to consider is the way in which scientific ideas have been used, or misused, to support various political views. For example, do people of different racial groups have innately different I.Q.s and, if so, does this justify discriminatory social practices? Another popular issue is: what is the basis of the differences between the performance of boys and girls? It is important to point out that the methods of scientists and others are different in many respects, and it may not be appropriate to judge the conclusions of research in one field by the criteria of another.

STUDY ITEM

13.31 The pecking response of herring gull chicks

This example is chosen largely for its historical importance, and for the way in which the conclusions of the original workers have been modified by subsequent research. The main objection to the original study is that it envisaged the Innate Releasing Mechanism (IRM) as a complex, genetically encoded picture of the environment which was somehow present and complete in an animal's brain at birth. Hailman's work shows that such a concept is unnecessarily complex, and that it is learning that is responsible for the development of complex configurational pictures of the environment, in this case of the parent's head.

Questions and answers

a ***Why was it important to use 'inexperienced' chicks?***

To ensure that only innate behaviour is studied. Inexperienced chicks would be less likely to have established behavioural responses. Unfed chicks are more likely to be highly motivated.

b ***Suggest reasons for the drop in responsiveness during repeated stimulation. (The term 'negative conditioning' was used for this process by Tinbergen and Perdeck. An alternative term for it is 'habituation'.)***

The chick responds initially but no food results. The response therefore wanes.

c ***Why was it not necessary to keep to the 30 second period in this case?***

In this case two models (or one model with two parts) were in competition and were presented simultaneously. A comparison can be made in terms of the number of responses elicited regardless of time, since this will be the same for both.

d ***Why was a naturally coloured bill used as the standard model?***

To act as a standard against which to compare those with a spot. It will also act as a control.

e ***To what extent do the results support or contradict the hypothesis on which the experiment was set up?***

The results do not completely support or contradict the hypothesis. The greater response obtained for black suggests that red was not acting as a colour but merely as a contrast in intensity with the yellow bill. Black may have got more responses because it contrasted more strongly.

f ***How could this have affected the results?***

If the red bill is presented, the chick pecks at it, but no reward follows. The stimulus 'bill with red spot' may lose its effectiveness in eliciting a response from the chick. This can be regarded as an example of negative conditioning, or we can say that the chick has become habituated to a stimulus that does not lead to the production of food, and it therefore no longer responds.

g ***On what grounds were the investigators justified in setting up a new experiment on this subject?***

A new hypothesis has been suggested in answer to question **e**, explicable in terms of the answer to question **f**. This must be tested.

h *Is it reasonable now to conclude that the red patch is the stimulus that releases the pecking response?*

Yes, but colour spots other than red are effective in releasing the response. It does seem that the contrast of colours between those of the spot and the bill is also a factor.
(Students should realize that the next experiment described tests this hypothesis.)

i *What was the hypothesis on which this experiment was based?*

A contrast of colours play a part in the pecking release mechanism.
(What results could be expected if the hypothesis is valid?)

j *What now appear to be the features that act as stimuli releasing the pecking response? Can these be termed 'dominant' or 'exclusive' influences?*

Both contrast and colour seem to act as releasers. Strictly speaking, the colour patch could not be called a 'dominant' or 'exclusive' stimulus releasing the chick's begging response until other possible stimuli associated with the parent (for example, the shape and the background colour of the bill) had been investigated.
(Tinbergen and Perdeck investigated the general background colour of the bill and found that although yellow, the natural colour, received no more responses than, say, blue or green, red bills elicited twice as many responses as any other colour. This they took to be strong confirmatory evidence that the red spot acts through its colour as such as well as a contrasting feature. Students might be asked to suggest an experiment to follow up this point: compare a red bill with the standard dummy – what results would be expected?)

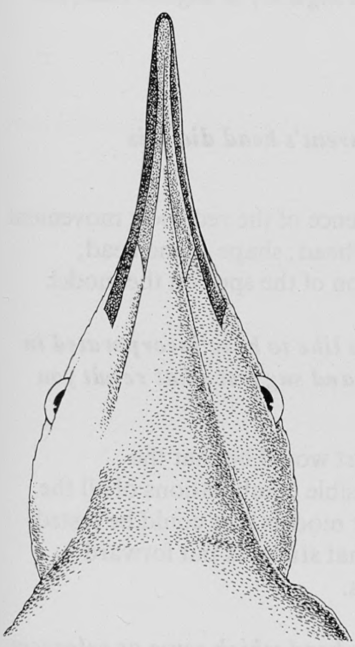

Figure 40
A parent gull's bill, as seen by the chick at first feeding.

k *What explanation might be given for this unexpected result?*

At the very first feedings the chick will view the adult's bill from underneath, and thus see its very narrow ventral side (*figure 40*). Hence the high response to it.
(Even though the thinness of the beak is of consequence only in the first feedings, it is interesting to note that the chick's high sensitivity to a long thin bill remains long after its function has been fulfilled.)

l *Describe methods which could have been used to obtain these results.*

Examples are:
1 Successive tests with different stimuli for 30 second periods.
2 Choice tests, presenting two or more stimuli together.
3 A combination of (**1**) and (**2**).
Using this framework, bills could be presented at varying angles, 'degrees of nearness', and so on.

m ***What other features of a gull's head may have been investigated with negative results?***

Three-dimensional features.
(When Tinbergen and Perdeck tested this they found that the parent's head does not provide any stimuli of a three-dimensional character – with the possible exception of 'nearness' – that flat dummies cannot provide.)

n ***What is meant by this phrase?***

The authors are not, in fact, contradicting their findings. This is merely a statement to the effect that the shape of the bill did not evolve specifically as a releaser stimulus, as seems probable with the red spot. □

STUDY ITEM
13.32 A critical evaluation of the herring gull experiments

Questions and answers

a ***What do you suppose is meant by 'calibrating the standard model'?***

Models 1, 2, and 3 establish a baseline or control, against which the effectiveness of the other models in eliciting pecking can be measured. Clearly, if there were significant differences between these models the usefulness of models at all in this type of experiment would be in doubt, especially if the standard model was very different from the real head.

b ***Which models elicited a pecking rate as high as, or higher than, the natural head?***

Models 2, 3, 5, 6, 10, 11, 12, 13, and 14.

c ***How many different attributes of the parent's head did this experiment investigate?***

Seven: shape of the bill; presence or absence of the red spot; movement or lack of movement; orientation of the head; shape of the head; colour of the head, bill, and spot; position of the spot on the model.

d ***What further models, if any, would you like to have incorporated in the experiment? Explain your answer, and suggest what result you would expect.***

Of course, the general criticism of a purist would be that the experiment does not incorporate all possible combinations of all the variables. There are innumerable further models that could be tested, but the important thing to establish is that students put forward suggestions based on specific hypotheses.

e ***Summarize the features of the parent's head which serve as releasers for the chick's pecking behaviour. Compare these with Tinbergen's earlier conclusions (page [S]410).***

1 Long thin beak, vertically orientated.
2 Red spot, strongly contrasting with background colour.
3 Movement.

There is, then, broad agreement between Tinbergen and co-workers, and Hailman on this point.

f ***Hailman concluded that, of the models in* figure [S]298, *only the configuration model (15) supported the innate hypothesis. Why do you think he reached this conclusion?***

Only the response to the configuration model provided any reason to suppose that the newly hatched chick is endowed with a complex mental picture of the parent's head and bill, rather than a tendency to respond to discrete stimuli. Recent work on the retina has shown that a moving red spot is an ideal stimulus for one class of receptors in the visual system, while horizontally moving vertical bars are specifically recognized by another group of receptors.

g ***Which model (or models) is most likely to move like a real gull's head?***

Models A and C.

h ***Which bill tip moves through the largest arc?***

Model B.

i ***Which red spot moves through the largest arc?***

Models A and B are equal.

The reason for the greater effectiveness of model B compared with A in eliciting responses, despite the arc described by the red spot being the same in both, was attributed to the additional effect of the movement of the bill (the bill was called a secondary releaser). □

Imprinting

This topic always arouses great interest, perhaps because of the appeal of the image of Lorenz, or oneself, being followed around by ducklings. There might be project work here, for example, a comparative study of hand-reared, bottle-fed lambs and their normally mothered contemporaries.

Questions and answers

a ***List familiar species which you might suppose, from what you have read, would show imprinting behaviour. Would you include humans among these? Give your reasons.***

Imprinting occurs in cows, horses, goats, sheep, guinea pigs, deer, chicks, ducklings, and goslings, but not in dogs, cats, pigs, hamsters, gerbils, rats, and mice. One would not include humans; we are not at all precocial, indeed we have an unusually long period of infant dependency.

b *Some practices in animal husbandry involve the fostering of young by a parent of another species. For example, bantam hens are often used to rear non-bantam chicks, and lambs are often hand-reared. What might the consequences of these practices be?*

Disturbances and possible abnormalities of social behaviour with other members of their own species, and possible subsequent problems with sexual behaviour later. Quite a few endangered birds are reared in captivity in incubators or by foster species. Great care has to be taken to maintain the least possible contact between the young and the humans or foster parents to ensure that, when released, the birds do not approach humans too readily or try to mate with the wrong species.

13.4 Studying the functions of behaviour

Assumption

1 A general understanding of natural selection, including the appreciation that it is the individual, as the 'carrier' of genes, that is the unit on which natural selection acts. A widespread error is the notion that natural selection favours characters that are 'good for the species', that is, group selection.

Principles

1 That determining the function of a behaviour pattern is not simply a matter of inventing plausible arguments, or 'hand-waving' explanations, but involves the formulation and testing of hypotheses, by observation or by experiment.

2 That investigating function involves finding, or experimentally creating, a variation in the expression of a character, and correlating that variation with a variation in individual fitness.

Determining function by observation

Question and answer

a ***List as many possible functions as you can think of for territorial behaviour.***

The many possibilities should include:
1 The reduction of competition between members of the same species for various resources, such as food, water, nest sites, and mates.
2 The reduction of predation, possibly by increasing the spacing out of individuals or their young, or both.
3 The provision of a familiar home range in which sources of food and water, hiding places, escape routes, *etc.* are known.
4 The reduction in the density of individuals, minimizing the spread of parasites and pathogens.

STUDY ITEM
13.41 Territorial behaviour in great tits

Questions and answers

a ***Do these data support the hypothesis that, for great tits, territorial behaviour is a defence against predation?***

Yes. Clearly, the greater the distance to the nearest neighbour, the less likely are eggs and chicks to be taken by at least one predator, the weasel. However, it should be remembered that weasels are not the only predators of great tits, and so a general statement about predation cannot be made on the basis of this information. Also, these data provide no information about numbers of chicks surviving in relation to the size of a territory. Only if those great tits with larger territories successfully rear larger broods than those with smaller ones can one demonstrate the survival value of a large territory. It is possible, for example, that birds with very large territories spend so much time defending them that they have insufficient time to feed their chicks. Clearly, predation is only one aspect of a very complex system.

b ***Can you think of any factor that might impose a limit on the size of territory a great tit would defend?***

Defending a territory requires time and energy in terms of song output and territorial disputes at the border. The length of a territory's perimeter will increase faster, as a function of radius, than the area. Thus the energy costs of defending the boundary will increase faster than the benefits afforded by the area contained within the boundary.
☐ At some point, costs and benefits will be in balance.

STUDY ITEM
13.42 Territorial behaviour in guillemots

Questions and answers

a ***How would you explain this result?***

The greater the distance between nests, the greater the losses due to predation. This occurs because predators are deterred by dense groups of guillemots, all defending their own nests, but have relatively little trouble removing eggs or chicks from more isolated nests. In many colonial species, it is an advantage to nest in the middle of a colony rather than on the periphery. This parallels effects in herding animals, like wildebeest, where predators tend to wait for a straggler to appear before mounting an attack.

Great tits and guillemots both show the influence of predation on the size of a territory, but the effect acts in opposite ways in the two species.

b ***From what you have read, compile a list of all the possible functions of territorial behaviour. Compare your list with the one you drew up in answer to question a on page [S]415.***

See the answer to question **a** on page 326. Individual students should appreciate how their own ideas have changed, and have perhaps become less simplistic. □

Determining function by experiment

STUDY ITEM

13.43 The removal of eggshells in black-headed gulls

Another classic experiment by Tinbergen.

Questions and answers

a ***List as many reasons as you can to explain why the removal of eggshells could be adaptive.***

1 The sharp edges of eggshells could injure the chicks.
2 The shell membranes and remnants of the embryonic membranes inside the shell might putrify and become a source of infection.
3 The broken eggshell might destroy the camouflage of the nest.
Tinbergen tested hypothesis **3**.

b ***How would you test whether the difference in survival rate between nests with and without eggshells could be due to chance?***

The null hypothesis assumes that the survival rates would be the same in the two conditions. The expected values would therefore be the overall mean value, that is: $\frac{21 + 47}{2} = 34$ eggs surviving. These can then be used in working out the value of chi-squared.
The null hypothesis here is that there is no difference between the survival rates of eggs in the two kinds of nest (those with and those without eggshells). This is tested by using a χ^2 test on a 2×2 contingency table, derived from table (S)52. The first step is to convert the 'Number of eggs laid out' column to 'Number of eggs lost'. Then expected values for each result must be worked out.

$$\text{The expected value (E)} = \frac{\text{row total} \times \text{column total}}{\text{total number of observations}}.$$

The complete table should thus be as follows.

	Number of eggs lost	*Number of eggs surviving*	*Totals*
Nests with shell	39 (26.0)	21 (34.0)	60
Nests without shell	13 (26.0)	47 (34.0)	60
Totals	52	68	120

The formula for χ^2 is: $\chi^2 = \sum \frac{(O - E)^2}{E}$

Thus, taking the values from the table:

$$\chi^2 = \frac{(39-26)^2}{26} + \frac{(21-34)^2}{34} + \frac{(13-26)^2}{26} + \frac{(47-34)^2}{34}$$

$$= 6.5 + 4.97 + 6.5 + 4.97$$

$$= 22.94$$

For a 2×2 table, degrees of freedom = 1. From a χ^2 table, we find that the probability of obtaining a value of 22.94, when df = 1, is less than 0.001. Therefore, the null hypothesis is rejected.

c ***Do these results provide support for Tinbergen s hypothesis?***

Obviously, yes.

d ***Compare these results with those in table [S]52.***

To do this the values in table (S)52 should be expressed as percentages: the number of eggs surviving in nests with shell, 35 %, and in nests without shell, 78 %. The farther the eggshells are from the nests, the less likely it is that predation will occur. Note that even when the eggshells are placed 100 cm from the nests, fewer (68 %) survive than at nests where there are no shells at all (78 %). This suggests that gulls should carry their eggshells a long way away from their nests. Students could draw a graph of the survival percentage against the distance from nest to eggshell.

e ***What further information do these results provide about the function of the removal of eggshells?***

That, to be fully effective in reducing the risk of predation, it is necessary to remove the eggshell some distance, certainly well over 1 metre. □

STUDY ITEM
13.44 Red-winged blackbirds

Questions and answers

a ***How would you distinguish between these two hypotheses experimentally?***

By changing the colour of the epaulets. See subsequent text for details.

b ***What controls do you think are necessary in an experiment of this kind?***

Two important things to control for are:

1 the influence of catching, handling, and dyeing, as described in the text, and

2 that both groups are matched for previous success in holding territories and attracting females.

c ***What statistical test would you use to see if the difference between the experimental and control males is significant?***

The chi-squared test.
The null hypothesis here is that there is no difference between the two groups of male blackbirds (those with black and those with red epaulets). The χ^2 test is applied directly to table (S)54, the first step being to work out the expected values for each result.

$$\text{The expected value (E)} = \frac{\text{row total} \times \text{column total}}{\text{total number of observations}}$$

The expected values are included in brackets in the following table.

	Experimental males with black epaulets	*Control males with red epaulets*	*Totals*
Number of males that lost territories to other males	25 (15.3)	3 (12.69)	28
Number of males that continued to hold their territories	16 (25.69)	31 (21.31)	47
Totals	41	34	75

The formula for χ^2 is: $\chi^2 = \sum \frac{(O - E)^2}{E}$

Thus, taking the values from the table:

$$\chi^2 = \frac{(25 - 15.3)^2}{15.3} + \frac{(3 - 12.69)^2}{12.69} + \frac{(16 - 25.69)^2}{25.69} + \frac{(31 - 21.31)^2}{21.31}$$

$$= 6.15 + 7.40 + 3.65 + 4.41$$

$$= 21.61$$

For a 2×2 table, degrees of freedom = 1. From a χ^2 table, we find that the probability of obtaining a value of 21.61, when df = 1, is less than 0.001. Therefore, the null hypothesis can be rejected. In other words, the difference between the experimental and the control males is highly significant.

d ***Do these results support hypothesis 1 or hypothesis 2?***

Hypothesis 2.

e ***Why did Smith dye the epaulets of the control males red when they were already red?***

It is important to keep everything in a control group the same as in an experimental group, except for the one aspect which you are testing. In this case the epaulets were dyed red because of possible effects of the chemical composition of the dye. ☐

The function of bird song

Questions and answers

a ***What other functions might bird song serve?***

The list of *possible* functions could be very extensive. Bird song, however, *has* been shown to serve the following functions:

1 Proclaiming territorial ownership.
2 Attracting mates.
3 Announcing discovery of a food source.
4 Alarm calls in response to predators.

b ***What would you regard as good evidence that the function of male song is to attract females?***

The best evidence would be a correlation between the amount of time for which males sing and the number of mates which they acquire. Another possibility is that muted males should be unable to obtain mates. This could, however, be due to the mutilation and interference involved in such an experiment.

STUDY ITEM

13.45 The song of white-throated sparrows

Questions and answers

a ***What explanation can you give for the stronger response to distant rather than nearby songs?***

There are two possible explanations, one causal and the other functional:

1 A causal explanation: the test birds have heard their neighbours' songs much more often than the distant songs and have habituated to them.

2 A functional explanation: known neighbours are less likely to be a serious threat to a territory owner than strange males, who might be attempting to usurp the territory.

b ***Does this result support the explanation you gave in answer to question a? If not, how do you now explain the two sets of results?***

This result supports the causal answer given above to question **a**, that is, birds seem to habituate to songs which they hear often.

c ***How, in terms of your explanation, can you explain the response of birds to their own songs, as shown in* figure [S]304?**

They will be very familiar with their own songs, from singing them frequently. However, hearing them from a different source will be a highly unfamiliar experience. Hence the intermediate effect.

d *Do these results support the hypothesis that birds are able to recognize one another individually on the basis of their songs?*

Not entirely. The results are consistent with such a hypothesis, but do not exclude the possibility that birds recognize general categories such as 'neighbour' and 'stranger'. □

STUDY ITEM

13.46 The song of the great tit

Questions and answers

a *What do you suppose was the rationale for the control sound area?*

To control for the possibility that *any* sound might scare off birds.

b *What do these results tell you about the function of the male great tit's song?*

That it acts as a deterrent to other males seeking to set up territories; song acts as a 'keep out' signal. This does not exclude other functions, in particular that song attracts females, as discussed earlier.

c *What does a comparison of the occupation of the three areas tell you about the respective effects of the great tit's song and the sound of a tin whistle?*

The tin whistle sound is no more effective as a 'keep out' signal than no sound at all. This establishes that it is the particular quality of the great tit's song, as opposed to any noise, that potential invaders respond to.

d *What do these results tell you about the function of a large repertoire of songs, as opposed to a single song?*

That a large repertoire is more effective than a single song as a 'keep out' signal.

e *In what way could this story be analogous to the behaviour of male great tits?*

Great tits might interpret a male with a large repertoire as being, not one, but several territorial males. Alternative hypotheses are that habituation to a large repertoire will be slower than to a single song, and that only the most powerful or most aggressive males produce many songs. □

Social behaviour

Practical investigation. ***Practical guide 4*, investigation 13E, 'Social order in hens'.**

PART II The *Practical guide*

The investigations related to this chapter appear in *Practical guide 4, Co-ordination, response, and behaviour.*

It is **most important**, whenever live animals are used, to treat them in such a way that they are not under any stress. Whenever possible, they should be returned to their natural environment at the end of the investigation.

INVESTIGATION 13A Turning behaviour in woodlice

ITEMS NEEDED

Woodlice, 40 in Petri dish 1/group

Bench lamp 1/group
Brush, fine artist's 1/group
Graph paper
Maze with 8 alley blocks 1/group
Petri dish 1/group
Stopclock or stopwatch 1/group

(*Study guide* 13.2 'Studying the causation of behaviour'.)

A causal explanation of the behaviour of woodlice in a maze is investigated.

Assumptions

1 A knowledge of the χ^2 test.
2 An understanding of the term 'median'.

Principles

1 The direction that a woodlouse turns is influenced by the direction of the previous turn.
2 The influence is such that a woodlouse is more likely to turn in the opposite direction to that of the previous turn.
3 The influence of one turn on the next is reduced if the distance between the turns is increased.

Porcellio scaber is recommended, though other species of woodlice should also be suitable. The width of the maze is designed only for average woodlice, so very large or small ones should be rejected. Woodlice that fail to move or stop completely in the maze should also be rejected.

If woodlice are in short supply fewer numbers can be used and the results pooled for stage 3.

The maze is 20 cm long (from point V to point Z) and 20 cm wide (from point A to point A′). The alleys are 8 mm wide. The maze should be made of Perspex with polystyrene blocks.

Questions and answers

a ***Do the results indicate that the direction of a forced turn influences the direction of a subsequent free turn?***

Yes.

b ***If your answer to question a is yes, is the influence in the same direction as, or the opposite direction to, that of the forced turn?***

Woodlice are more likely to turn in the opposite direction to that of the forced turn.

c *Does the direction of a free turn vary with the distance between the forced and free turns?*

Yes. As the distance between the forced and free turns increases, fewer opposite choices should be made until eventually turns should be made randomly.

d *Compare the results of stages 4 and 6. Does the influence of one turn on the next vary with the distance between the turns or with the time which elapses between the turns?*

Delaying the woodlice at point D is likely to have a similar effect to increasing the distance between the forced and free turn points. However the delaying technique is questionable since it involves introducing another stimulus, and so it is very difficult to say whether the increased randomness of the free turns is due to the woodlice 'forgetting' or being disturbed.

INVESTIGATION
13B The reproductive behaviour of the three-spined stickleback

ITEMS NEEDED

Sticklebacks, *Gasterosteus aculeatus*, male, in breeding condition 2/group
Stickleback, gravid female 1/group

Aquarium net 1/group
Aquarium tank (60 × 30 × 30 cm minimum), with gravel and water plants, including filamentous algae and string as nest-building materials 1/group
Glass sheet, 30 × 30 cm, opaque 1/group
Glass sheet, 30 × 30 cm, transparent 1/group
Mirror 1/group
Models of stickleback's shape and colour, or modelling materials

(*Study guide* 13.2 'Studying the causation of behaviour'.)

The behaviour of sticklebacks in an aquarium is observed. Other animals or filmed material can be used instead.

Assumptions

1 The ability to observe patiently and unobtrusively.
2 An appreciation of the waning of responsiveness that occurs if a stimulus is presented repeatedly.

Principles

1 Male sticklebacks establish and defend territories, within which they construct a nest.
2 The red coloration of breeding males acts as a releaser of aggressive behaviour, much of which involves the use of ritualized movements.
3 Courtship follows a sequence of events, each with its own releaser, one function of which is to reduce the likelihood of aggression.
4 Displacement activities may be observed during courtship or aggressive encounters.

It is easy to keep sticklebacks in aquaria, after catching them early in the year from ditches or ponds. Handle the fish with care, since even minor scratches allow fungi to grow on the skin. A stock tank containing freshwater at 8° to 10 °C with 8 hours of daylight (winter conditions) can be used until the fish are required. To bring them into reproductive condition, raise the temperature to 18 °C and the day-length to 16–20 hours. The males will soon develop the characteristic red coloration around their throats, at which stage they should be isolated. They should show territorial and nest-building behaviour almost immediately. Tanks should contain a 3 cm depth of sand or gravel and suitable water plants and nest-building materials; they should be placed out of direct sunlight. The fish are carnivorous and will accept *Daphnia* and other small crustacea or chopped earthworms. Gravid females will benefit from a rich diet of *Tubifex* worms.

Other fish that are easy to keep and have interesting courtship behaviour are the Siamese fighting fish, guppies, and various species of gourami. Zebra finches and *Drosophila* can provide alternative or additional work, as can films or film loops. With *Drosophila*, isolated male and female flies (virgins) should be placed together in a small specimen tube and observed. This could lead to work on sex attractants (see investigation 13C).

Questions and answers

a ***What processes are involved in building a nest? What discrimination does the male show during nest-building?***

The male sucks sand into his mouth, to form a shallow depression, spitting it out in a different place. Discrimination is shown in the selection of nest material; the fish tends to pick up small pieces of plant material or string and release them again immediately. Only material that does not rise to the surface after it is torn loose is brought back to the nest site. The male pushes the material into position with his snout and glues it together with a kidney secretion. Sand may also be added. The male then bores a tunnel through the nest, in which the eggs will be laid.

b ***In what ways do the appearance and behaviour of a male change when***
1 it sees another male, or
2 another male enters its territory, or
3 it sees its own reflection?

It will go redder, raise its spines, and swim vertically with head down (threat posture); it may charge and attempt to bite. Aggression should be shown in all three situations, indicating the importance of visual stimuli, but will be greater when the territory is entered and the nest approached. A stickleback may flee from a foreign territory into its own. The ritual nature of the 'fighting' should be noted.

c ***What feature of a male seems to be the most significant in releasing aggressive behaviour?***

The red colour, although experiments with models may be inconclusive. The concept of a sign stimulus could be introduced here.

d ***What advantages are gained by the male and the species in general as a result of the establishment and defence of territories?***

The male will avoid competition with other males. Territories ensure spatial separation of nests, reducing overcrowding and competition between the offspring. If space is limited, the strongest males will reproduce. Male sticklebacks also chase other organisms out of their territory, for example, newts.

e ***Describe the movement of a breeding male towards a receptive female. Summarize the responses of courtship in the form of a chart as shown on the left side of* figure [P]32.**

The male swims towards the female in a zig-zag dance, which is believed to have evolved from the ritualization of intention movements to attack and flee. The sequence of events in a successful courtship is as follows:

female appears
male's zig-zag dance
female turns to male
male leads to nest
female follows
male points to nest entrance
female enters nest
male quivers against rump of female
female lays eggs
male enters nest and fertilizes eggs

f ***What stimuli appear to be important in releasing the various courtship responses, for example, the zig-zag dance of the male or egg laying by the female?***

The various responses each have their own releaser, for example, the swollen shape of the female is an important stimulus for the zig-zag dance and the prodding of the female's rump by the male stimulates egg laying. These and other suggestions could be tested experimentally.

g ***Did any of the behaviour patterns of the fish take place out of context? Describe the occurrence of these apparently irrelevant or displacement activities.***

Displacement fanning (normally done to aerate the eggs in the nest), sand digging, or glueing may be seen during aggressive encounters or courtship, particularly when the male is in a situation producing conflicting tendencies, for example, to attack and flee.

INVESTIGATION
13C Sex pheromones in the Mediterranean flour moth

ITEMS NEEDED

Ephestia kühniella, female 1/group
Ephestia kühniella, male 5–10/group

Ethoxyethane (diethyl ether)

Aquarium tank 1/group
Forceps 1/group
Gauze or nylon pieces 3/group
Glass sheet, to cover 1/group
Mortar and pestle 1/group
Pipette 1/group
Pooter 1/group
Rubber bands 3/group
Scalpel 1/group
Specimen tubes 3/group
Watch-glasses 4/group

(*Study guide* 13.2 'Studying the causation of behaviour'.)

The function of pheromones as sexual attractants is investigated.

Assumption

1 An understanding of the term 'pheromone'.

Principles

1 Chemical communication can occur *between* organisms as well as *inside* them.

2 Male *Ephestia* moths respond to an airborne chemical released from the tip of the abdomen of female moths.

3 This chemical acts as an attractant and is termed a sex pheromone.

Ephestia kühniella can easily be cultured in the laboratory. The culture medium used is a mixture of wholemeal flour, yeast, and propane-1,2,3-triol (glycerol), mixed together thoroughly in the ratio 10:1:2 by mass. All glassware and the wholemeal flour should be heat sterilized at 105 °C for 2 to 3 hours. A pupation site (a roll of heat sterilized, corrugated cardboard) and a drinking fountain (a specimen tube containing moist cottonwool) should also be included. A jam jar, Kilner jar, or other glass container is suitable, and should be covered with muslin. A suitable arrangement is shown in *figure 41*.

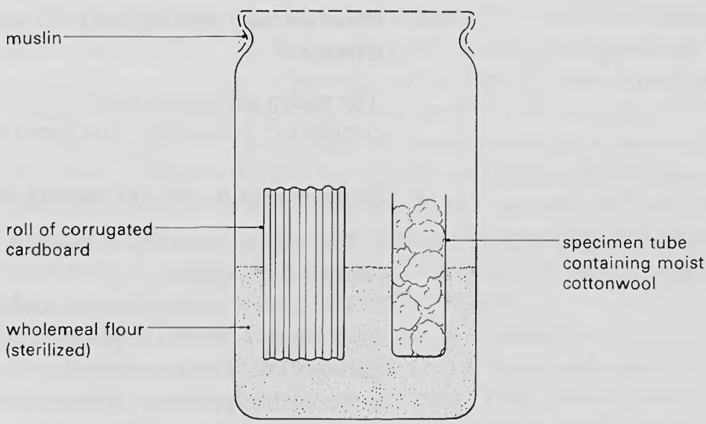

Figure 41
A culture of *Ephestia kühniella* in a jam jar.

At 25 °C the life cycle of the moths lasts 4 to 6 weeks. Sub-culturing can easily be done by transferring the roll of cardboard to a fresh jam jar. The release of a pheromone and the responsiveness of the males to it are greatest just before dawn. It is therefore essential to keep the cultures under an artificial light regime, so that 'dawn' occurs when the organisms are required for use. A light regime of 16 hours light and 8 hours darkness will also help to prevent diapause.

Sexing is best done with the larvae, several weeks before the adults are required. The testis is easily visible as a red blob on the dorsal side of the male, about two thirds of the way from the head end. Sufficient numbers of males and females should be separated and kept under the same conditions as the stock culture until the adults emerge.

The moths can be gently captured in pooters of wide bore (for example, 1 cm). If anaesthetization is necessary, carbon dioxide should be used.

Questions and answers

a ***How do the male* Ephestia *moths respond to the presence of a female?***

The males will be attracted to the tube containing the female moth and should show little response to the control. The female will be seen raising her abdomen and extending the tip as the pheromone is released. This is known as calling.

b ***To what types of stimuli could the male moths be responding?***

Visual, auditory, chemical, or any combination of these.

c ***How far do the results of stage 10 help you to decide what type of stimulus the moths are responding to?***

The males will be attracted to excised female tips. This suggests that the stimulus is a chemical one, but the possibility that the males are responding to some visual cue cannot be ruled out.

d ***What do the results of stage 13 suggest about the nature of the stimulus?***

The males will be attracted to the extract and may show courtship or copulatory behaviour. The stimulus must be a chemical one.

e ***Suggest reasons for the waning of the males' response to the extract.***

1 The extract could be exhausted, in which case a fresh drop should restore the response.

2 The males' responsiveness could decrease with time (which it will do after 'dawn') or with exposure to the chemical, that is, true waning or habituation. This could be due to sensory adaptation or some change in the neural pathways of the moths. It is worth pointing out that in the presence of a real female the males will receive additional (tactile) stimuli which will increase the chances of copulation.

f ***What further experiments should be done to investigate the mechanisms underlying this behaviour?***

The site of the male's sense organs could be investigated, for example, by carrying out experiments after the removal of the antennae. The triggering of the release of a pheromone could be investigated by comparing the effectiveness of excised tips or extracts from females that either have or have not been exposed to males.

INVESTIGATION
13D Associative learning in mammals

ITEMS NEEDED

Small mammals, ideally of the same age, kept under identical conditions, in separate cages 2/group
Water and food for the mammals
Bedding sawdust
Double choice learning box 1/group
Stopclock or stopwatch 1/group

(*Study guide* 13.2 'Studying the causation of behaviour'.)

Small mammals are used to investigate the effect of previous experience on learning.

Assumptions

1 An appreciation of what is meant by the term 'associative learning', that is, the formation through experience of an association between a stimulus and a response.
2 The ability to handle small mammals carefully, and an awareness that they may respond unpredictably to stimuli that are not obvious to the observer, especially when placed in an experimental situation.

Principles

1 The response to a stimulus can be learned, that is, the number of responses made increases with the exposure to the stimulus.
2 One type of associative learning depends on the animal's response producing a favourable environmental effect or 'reward', for example, the presence of another animal.
3 The reward reinforces the behaviour, increasing the probability of the appropriate response and decreasing that of other responses.
4 Such learning is of adaptive value. An animal's behaviour will be modified so as to bring about favourable environmental effects.

Gerbils are possibly the easiest mammals to handle and keep, though mice and rats should give equally good results. The apparatus could be modified to suit any vertebrate that is kept by the school, including aquatic ones, though learning times are likely to be longer.

The apparatus can easily be constructed in a wooden box or aquarium, using hardboard to make the partitions and the doors. Tough cardboard could be used instead, as the animals will not be in the apparatus long enough to cause damage by gnawing. The doors can be suspended by means of sticky tape, or pins driven into the edges of each door and resting on other pins bent upwards.

A simple T-maze could be used as an alternative.

The animals must be kept in isolation from each other for at least forty-eight hours before the experiment. This should ensure that the presence of a companion (of the same or opposite sex) acts as a reward.

Questions and answers

a ***Compare results for the experimental and the control animals. What do the results indicate as far as the time taken to make a choice is concerned?***

With the experimental animals there should be a relatively steady drop in the time taken to make a choice for either side, until a minimum time is reached (about 5 seconds). The control animals may show some improvement, particularly over the first few trials, but there should be a clear difference after twenty trials.

b ***What do the results indicate as far as choices for chamber B are concerned?***

The experimental animals should show an increased preference for chamber B, though occasional entries into chamber C will still occur. The control animals should show no such preference.

c ***Explain as fully as you can the differences between the experimental and control results.***

The experimental animals learn to pass through the doors and to select the one leading to chamber B. This behaviour is reinforced by the reward. This reinforcement is absent in the controls, though they may learn to pass through the doors initially, probably because the opportunity to explore new territory is reinforcing.

d ***Learning of this type is sometimes known as operant or instrumental conditioning. To which features of the experiment would you apply the following terms?***

1 Conditioned stimulus.
2 Conditioned response.
3 Reinforcing stimulus.
4 Unconditioned response.

1 The doors, or specifically the door leading to chamber B.
2 Passing through the doors or door.
3 The animal in chamber B.
4 Attempting to sniff or touch the animal in chamber B.

e ***Explain how this type of learning might be of value in the wild. Can you think of any advantage to the animal in continuing to make occasional entries into chamber C?***

This would enable animals to learn the route to be taken in order to reach a companion. Although the experiment is a highly artificial situation, small mammals will often be faced with choices when they encounter obstacles in a heterogeneous environment. Choices that are subsequently reinforced are more likely to be repeated in the future. However the occasional 'wrong' choice would be of value in an environment that is subject to change.

INVESTIGATION 13E Social order in hens

ITEMS NEEDED

Hens or other small social animals (see opposite), marked 4–5/group
Water and food for the animals

Observation pen (for size see opposite) 1/group

And/or
Film material of social order in hens

(*Study guide* 13.4 'Studying the functions of behaviour'.)

The observation of a small group of hens, at first hand, or on film.

Assumption

1 An appreciation of the terms: 'dominance'; 'submission'; 'threat'; 'appeasement'.

Principles

1 Social order is established through behavioural signals that indicate dominance or submission.

2 Social order confers certain advantages for the survival of the group.

It is recommended that practical work of some type is attempted. It may be possible to borrow a group of hens for a few weeks. They should be kept initially in separate cages out of sight of each other and then observed in a pen of 3 × 3 m. If small mammals are used, a pen of 0.5 × 0.5 m is sufficient. It is worth attempting work on social order or agonistic behaviour with any animal that lives in a group and is easy to maintain and observe in school, for example, mice, zebra finches, Japanese quail, Siamese fighting fish, or red swordtail fish. It is essential that the animals can be identified individually.

Films or film loops showing social order in hens can be used as an additional or alternative resource.

Questions and answers

a ***Which behaviour patterns are associated with the following:***
1 threat;
2 attack;
3 appeasement;
4 escape?

1 Threat: a posture with the tail erect, the head held high, feathers raised, and attempted mounting of the other animal.
2 Attack: pecking, scratching, jumping, and wing flapping.
3 Appeasement: a posture with the tail down and the head held low, that is, crouching, and freezing in position.
4 Escape: turning away from the other animal.

b ***Which patterns are shown by dominant animals and which by submissive ones?***

Dominant animals show behaviour associated with threat and attack, but submissive animals show appeasement and escape behaviour.

c ***How far is it possible to work out the social order or hierarchy of the animals?***

This will depend on the results. It may not be possible to work out a linear rank order.

d ***Is the social order maintained in an established group?***

The likely answer is yes, though the rank order may be subject to change as a result of interaction during the intervening period.

e ***Is the social order correlated with success in feeding when food is scarce?***

Some correlation may be detected, depending on the results.

f ***What do you think may be the function of an established order in a group of animals in the wild?***

Once the order is established, actual fighting is greatly reduced. Interactions become more ritualized (threat and appeasement postures), and it is worth stressing the evolution of displacement activities and intention movements into social signals that indicate dominance or submission. With needless fighting reduced, the animals will have more time for feeding, will gain in mass, and will produce more offspring.

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CHAPTER 14 THE HUMAN BRAIN AND THE MIND

A review of the chapter's aims and contents

1 One cannot begin to understand human behaviour, and the working of the mind, without a knowledge of the basic anatomy and physiology of the brain. It is therefore with this that the chapter begins.

2 Some of the methods used in elucidating brain function are explained. Often such studies draw upon clinical observations for their starting points.

3 In describing the structure and working of individual parts of the brain, one must not overlook the fact that it works as an integrated whole.

4 To study the working of the mind, psychological and behavioural studies are made from which conclusions can be drawn, though with some caution. Many areas of this elusive subject are controversial and open to alternative explanations. Students are encouraged to put forward their own ideas.

PART I The *Study guide*

14.1 The human brain

This section deals with the basic anatomy and physiology of the brain.

Principles

1 The human brain is the most complex structure of which we are aware. Even its gross anatomy is difficult to grasp and its microscopic organization and interconnections are an enormous challenge to our understanding.

2 Some early embryology of the brain has been included to help the student to understand the relative positions of the structures, especially the cerebral hemispheres.

3 A distinction between the physiological and psychological approaches to brain research is drawn.

4 The origins of the central nervous system as a simple reflex centre at the head end of invertebrates serves to emphasize the role of the brain primarily as an integrating centre responding to ever more complex stimuli and producing ever more complex responses as the evolutionary path is followed.

5 The descriptive subdivisions have been made on an anatomical basis. In some cases, for example, the cerebellum, this coincides with a clearly delineated function. Other cases, for example, the pons and the medulla, are clear anatomical entities in which are found, or through

which pass, parts of many physiological systems. The most elusive brain subdivision is the reticular formation, whose importance is now beginning to be recognized.

STUDY ITEM

14.11 The evolution of the brain

The purpose of this exercise is to encourage students to see the brain in a general evolutionary perspective and to appreciate its probable origins. An elementary knowledge of the organization of the nervous systems of invertebrates, such as annelids and arthropods, is assumed.

Questions and answers

a ***Why should the evolutionary development of the brain be associated with movement in one direction?***

Animals which move in one direction have a leading end which is equipped with sensory receptors for probing the environment ahead. Associated with these receptors are clusters of neurones or 'ganglia'. These act as reflex centres to link sensory input to appropriate motor activity.

b ***Which of the following functions of an animal's brain are associated with movement in one direction? Explain your selection.***
Thought/Vision/Balance/Co-ordination of feeding/Intelligence/Emotions/Hearing/Memory

Vision, co-ordination of feeding, and hearing. It is functionally logical that an animal's eyes, feeding structures, and ears should be at its leading end.

c ***The development of a head and centralization of nervous tissue are believed to be phylogenetically ancient features. What observation supports this idea?***

Virtually all animals have a central nervous system of some kind. The cnidarians are one of the few groups not to have a central nervous system and it is interesting that they are radially symmetrical and have no leading end. □

General structure of the human brain *and* The functions of different parts of the brain

Questions and answers

a ***It is commonly said that smells are particularly evocative of memories and the accompanying emotional overtones. Suggest an explanation for this.***

This common observation suggests that there may be some integration of the sense of smell with the new limbic functions.

b ***Why is the surface of the cerebral hemispheres convoluted?***

The folding is necessary to accommodate the large area of the cortex within a reasonably sized skull.

c ***What sort of observations would need to be carried out to establish this?***

Two types of approach are possible:
1 Patients with localized lesions of parts of the cerebral hemispheres may show specific sensory impairment.
2 Recording electrodes, connected to an oscilloscope, could be placed in contact with different areas of the brain and then stimuli applied to the eye or ear.

STUDY ITEM
14.12 The malfunction of the basal ganglia

Principle

1 An important approach to determining the functions of different parts of the brain is to correlate the degeneration of particular areas, which occurs in certain brain diseases, with behavioural symptoms.

Questions and answers

a ***What can you deduce about the cause of Parkinson's disease?***

The two changes associated with Parkinson's disease are degeneration of certain cells in the mid-brain (the substantia nigra) and low levels of dopamine in the basal ganglia. Since it is known that there are connections between these structures, the most likely explanation is that there is a dopaminergic pathway from the substantia nigra to the basal ganglia, and that the degeneration of this pathway leads to Parkinson's disease.
The cell bodies are located in the substantia nigra and show degeneration. The dopamine is actually synthesized and stored in, and liberated from, the nerve endings in the basal ganglia. Degeneration of the cell bodies leads to degeneration of the axons and a fall in the level of dopamine in the basal ganglia.

b ***Can you suggest possible mechanisms for this?***

Any of the following possibilities could cause the drug to produce the effect:
1 Toxic effects on the substantia nigra cells.
2 Interference with the synthesis of dopamine.
3 Prevention of its release.
4 Blockade of dopamine receptors (this is the actual mechanism).
5 Stimulation of the mechanism which dopamine normally inhibits.

c ***What does this tell you about the normal functioning of the basal ganglia?***

The symptoms of Parkinson's disease appear when there is a loss of dopaminergic neurones, or a depression of dopaminergic input to the basal ganglia. These symptoms can be alleviated by blocking cholinergic pathways. This suggests that the normal functioning of the basal ganglia requires an appropriate balance between the activity of dopaminergic and cholinergic neurones.
The dopaminergic neurones inhibit cholinergic neurones in the basal ganglia. Thus loss of the dopaminergic pathway leads to overactivity of the cholinergic neurones.

d ***Does the observation that a particular effect is produced by stimulating a particular area of the brain necessarily mean that this area is responsible for producing this effect in the normal functioning state?***

Such experiments cannot distinguish between the effect of stimulating (or destroying) the fibres passing through the area and the stimulation of fibres terminating there, and the stimulation of nerve cell bodies in the area. □

Question and answer

a ***If an animal's reticular formation is stimulated electrically, the animal becomes aroused. In contrast, bilateral lesions of the reticular formation result in the animal becoming less vigilant. What does this suggest about one possible function of the reticular formation?***

The reticular formation is involved in the processes which regulate the sleeping/working cycle.
This aspect of the reticular formation's function is also possibly linked to changes in sensorimotor integration, as the outcome of sensory input is very different in different states of consciousness. The reticular formation may also play a role in generating the 'body image' and self-awareness.

STUDY ITEM
14.13 The function of the cerebellum

Principle

1 Another approach to the study of brain function is to correlate lesions of particular areas, associated with head wounds, with changes in sensitivity or motor co-ordination, or both. Such studies also provide an insight into how new sensorimotor skills are mastered.

Questions and answers

a ***What simple tests of cerebellar function can you think of?***

The test must reveal the ability to make rapid, precise, and smooth movements. The ability to touch the nose with the eyes shut is an example, and another is the ability to point to a series of objects in rapid succession.

b ***What does this tell you about what the cerebellum normally does?***

The soldier described the need for him to break down movement into components and tackle each bit separately by consciously directing it. The cerebellum is normally able to put together the appropriate instructions in response to a general command such as 'sign your name' or 'change gear'.

c ***What do you have to do for the first few times when you try a new skill?***

When you try a new skill for the first time you must act rather like the soldier described above. Movements are slow and each step has to be thought out and consciously monitored.

d ***What do you think has happened when you become able to perform the movement 'automatically'?***

Repetition allows the cerebellum somehow to 'lay down' the 'programme' for carrying out the movement. The more unconsciously a movement can be carried out, the more skilful it becomes. When a movement can be performed automatically, the entire co-ordinated sequence of movements has been 'built into' the cerebellum. □

Practical investigation. *Practical guide 4*, investigation 14A, 'Sensorimotor skills in humans'.

14.2 The mind and consciousness

Principles

1 Memory, intelligence, emotions, personality, and sleep are psychological aspects of brain function.

2 Mystical and religious experience, creativity, and extra-sensory perception, although less amenable to scientific investigation than the more physiological aspects of brain function, are of interest to students and it is hoped that they may generate lively discussion.

Questions and answers

a ***What conclusions would you draw from this simple experiment?***

The experiment shows that we live in two worlds: the external world of sense perception and the internal world of the mind. The students should also notice that we can attend to different aspects of the outside world by changing our attention from sight to hearing.

b ***What function of the brain is being used when we 'hear' and 'see' thoughts with our eyes closed?***

Memory. The students may not have realized that even when our eyes are open the picture we see or the sounds we hear only have meaning because we call on the memory to make sense of the information presented to our brains. The brain continually analyses the information it receives from the sense organs, and by comparing it with previous patterns of information stored in the memory constructs for us a picture of the world in which we live.

Practical investigation. ***Practical guide 4*****, investigation 14B, 'Perception'.**

c ***What advantages does the dualist theory have over the reductionist one?***

Intuitively, the dualist model has much appeal, as it suggests that consciousness, or the mind, is not limited to a brain, and that it may exist independently of the brain after death. It also allows for an explanation of the action of the mind at a distance, for example, telepathy, or precognition. The trouble is that the dualist theory puts the mind in a special category, so that it can never be properly examined. As the mind is remote there has to be some magical way by which the brain can contact it, and this is difficult to explain.

d ***What are the advantages and limitations of the holistic theory?***

Clearly, this view is the most comprehensive, as it suggests that the mind is a fundamental property of the universe and is not limited to the brain. It would also suggest that the mind may exist in many different forms and at many different levels of complexity within the universe. Although this theory is attractive, it is unfortunately not precise enough to be tested by science, and so it remains at present a matter of belief rather than a scientific hypothesis. The idea of mind and matter being part of the same aspect of nature is contained in the Buddhist religion.

e ***Which of these theories do you personally find the most credible, and why?***

The answer will depend on all sorts of personal factors – scientific, religious, and emotional. In analysing the reasons for their choices the students might be encouraged to distinguish between scientific and non-scientific arguments.

f ***Why is it necessary to have a mind?***

One theory is that it is an organ which has been developed to make a model of the outside world. This model is being constantly updated by the brain, which can then make predictions about the world's future behaviour. This model therefore allows the organism to adapt itself to the changing circumstances of its environment.

g ***Can you think of any people who you would say are mainly biased either towards activities of the left hemisphere, or towards those of the right hemisphere? Do you know any truly balanced people?***

People biased towards the left hemisphere use mainly reasoning and verbal abilities – for example, lawyers, accountants, linguists, and

historians. People biased towards the right hemisphere use mainly visio-spatial skills – for example, artists, draughtsmen, engineers, and people who manipulate objects in space, for example, crane drivers, aircraft pilots, and racing drivers.
Obviously the answer to the third question depends on the acquaintances of the student. Students should support their allegations with reasons based on the preceding text.

STUDY ITEM
14.21 A split brain experiment

Principle

1 Conclusions about the functioning of the cerebral hemispheres can be drawn from a set of data.

Questions and answers

a ***How do the performances of the left and right hands compare?***

Both hands made an equal number of mistakes but they differed in the kinds of mistakes they made.

b ***What conclusions may be drawn from the results of this experiment?***

Two main conclusions may be drawn:
1 In pattern-forming each hemisphere deals with a particular skill.
2 Neither hemisphere on its own can complete the task correctly. For a correct performance both hemispheres must work together.

c ***If a concert pianist were to have a split brain operation, what do you think would be the consequences on his ability to play the piano?***

He would probably be able to play competently with either his left or his right hand, but would be unable to play with both hands at once. □

Questions and answers

a ***Why is 'attention' necessary?***

It focuses the mind on important features of the environment and prevents the brain from being flooded with irrelevant information.

b ***How does 'attention' increase efficiency?***

It allows concentration. Amongst animals this is particularly important in hunting prey or escaping from predators, or both, and therefore has immense survival value.

Memory and learning

Questions and answers

a ***Make a list of as many brain functions as you can think of for which memory is required.***

Our ability to think complex and abstract thoughts; our ability to use imagery; the development of language; our concept of time; and our sense of personal identity. All these (and many other human attributes) stem from our vast capacity for storing information.

> **Practical investigation. *Practical guide 4*, investigation 14C, 'Memory'.**

b ***Why are some people good at remembering words, while others are better at recalling pictures?***

Different memories (verbal and visio-spatial) are filed by different sides of the brain, and one side may be more efficient than the other.

Intelligence

> **Practical investigation. *Practical guide 4*, investigation 14D, 'Intelligence'.**

STUDY ITEM

14.22 Does intelligence change with age?

Principle

1 In investigating a human attribute as elusive as intelligence conflicting results are often obtained.

Questions and answers

a ***How do you think the data were obtained?***

The data could have been obtained in two ways:
1 By measuring the intelligence of a group of people at different times during their lives. Obviously this would be a long-term study, in this case carried out over a period of almost 60 years.
2 By measuring the intelligence of cross-sectional samples of people of different ages. This is, in fact, how the investigation was carried out.

b ***What conclusion would you draw from the data?***

That intelligence, as measured by this particular test, rises between the ages of 10 and 20 years, and then gradually declines.

c ***Do the results of this investigation support the conclusion drawn from the data in* figure [S]316*?***

No. On the contrary, the results appear to conflict.

d ***What do the results suggest about the relation between age and intelligence?***

They suggest that between the ages of 20 and 70 years intelligence, as measured by this test, rises.

e ***Suggest possible explanations for the apparent contradiction between the data in* figures [S]316 *and* [S]317.**

Possible explanations might be:
1 that the tests, being different, measured different aspects of intelligence;
2 that the data were collected in different ways, with the first involving samples of people, and the second involving individuals;
□ 3 that the individuals in figure (S)317 practised between the tests.

Questions and answers

a ***What sort of investigations would need to be carried out to find the relative contributions of heredity and environment to intelligence?***

Several approaches are possible but the most fruitful have been 'twin studies'. I.Q.s have been measured of identical twins reared together, identical twins reared apart (adopted), and non-identical twins reared together. It has been found that identical twins, even those who are brought up separately, have almost the same I.Q.s. On the other hand, non-identical twins reared together in the same environment may have markedly different I.Q.s.

b ***Of what use might I.Q. tests be to society and what are their possible dangers?***

It is clear that in general terms they are useful predictors of examination performance. However, it is arguable whether such information is of much use to society and in any case there are numerous exceptions. There have been many cases of people with relatively low I.Q.s gaining places at university and achieving good results.

c ***Why does intelligence, as we have defined it here, not necessarily correlate with success as a human being?***

It may be because our definition of intelligence is too limited, or because our ability to measure it – in all its dimensions – is inadequate. Certainly many other qualities besides those that are measured in standard I.Q. tests contribute to success, for example, perseverance, imagination, and enthusiasm.

Practical investigation. *Practical guide 4*, investigation 14E, 'Emotion'.

Questions and answers

a ***Do you consider the 'fight or flight' response to be a useful aspect of human emotion?***

It is easy to see the usefulness of this 'emergency function' in a more primitive society, but it seems to have lost much of its adaptive value in a contemporary society where neither fight nor flight are the normal or socially desirable consequences of emotional arousal.

b ***In what circumstances might the 'fight or flight' response be disadvantageous?***

Extreme arousal is counter-productive in terms of an emergency response. A certain level of anxiety, for example, may make a student feel alert and stimulated so that he or she performs well in an examination. In other words it produces efficiency, although if one is excessively anxious one's performance will deteriorate. The best performance occurs at an optimum level of arousal, and not at a maximum level where behaviour becomes disorganized and inefficient. It can be argued that in extreme arousal the mechanism may be functioning outside its normal evolutionary limits and so breaking down.

STUDY ITEM

14.23 Investigating the cognitive theory of emotion

This exercise serves as an example of the kind of enquiry which is carried out on human behaviour. It points up some of the difficulties inherent in working with human subjects and illustrates the importance of having the necessary controls.

Questions and answers

a ***How do you think the subjects' emotional states could be assessed?***

The subjects' behaviour could be watched by an observer, and afterwards the subjects could be asked to rate their own levels of emotional arousal.

b ***Which subjects belonged to the experimental group as opposed to a control group?***

Those in both the angry and euphoric conditions who had been injected with adrenaline but had not been told of its real side-effects, and so could not attribute their feelings to the injection.

c *If the cognitive theory of emotion is valid, what results would you expect?*

The subjects in both the euphoric and hostile situations would be expected to experience the same general level of emotional arousal, but the type of emotion – elation or anger – would depend on which of the two atmospheres they had been exposed to.
(In fact the results were as predicted above. The largest increase in arousal was shown by the subjects who had been injected with adrenaline but had not been told of its real side-effects; this suggests that the intensity of the emotion which they experienced was due to a general sympathetic arousal.) ☐

STUDY ITEM

14.24 (Essay)

Does it matter what emotions we allow ourselves to feel?

There are obviously many ways of tackling this question but basically the answer is yes, it does matter, because styles of thinking can greatly influence our mental and physical health. There is evidence that the repeated experience of 'negative' emotions – anger, frustration, or sadness – can damage one's health. People who habitually respond to a situation by becoming angry and frustrated are more likely to develop high blood pressure and cardiac problems. It pays to take a more relaxed view of life and not allow ourselves to be worked up by trivial problems. Continual self-criticism and the thinking of sad thoughts have a bad effect too. If we constantly think sad thoughts, this produces changes in the brain chemistry which alter the way we see the world, so that it becomes a gloomier place in which to live. This in turn increases the likelihood of our becoming even more sad and perhaps eventually developing a depressive illness. It is important to realize the value of positive thinking.

When one is asked to do an unpleasant task one can approach it in a negative way, so that feelings of resentment and anger build up, altering the body physiology and maybe even sending one's blood pressure up; or one can approach it in a positive way, so that even though the task may not be enjoyable, one does at least make sure that one's negative emotions are kept under control. ☐

Personality

Question and answer

a ***Not everyone is happy with such a simple system. What are its limitations, and what alternative can you offer?***

The most obvious limitation is that it suggests that personality consists of only two dimensions. An alternative system would show personality as consisting of many dimensions.

The Minnesota Multiphasic Personality Inventory uses scales classified under 25 headings including mental and physical health, education and occupation, and sexual, social, religious, and political attitudes. Each of these scales is determined by a set of questions which define how you behave on that scale. Like the Eysenckian personality inventory, these questions have been chosen so that they relate to one dimension only. Hidden amongst the questions are some which are designed to detect whether or not you are being truthful, for example: 'Have you ever cheated?' Because everyone has cheated at something at some time or another, a 'no' answer to such a question is an indication that the person's answers to other questions may not be 100 per cent truthful, and this will be taken into account when the score is assessed.

Sleep

Questions and answers

a **_Bearing in mind the neural and other physiological events that take place during sleep, why do you think we need sleep?_**

As yet not all the reasons why we need sleep are understood. It used to be thought that the body and brain rested during sleep, but recent studies have shown a very different picture of sleep. Slow wave sleep seems to be related to body repair; after exercise and in diseases such as thyrotoxicosis in which the metabolic rate is increased, stage 4 sleep is prolonged. REM sleep seems to be related to learning and the way the brain deals with the organization of daytime experience. REM time is highest in infants and young children and lowest in old age. Animal experiments have shown that in some cases REM deprivation can lead to faulty learning.

b **_What function or functions can you suggest for dreams?_**

There are many theories. One of them suggests that dreaming is simply the brain organizing daytime experience. Another suggests that it gives the brain an opportunity to run through and 'rehearse' important behaviour, for example, fighting, eating, and copulating, while the body is 'paralysed'; or it may focus the mind on important issues. Earlier psychoanalytical theories maintained that dreams should be thought of as symbols of our suppressed wishes and conflicts. The Russian mystic P. D. Ouspensky said that dreams were of three types: (1) the common or ordinary dream; (2) the rarer, symbolic dream which gave guidance; and (3) the very rare dream through which came great insight and creativity.

Practical investigation. ***Practical guide 4*, investigation 14F, 'Sleep'.**

Questions and answers

a ***What do you consider to be the essential pre-requisites of creativity?***

The creative side of our nature is a fundamental property of the mind. We all have it, but it needs to be recognized and trained, a training best described as abandoning our preconceptions and opening the mind to new ideas. The creative process is a strongly intuitive one, in which the mind is able to take leaps and make connections, but which is based on a previous period of work and preparation so that all the information needed to solve the problem is there.

b ***Give an example of a biological discovery which, in your opinion, involved an important component of creativity on the part of the discoverer.***

Examples might include the circulation of the blood (William Harvey), the theory of evolution (Charles Darwin), the laws of inheritance (Gregor Mendel), the microbial basis of disease (Louis Pasteur), the tricarboxylic acid cycle (Sir Hans Krebs), and the double helical structure and replication of DNA (James Watson and Francis Crick). All involved intuitive leaps of the mind. Obviously many other examples could be given.

c ***Now see if you can do the same thing with an artistic achievement such as a painting, piece of music, book, play, or film.***

The difficulty is to decide what constitutes a genuinely *creative* work. One useful test is to ask if the work leans heavily on preceding works or contains a major component of originality.

Psychic experience

Practical investigation. *Practical guide 4*, investigation 14G, 'Extra-sensory perception and psychokinesis'.

PART II **The *Practical guide***

The investigations related to this chapter appear in *Practical guide 4, Co-ordination, response, and behaviour.*

INVESTIGATION
14A Sensorimotor skills in humans

ITEMS NEEDED

Drawing pins or adhesive tape
Graph paper
Hardboard sheet, 30 cm × 30 cm 1/2
Mirror, plain, 15 cm × 15 cm 1/2
Paper, plain and graph
Pencil 1/2
Retort stand and clamp 2/2
Ruler 1/2
Star figures, 5-pointed 20/2
Stopclock or stopwatch 1/2

(*Study guide* 14.1 'The human brain'.)

An exercise involving mirror drawing and the effect of experience on the performance of a skill.

Assumption

1 A knowledge of the existence of receptors (proprioceptors) which provide information on the position and movement of the limbs.

Principles

1 Mirror drawing involves learning a new pattern of co-ordination between hand and eye.
2 Learning depends on information about previous performances, that is, experience.

The experimental set-ups are very simple. Sufficient star figures must be duplicated, as shown in *figure 42.*

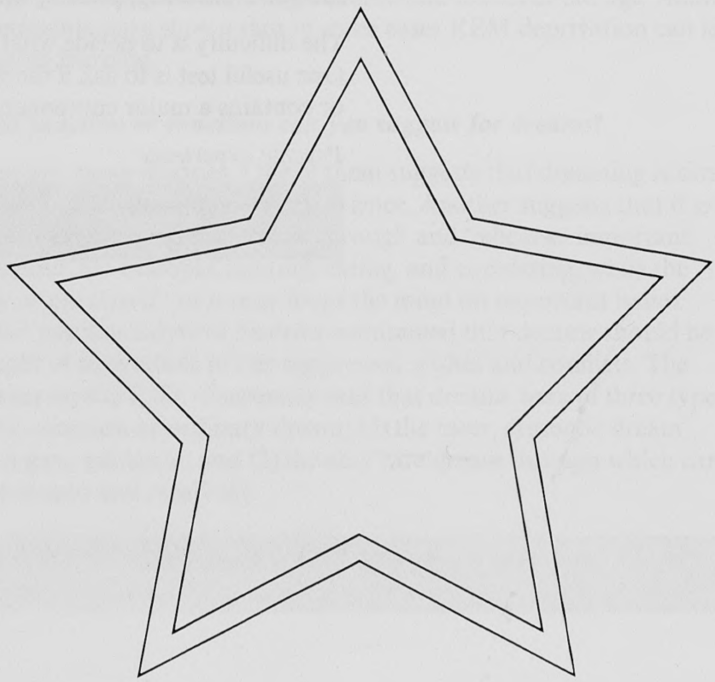

Figure 42
A five-pointed star figure suitable for use in the investigation on mirror drawing.

Questions and answers (Part 1)

a ***What do the graphs indicate about the speed at which you can learn this type of skill?***

The graphs should be in the form of a typical learning curve, showing a rapid decrease in the time taken and the number of errors made over the first few trials, until the lowest values are reached and no further improvement is made.

b ***How much variation in the rate of learning is there in the class?***

Considerable variation should be shown, both in the rate of learning and in the best performances achieved.

c ***How could you find out if the skill you have gained on this particular drawing makes it easier to produce a different mirror drawing?***

This question concerns the transfer of learning from one situation to another. To test this the subject could attempt to write a short word, for example, 'cat', while looking in a mirror. If learning is transferred completely there should be no improvement in performance and a learning curve will not be produced. In practice it will probably be found that the ability to draw around the star figure does not help in writing the word. However, a test involving drawing around a 6-pointed star figure will show a transfer of learning. This could form the basis of further project work. It is also possible to investigate the effect of varying the time interval between trials, for example, compare the rates of learning of subjects undergoing one trial per day with those undergoing all the trials in one day.

Questions and answers (Part 2)

a ***Did these experiments indicate that knowledge of the results enhanced subsequent performances?***

Yes.

b ***What other information must be available to the brain, in all these experiments, if performance is to be improved?***

Information obtained by kinaesthesis must be used; that is, knowledge of the position and movement of parts of the body. Proprioceptors in the muscles, tendons, and joints provide the brain with a position feedback from the environment, so that we can sense where our limbs are in space even with our eyes closed. The information on the effectiveness of performance combined with this kinaesthetic sense enables performance to be improved.

c ***How might performance be affected if you ran a series of 50 trials or more without a break?***

The performance will deteriorate owing to muscle fatigue or the subject's boredom.

d ***On what occasions are the results of these investigations used in everyday life?***

Riding a bicycle or driving a car involve sensorimotor skills that are learned in a similar way to the simple experimental tasks and in these cases knowledge of the results of performance is vital. Knowledge of the results may also improve intellectual tasks, such as performance in tests and examinations.

INVESTIGATION
14B Perception

ITEMS NEEDED

Millimetre rulers 1/1
Paper, plain and graph

(*Study guide* 14.2 'The mind and consciousness'.)

An examination of visual illusions

Assumption

1 An appreciation that while sense organs receive stimuli from the environment it is the brain that interprets or perceives this sensory information.

Principle

1 Visual illusions arise when the brain receives ambiguous cues that can be interpreted in different, distorted, or misleading ways.

The suggested work is simple and straightforward. Other examples of visual illusions could be included.

Questions and answers

a ***Describe how the brain is confused or misled by the illusions shown in* figure [P]40.**

1 The vase illusion. A spontaneous reversal of the object and its background occurs because the differentiation between object and background is unclear. The drawing is seen as either a vase or two faces in profile.
2 Concentric circles. The three circles are geometrically perfect; this can be checked with a compass. The apparent distortion is caused by the angles which the straight lines make with the circles, giving the perceived effect of circles with flattened sides. In a similar way, the straight lines seem to be broken where they intersect with the circles.
3 Reversible steps. The drawing is ambiguous, so that the steps appear to be going either up or down. The brain is confused and alternates between the two possible interpretations.

b ***What additional cues might resolve the ambiguities in the drawings?***

Shading the vase or drawing flowers in it, for example, would enable the brain to isolate the vase as the perceived object. Shading will not help resolve the reversible steps illusion since depth cues are already given.

c ***What do your results suggest about the effect of adding arrows to the measured lines?***

The estimated sizes of lines >———< are likely to be longer than their actual sizes. The estimated sizes of lines <———> are likely to be shorter than their actual sizes.

INVESTIGATION
14C Memory

ITEMS NEEDED

Stopclock or stopwatch 1/class
Tray of 15 cards with names written on them 1/class
Tray of 15 cards with numbers written on them 1/class
Tray of 15 objects 1/class
Tray of 15 photographs of well-known people 1/class
Tray of 15 cards with the names of the well-known people written on them 1/class
100 word story (see below)

(*Study guide* 14.2 'The mind and consciousness': Memory and learning.)

This investigation tests the recall of information by the brain.

Assumption

1 The knowledge that the two hemispheres of the brain are dissimilar; the dominant hemisphere (the left hemisphere in right-handed people) is specialized for verbal and numerical skills, and the non-dominant hemisphere (the right hemisphere in right-handed people) is specialized for visio-spatial skills.

Principles

1 Because different memories are filed by different sides of the brain, one side may be more efficient than the other. This explains why some people are better at remembering words, while others are better at recalling pictures.

2 Memories filed by one hemisphere may be supplemented by memories filed by the other hemisphere.

The trays of items will have to be prepared in advance. It is important that the 'famous faces' are known to all the students.

The 100 word story is included below, together with the method of scoring.

It was a rainy Sunday afternoon at about 3 o'clock when the diesel train from the old mine passed by the rotting tree-trunk beside the line. A red squirrel hunting for nuts and berries looked up, frightened by the noise. It ran, slithering down the bank, until its paws reached the water. Then with two leaps it crossed the sluggishly flowing stream to reach the dangling branch on the far side. Clinging to the bough, it swung itself onto the dry leaves of the bank. At last it was home, and it dashed into its burrow, its heart fluttering and tail quivering.

Total score: 50

Method of scoring

Read the story aloud once. Then the class should immediately write down as much as they can remember. What they have written must then be covered over. After more than five minutes, but less than ten, they should again be asked to rewrite the story from memory. It is important to mention that marks are given for the actual words used. The scoring of both versions is based on the number of underlined words that are reproduced. To calculate the percentage recall divide the score for the second version by that for the first version and multiply by 100.

The reason for presenting the result as a percentage of the first version is that it shows the amount of information which went into memory. From this the decline can be measured. The score for the first version is a measure of attention and intelligence as well as of memory; the percentage score is more a measure of memory alone.

Some latitude in marking is permissible. The words need not be in the right order, and close synonyms should be allowed.

Questions and answers

a ***Did you find that some people scored better on some tests compared with others? Was there a difference in the scoring between males and females?***

Some people may score better on objects and faces, and others on names and numbers. Males tend to do better on the former items, and females on the latter ones.

b ***Which tests indicate dominant hemisphere functioning and which indicate non-dominant hemisphere functioning?***

Tests on objects and faces indicate non-dominant hemisphere functioning (visio-spatial skills), and tests on names and numbers indicate dominant hemisphere functioning (verbal and numerical skills).

c ***Can you explain your answer to question a in terms of the efficiency of the two cerebral hemispheres?***

Since the two hemispheres are specialized as far as memory is concerned, if one hemisphere is more efficient than the other, then this could account for the differences mentioned in answer to question **a**. Students may be able to suggest which of their cerebral hemispheres is the more efficient in the light of their results.

d ***Account for the results of the test using the second tray of names (stage 5).***

The class is shown names of the 'famous faces' which they have already seen. There should be a higher proportion of correct recall this time, as the memory should have been supplemented by the non-dominant hemisphere's memory of the faces.

e ***Is there any evidence from stage 7 that you remembered some parts of the story better than others?***

When trying to remember a sequence of words or numbers one tends to recall the first and the last few better than those in the middle.

INVESTIGATION
14D Intelligence

(*Study guide* 14.2 'The mind and consciousness': Intelligence.)

In this investigation the skills which seem to be reliable indications of intelligence are tested.

Principles

1 Intelligence tests can be used to measure particular mental skills.
2 Creative thinking can be assessed by looking at the variety of new or different solutions to a problem.

The solutions to the intelligence tests are given below.

1 ? = 8
Each pair of numbers in opposite sectors of the octagon add up to 19.

2 ? = 0
In the rhombus the sum of the two numbers at the top equals the sum of the two numbers at the bottom; similarly the number at the top of the triangle equals the sum of the two numbers at the bottom. If the sum of the two numbers at the bottom of the pentagon equals the sum of the three numbers at the top, then ? = 0.

3 The odd one out is fourth from the left. It is the only shape that will not fit into the others.

4 The corresponding shape is second from the left. It differs from the oval in as many features as the rectangle differs from the hexagon.

Questions and answers

a ***Do the results of the intelligence tests follow any pattern? Is there a difference in the performance of the class between boys and girls?***

Boys usually tend to do better on visio-spatial (right hemisphere) skills and girls on numerical (and verbal) (left hemisphere) skills.

b ***Do you have any criticisms of the tests?***

Alternative answers could be given to at least some of them. The class might like to think of possible alternative answers. If these are scored as 'wrong' in an I.Q. test, a misleading result could be given.

c ***How does conventional thinking differ from creative thinking as shown by* figure [P]42*?***

The conventional thinking process keeps the same basic shapes and only alters the sequence. The creative thinking process reduces each shape to its three basic elements, giving a vastly increased number of possible combinations and therefore solutions (see *figure 43*). The quality of thinking is measured by the end result. The list of different uses of a brick can be assessed in a similar way; the more widely they diverge from conventional thoughts about bricks, the more creative the thinking process.

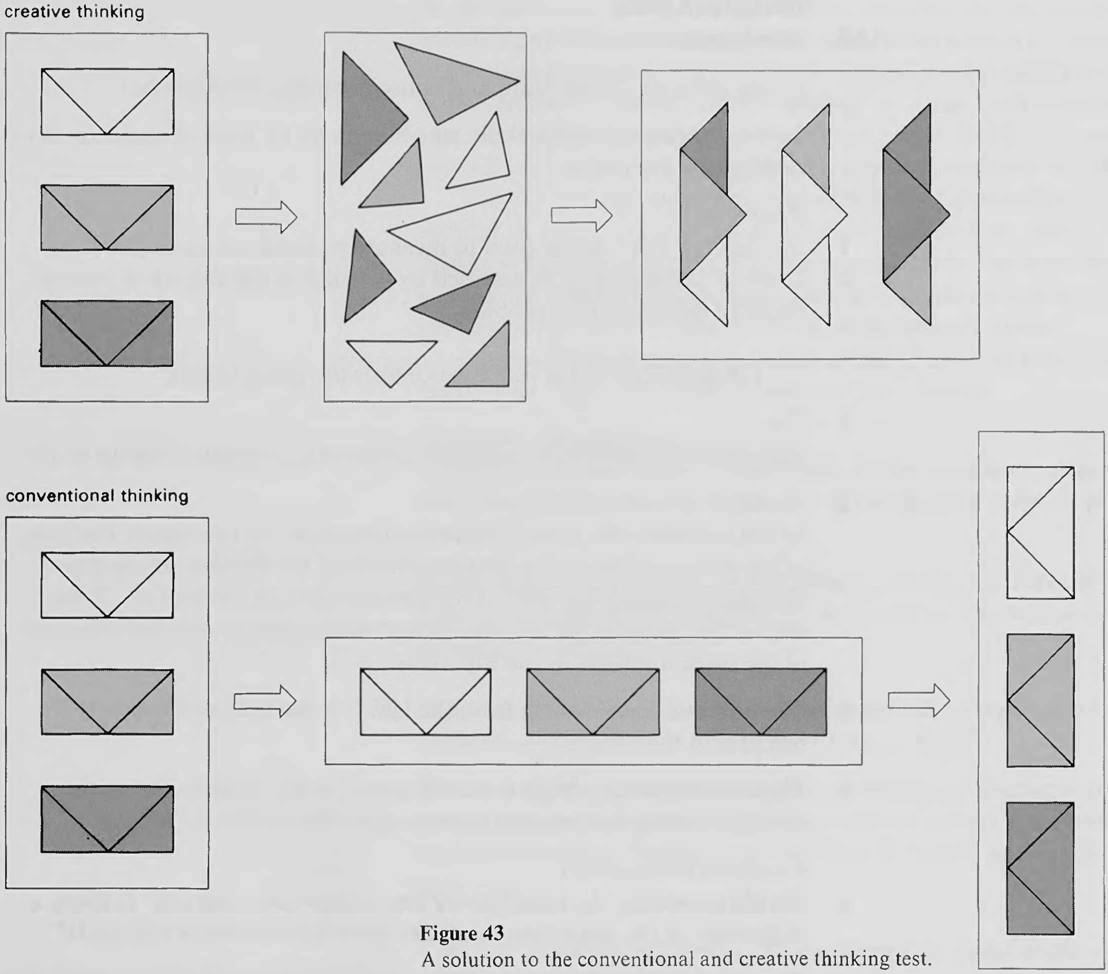

Figure 43
A solution to the conventional and creative thinking test.

INVESTIGATION
14E Emotion

ITEMS NEEDED

Stopclock or stopwatch 1/2
Suitable stimuli (see below)

(*Study guide* 14.2 'The mind and consciousness': Emotion and feeling.)

Certain physical signs are used to indicate the state of arousal of the brain.

Principles

1 The bodily arousal which is observed in emotion is characterized by an increased pulse-rate, an increased activity of the sweat glands, a withdrawal of blood from inessential organs, and an increased pupillary size. These changes prepare the body for action.
2 Bodily arousal is controlled by the autonomic nervous system (sympathetic branch) and the endocrine system.

Once the students have established the base levels for the three parameters, subject them to an unexpected, loud bang, for example, the board rubber on the blackboard. They should measure the changes in arousal immediately; pulse-rate is the simplest and most accurate one to use. A sad or horrifying story could be read to the class to increase arousal, or they can be asked to watch a suspense film and take their pulse-rate when they feel themselves becoming most aroused.

Questions and answers

a ***To what extent are the three parameters (the pulse-rate, the skin response, and the pupillary size) correlated in different people?***

People who normally have a high pulse-rate should also have cold, sweaty hands and wide-pupilled eyes, and *vice-versa.*

b ***How do the three parameters change during bodily arousal?***

The pulse-rate should increase, the hands should become colder and sweatier, and the pupils should dilate.

c ***What mechanisms might underlie the observed changes?***

Bodily arousal is mediated by the sympathetic branch of the autonomic nervous system and the hormonal secretions of the pituitary and adrenal glands. The heart-rate and the sweat glands' activity are increased, arteriole constriction diverts blood from inessential organs, and increased pupillary size possibly improves efficiency of vision.

INVESTIGATION
14F Sleep

ITEMS NEEDED

Clinical thermometer 1/1

(*Study guide* 14.2 'The mind and consciousness': Sleep.)

This investigation is an attempt to establish a pattern of night and day rhythms.

Assumptions

1 An understanding of the different phases of sleep, that is, slow wave and REM sleep.
2 A familiarity with the main theories of dreaming.

Principles

1 It is usual to awake from a period of REM sleep.
2 Body temperature and therefore physiological efficiency varies throughout the day.

This work requires records to be kept conscientiously over a period of a week.

Questions and answers

a ***Which phase of sleep do you usually wake from in the morning?***

REM sleep.

b ***Which theory of dreaming seems to fit your own experience?***

This will depend on the dreams. Theories of dreaming are discussed in the *Study guide.*

c ***Do the graphs of body temperature show any common pattern? If so, does this relate to the time when individuals feel at their best or function most efficiently?***

About half the population feel a regular daily rhythm in their lives. Some feel best on waking (the 'larks'), while others are at their peak later in the day (the 'owls'). The 'larks' will show a peak level of body temperature earlier than the 'owls'; by about 2 pm the levels will be equal and from about 6 pm the body temperature of the 'larks' will start to fall more rapidly than that of the 'owls'. The higher the body temperature (within optimum physiological limits), the more efficiently a person functions. 'Larks' are therefore at their most efficient during the morning and less efficient during the afternoon.

INVESTIGATION
14G Extra-sensory perception and psychokinesis

ITEMS NEEDED

Set of ESP cards 1/class
Six-sided die 1/class

(*Study guide* 14.2 'The mind and consciousness': Changing the way we see the world.)

Simple experiments on extra-sensory perception are carried out.

Assumptions

1 An understanding of the terms 'clairvoyance', 'telepathy', and 'psychokinesis'.
2 The ability to use the χ^2 test.

Principles

1 Simple experiments can be used to test for the existence of ESP and psychokinesis.
2 Though some experiments have suggested that the mind can act at a distance, there is considerable controversy in this field of science.

Set of ESP cards. This is easy to make. 25 cards are required in all, 5 of each type. The usual designs are: a cross, a star, three wavy lines, a circle, and a square. It is suggested that the experiments are conducted with the class as a whole.

It would be instructive, perhaps if one is repeating experiments, to set up a deliberate cheat, to show:

1 how easy it can be to cheat unless experimental conditions are stringent, and
2 how eagerly most people accept 'good' results because they are so much more interesting than 'bad' ones.

This can be done with an accomplice in the class for the ESP experiments. For example, the teacher could signal discreetly, using a pre-arranged code indicating the position of a finger or foot as he or she turns over the cards.

Another experiment called 'remote viewing' can be tried. The class is divided in half. One half goes to a nearby local feature and looks at it intently from the same viewpoint, endeavouring to send back details to the rest of the class. Meanwhile the rest of the class draw what they see in their minds. If this experiment is attempted, emphasis should be placed on how best to evaluate the results scientifically.

Questions and answers

a ***What do your results suggest about the existence of ESP and psychokinesis?***

This will depend on the results actually obtained and the χ^2 analysis. The experiments should be replicated if firm conclusions are to be drawn.

b ***What is the difference between stage 1 and stage 2?***

In stage 1, no one was consciously aware of the card sequence, while in stage 2, the person turning over the cards was possibly transmitting information and so this is telepathy rather than clairvoyance.

c ***If some people seem to be particularly good at influencing the die at home, can they repeat their success in class? If not, why do you think they fail?***

Possible reasons include:
1 Cheating (see above).
2 Experimental bias (for example, were any 'bad' results ignored or discarded?).
3 The class has an inhibitory effect on the individual.

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APPENDIX THE USE OF ANIMALS AND PLANTS IN SCHOOL SCIENCE

Introduction

This joint statement from the Association for Science Education, the Institute of Biology, and the Universities' Federation for Animal Welfare is the result of discussions, held over several months, in response to numerous requests for guidance from teachers, advisers, and members of education committees and governing bodies. It is offered in the hope that those responsible for the teaching of science will find it helpful in deciding on practices and policies.

Living material in schools

Biology is the scientific study of living organisms and, as in all science education, emphasis is placed on direct, personal, practical experience. To try to teach the science of life without making use of living plants and animals is a contradiction in terms. The educational and biological justification for using living and once-living material in schools has been explored in detail in a number of publications (Wray, 1974; RS/IOB, 1975; Kelly, 1976).

For these well established biological and educational reasons, pupils should be given the opportunity of observing living plants and animals in their natural habitats. Where this is not possible, living material should be brought into the classroom. This can pose ethical problems of conservation and humanity as well as practical ones of management and hygiene.

Animals and plants in schools should be kept in the peak of condition at all times. Regular attention is essential including cleaning and maintenance; with immediate removal of dead and diseased material. Healthy stock is a reflection of the care and concern for living organisms which are a central theme of biological education. A school's attitude to the keeping of living material on the premises and its use in science lessons should be carefully thought out and, where possible, included in any statement of the school's aims for science education.

Dead material in schools

The investigation of dead animals and plants can be an adequate, sometimes preferable, alternative to the use of living material. Dissection – the internal investigation of dead animal and plant material – is a basic biological technique (RS/IOB, 1975). Usually, however, the term is restricted to the internal investigation of animals. Controversy has arisen in particular over the dissection of rodents and other small mammals. It has become incorrectly associated with vivisection, which essentially involves live animals and raises moral and humane issues that do not relate to dead material. No school is allowed to perform any experiment involving the internal investigation of *live* vertebrate animals.

The biological and educational aims of animal dissection (RS/IOB, 1975) can be summarized as follows:

a to gain knowledge and understanding of internal structures, and of variation between individual organisms of the same species;
b to gain personal experience of both the fragility and strength of fresh tissues;
c to improve learning through active involvement of the pupil;
d to appreciate the organism as an entity rather than as a collection of organ systems.

The point at issue is whether these aims are essential to the proper teaching of biology and whether dissection is the only way of achieving them. Charts, models, films, and other visual aids have their uses and are sometimes proposed as adequate substitutes but, not least because of their lack of personal involvement, they are not accepted as such by the majority of science teachers. Many of the arguments against whole animal dissection stem from a concept of 'animal rights'. Whether or not this has a sound philosophical basis, it is widely accepted that human beings have responsibilities towards animals. One argument for keeping live animals in schools is that contact with them is essential to the development of a proper understanding of their needs and so a proper attitude towards them.

It is generally agreed that any animal with which pupils may have been emotionally involved should not then be killed for dissection, but that animals should be obtained especially for this purpose. Before doing so, teachers should consider carefully why they are including a whole animal for dissection in the teaching programme, and pupils should be encouraged to discuss the moral, aesthetic, educational, scientific, and other issues involved. The use of abattoir material can largely fulfil the aims of animal dissection set out earlier.

Those responsible for science teaching in schools should consider sympathetically the feelings of staff and pupils who do not wish to undertake or watch dissection. Pupils should be made aware that some A-level courses include dissection and should be told whether or not this is a compulsory part of the examination. Pupils should also be encouraged to consider carefully the career implications involved in not doing dissection.

Animals and plants in rural science teaching

All that has been said above applies also to the use of animals and plants in rural science teaching. The killing of livestock is, however, a particular issue: the slaughter of animals reared for meat and the subsequent preparation of the carcasses for human consumption must be done by appropriate personnel in accordance with national regulations. Diseased farm animals must be treated by suitably qualified personnel and, where necessary, destroyed humanely, according to animal health legislation. Dead animals must be disposed of safely. Rural science teachers will obviously wish to maximize the efficiency of their stock-rearing and demonstrate good practice by keeping their animals in first class condition. The whole issue of the rearing of animals for food is a proper part of the rural science syllabus.

Summary

1 Biology is the study of living organisms and its teaching must include them wherever possible. Teachers should be able to justify this on biological and educational grounds, and be conversant with regulations and recommendations concerning the maintenance of living organisms in schools, including humane killing, where appropriate.

2 Material for animal dissection should be obtained specially for the purpose. Teachers must be able to justify the inclusion or exclusion of dissection on biological and educational grounds, and should be prepared to discuss these. For pupils below the age of 16 years, a demonstration dissection by the teacher will normally be sufficient.

3 Teachers and pupils who object to dissection should be made aware of possible career consequences. Dissection is already optional for several examination boards and teachers should consider changing boards if they or their pupils object to dissection in an A-level practical examination.

4 A school's views on the use of animals and plants, both living and dead, should be part of the general statement on the aims of science education which is made available to parents.

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Association for Science Education
Institute of Biology
Universities' Federation for Animal Welfare

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The editors and authors of the revision of Nuffield Advanced Biology, in consultation with teachers, schools, and experts in biological education, have produced a course to which students will respond with pleasure and interest. The basic work of the course is in the two new Study guides, **with experiments covered in separate, short** Practical guides.

This Teachers' guide **accompanies** Study guide I **and related** Practical guides. **Each chapter begins with a review of aims and contents. Following this, the first part of the chapter sets out the assumptions and principles underlying each section in the** Study guide **and repeats and gives answers to the questions. The second part deals in the same way with each practical investigation and its questions and includes a list of items needed. Both parts are thoroughly cross-referenced to the relevant students' books. The chapter ends with a bibliography.**

The first seven chapters are grouped under the title 'Maintenance of the organism'. They cover gas exchange and circulation in living organisms, cells and chemical reactions which take place in them, heterotrophic nutrition, and photosynthesis. The remaining seven chapters, entitled 'Control and co-ordination in organisms', deal with water relationships of individual cells and whole organisms, how organisms maintain a stable environment, control and co-ordination in organisms, their responses to stimuli, behaviour, and the human brain and the mind.

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