SYLLABUSES

FOR

SECONDARY SCHOOLS

BIOLOGY

(ADVANCED SUPPLEMENTARY LEVEL)

PREPARED BY
THE CURRICULUM DEVELOPMENT COUNCIL
RECOMMENDED FOR USE IN SCHOOLS BY
THE EDUCATION DEPARTMENT
HONG KONG
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PREAMBLE

This syllabus is one of a series prepared for use in secondary schools by the Curriculum Development Council, Hong Kong. The Curriculum Development Council, together with its co-ordinating committees and subject committees, is widely representative of the local educational community, membership including heads of schools and practising teachers from government and non-government schools, lecturers from tertiary institutions and colleges of education, officers of the Hong Kong Examinations Authority, and those of the Advisory Inspectorate and other divisions of the Education Department.

All syllabuses prepared by the Curriculum Development Council for the sixth form will lead to appropriate Advanced and/or Advanced Supplementary level examinations provided by the Hong Kong Examinations Authority.

This syllabus is recommended for use in Secondary 6 and 7 by the Education Department. Once the syllabus has been implemented, progress will be monitored by the Advisory Inspectorate of the Education Department. This will enable the Biology Subject Committee (Sixth Form) of the Curriculum Development Council to review the syllabus from time to time in the light of classroom experiences.

All comments and suggestions on the syllabus may be sent to:

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1. AIMS AND OBJECTIVES

This Biology syllabus is one of the three Advanced Supplementary (AS) level science syllabuses developed by the Curriculum Development Council to broaden the curriculum in Secondary 6 and 7. The others are the Chemistry and Physics syllabuses.

Aims

The broad aims of the AS level Biology course are to:

1. develop students' appreciation of the wonders of the living world; and to promote respect for all living things;
2. broaden and stimulate students' interest in learning biology; to encourage their worthy use of leisure; and to help them to acquire self-initiative in the study of biology;
3. develop students' power to think creatively, analyse critically and scientifically on biological issues and to make rational decisions and to communicate effectively;
4. develop students' observational, manipulative and experimental skills;
5. develop students' ability to retrieve appropriate information from proper sources; and to develop their confidence in self learning and to give them a sense of achievement;
6. develop students' appreciation of the importance of experimental and investigatory work in the study of biology;
7. enable students to acquire knowledge and understanding of basic biological principles;
8. develop students' awareness of and concern for biological issues in personal, social, environmental and technological contexts; and
9. prepare students to become responsible citizens in a changing world.

Objectives

The general objectives of the AS level Biology course are:

A. Knowledge and understanding

Students should be able to:

1. recall (*a*) biological facts,
   (*b*) biological terms,
   (*c*) biological concepts and principles, and
   (*d*) biological practical techniques;
2. recall some of the ways in which biological knowledge is applied in daily life;

3. locate, select and organise information from various sources; to present them in a clear and logical form; and to apply them to solve problems in familiar and unfamiliar situations;
4. recognise the need for measurements, to select appropriate instruments, and to recognise limits of accuracy of such instruments and performance;
5. recognise biological problems; such problems are often characterised by the presence of a range of interacting variables;
6. formulate working hypotheses and devise tests for them, using controls where appropriate;
7. interpret data and to interpolate and extrapolate from them; and
8. formulate generalisations in the light of both first-hand and second-hand evidence.

B. Attitudes

Students should acquire:

1. an interest and enjoyment in studying living organisms and their interrelationships;
2. a respect and feeling for living organisms;
3. a critical and inquiring mind;
4. an objective attitude towards evidence;
5. a positive attitude in discussing biological issues in personal, social, environmental and technological contexts;
6. an awareness that the body of biological knowledge is not static; and that experimental and investigatory work are important for its advancement;
7. an awareness of the need for appropriate safety procedures; and
8. an awareness of both the usefulness and limitations of hypotheses in making predictions and describing biological phenomena.

C. Practical skills

Students should be able to:

1. use instruments and apparatus to the limits of accuracy appropriate to a given problem;
2. observe and describe objects and phenomena accurately; and
3. perform common laboratory techniques and handle chemicals, apparatus and biological materials carefully and safely.

2. SYNOPSIS OF SYLLABUS

The syllabus is divided into seven sections:

I. The Structure and Functions of Cells

1. The general structure of cells
2. The chemical constituents of cells
3. Enzymes

II. Energetics

1. The ultimate sources of energy available to living organisms
2. Photosynthesis
3. Chemosynthesis
4. Organic compounds in plants as initial energy sources for heterotrophs
5. Respiration
6. Energy flow through the ecosystem

III. Genetic Control and Inheritance

1. The nature of the gene
2. Cell division
3. Heredity
4. Mutation
5. Genetics and society

IV. Regulation and Control

1. The need to maintain a constant internal environment in organisms
2. The movement of substances in and out of cells
3. The regulation of water loss in flowering plants
4. The blood system as a means of achieving a constant internal environment in mammals
5. Control and coordination in mammals involving
   (*a*) the nervous system
   (*b*) the endocrine system
6. Control in plants
7. The homeostatic role of the liver
8. The homeostatic role of the kidney

V. Variety of Life and Relations of Organisms with Their Environment

1. Diversity of organisms
2. The ecosystem
3. Populations
4. Evolution
5. Artificial selection

VI. Man and the Environment

1. Man's impact on the environment
2. Man's responsibility for environmental protection and conservation

VII. Man and Microorganisms

1. Microorganisms and food
2. Microorganisms and biotechnology
3. Microorganisms and diseases

3. ESTIMATED TIME ALLOCATION

A time allocation of four 40-minute periods per week each for Secondary 6 and 7 would be adequate to cover this syllabus. An estimate of the number of periods for individual sections of the syllabus is shown below:

Syllabus	*Number of teaching periods for*		
	Theory (T)	*Practical Work & Other Activities (P)*	*Total*
Section I	16	10	26
Section II	12	6	18
Section III	27	6	33
Section IV	24	10	34
Section V	15	13	28
Section VI	13	11	24
Section VII	15	10	25
Whole Syllabus	122	66	188

Although the estimate is rough, it provides some guidance on the depth of treatment required and the weight to be placed on the various sections of the syllabus. Teachers, in planning their courses, need not follow the estimate precisely. Adjustments can be made to meet the abilities, needs and interests of the students and the emphases of the teachers.

4. NOTES ON TEACHING

The curriculum places emphasis on the acquisition of general biological principles and their application rather than specific facts and examples. The topics outlined in the curriculum illustrate a close interaction of basic biological knowledge, modern technology and social issues.

Experiments and activities should form an integral part of the teaching process. The practical work aims not only to illustrate biological principles, but also to develop skills in experimental investigations and appreciation of scientific method. A good planning of questioning and discussion before and after an experiment allows effective learning of concepts and principles.

It is advisable for teachers to try out the experiments before giving them to the students as practical work with the purposes of

(i) obtaining appropriate results and knowing how to avoid misleading ones;

(ii) adjusting the concentration of the reagents used for a particular experiment;

(iii) selecting the appropriate biological materials;

(iv) becoming familiar with the difficulties that might be encountered by students; and

(v) uncovering any potentially dangerous situations.

As the experimental and activity approaches are used for the teaching of biology, safety in experimental work, field work and project work should be stressed whenever it is necessary. Teachers are advised to familiarise themselves with those parts related to the teaching of biology in the booklet, 'Safety in Science Laboratories' published by the Education Department.

The inclusion of a number of socially-related biological topics aims to arouse students' awareness of the application and contribution of biological knowledge in relation to our daily life. It also allows students to consider the moral and ethical aspects of the subject.

As this syllabus also aims in developing students' higher thinking abilities, communication skills and interest in biology, teachers are encouraged to employ various teaching strategies which involve students in active thinking, discussions, and class activities. Some suggested teaching strategies for teachers' reference are:

(a) Group discussion

Students can better understand scientific concepts if they participate actively in the learning process. Discussions on scientific observations or controversial issues provide a form of active learning. These activities encourage students to think critically and to communicate clearly with others.

The teacher should adopt the role of a facilitator during the discussion and collate the students' ideas at the end of the session. Sometimes it may be necessary for the teacher to guide students to arrive at certain decisions. Group discussions not only provide a good opportunity for developing students' higher cognitive and communicative abilities, they also affect their attitudes towards controversial issues (e.g. genetic engineering technology, AIDS and conservation).

(b) Project work

Students can study in depth a chosen topic through this method. It can be carried out either by individual students or in groups. Individual projects have the merit of training students to work independently. Group projects on the other hand will provide opportunity for co-operation among students and thus enhance the community life in schools. As reporting is a vital element in project work, students will be able to develop communicative skills.

The scale of a project may range from a simple collection of information (e.g. collection of newspaper cuttings related to greenhouse effect) to complicated projects including experimentation, data processing and presentation (e.g. project work on acid rain).

Teachers should play the role of an advisor in the choice and design of the projects. They should monitor the progress of the project, and give guidance and advice where appropriate.

(c) Visits

Organised visits to places (e.g. sewage treatment plant, and Kadoorie Farm) related to the contents of the syllabus will enhance the interest of the students. Visits are particularly valuable in enabling the students to visualise how theoretical principles can be put into practice.

Careful planning is crucial to a successful visit. Teachers should prepare their students adequately for the event, e.g. giving a pre-visit briefing, so that their students will know exactly what to look for during the visit.

It is more beneficial to students when the planning and preparation work of a visit is undertaken by the students themselves, provided that sufficient guidance and supervision are given by the teacher.

The teaching strategies suggested are just examples, and are in no way exhaustive. Teachers can find other suitable strategies in many resource books.

The teaching of this curriculum will be most fruitful if teachers are well familiarised with its aims and objectives, and if they employ a variety of teaching strategies to meet the requirements of the various topics.

5. SYLLABUS

The syllabus is organised into four columns:—'Topics', 'Explanatory Notes', 'Practical Work and Other Activities' and 'Periods'. The major contents of the syllabus are indicated in the 'Topics' column. The 'Explanatory Notes' column gives amplification of the content and indicates the depth of treatment required. The 'Practical Work and Other Activities' column indicates suggestions for experiments, demonstrations, discussion, debate and project work.

The number of teaching periods needed to cover the individual parts of the syllabus is suggested in the 'Periods' column for teachers' reference. The (T) stands for the suggested time allocation for the teaching of theory while (P) represents the number of periods suggested for the practical work and other activities. In places where there are practical work and other activities suggested but without (P) periods allocated, it is expected that the practical work and other activities are to be integrated with the teaching of theory in the same lesson(s), and vice versa.

The sequence of presentation of topics in the syllabus should not be regarded as a fixed teaching order. Individual topics need not be treated as separate entities, but should be studied as integral parts of the whole syllabus. Many ideas and principles which appear in more than one part of the syllabus would normally be treated in depth once only. Concepts developed in one section should be carried over into the teaching of other sections. Cross references are included where appropriate.

Practical work and other activities should form an essential element of the course. They should be provided for the students wherever appropriate to enable them to develop various skills and to complement principles and concepts introduced in the course. The practical work and other activities suggested in the syllabus are to assist teachers in planning learning activities. It is hoped that they will not inhibit in any way teachers planning other practical work and activities that will better achieve the teaching objectives. The numbers within parenthesis indicate notes for teachers which provide information for teachers' reference.

The attention of the teachers is drawn to the need for safety precautions in the laboratory and in the field, particularly in the safe and proper handling of apparatus, chemicals and biological materials, including living organisms.

Section I The Structure and Functions of Cells

Students should acquire an understanding of the application of the techniques used in studying cell structure and functions: electron microscopy, chromatography, dialysis and centrifugation. Practical details of these techniques are NOT required. Structural formulae for individual chemical compounds are NOT required.

Syllabus Topics	Explanatory Notes	Practical Work & Other Activities	Periods T	Periods P
1. The general structure of cells	An understanding of the relationship between structure and function as exemplified by the following: parenchyma, sclerenchyma, phloem, xylem, epithelia, blood cells and neurones.	Students should make temporary slide preparations (*1*) of plant and/or animal tissues (*2, 3*) (including simple staining). (The taking of human blood and cheek cells should NOT be carried out.)	2	3
	The structure of the following as seen under the electron microscope and, where possible, their functions in relation to structure: nucleus, nucleolus, cell membrane, cell wall, vacuole, chloroplast, mitochondrion, lysosome, ribosome, endoplasmic reticulum, Golgi apparatus and centriole.	Electron micrographs should be studied.	4	
2. The chemical constituents of cells The biological significance and chemical nature of:				
(*a*) Carbohydrates	Carbohydrates as a source of energy and as structural/storage materials. Monosaccharides: hexoses (glucose and fructose), pentoses. Disaccharides: sucrose and maltose. Polysaccharides: cellulose, starch and glycogen	Students should apply the following tests (*4*) to plant/animal tissues (*5*): 1. Benedict's test for reducing sugars 2. Iodine test for starch 3. Sudan test for lipids 4. Biuret test for proteins	2	3
(*b*) Lipids	Triglycerides as storage compounds. Phospholipids as components of membranes.			

Syllabus Topics	Explanatory Notes	Practical Work & Other Activities	Periods T	Periods P
(*c*) Proteins	Proteins as structural and functional materials. An understanding of the 3-dimensional shape of proteins: its ultimate dependence upon amino acid sequence and its significance in the functioning of enzymes. Amino acids, peptide bonds and polypeptide chain.		2	
(*d*) Nucleotides and nucleic acids	Mononucleotides: adenosine triphosphate (ATP) as an energy-rich compound. Dinucleotides: nicotinamide adenine dinucleotide (NAD) as a coenzyme. [Refer to Section II.5.] Polynucleotides: ribonucleic acids (RNAs) and deoxyribonucleic acid (DNA). [Refer to Section III.1 (*a*), (*b*).]		1	
(*e*) Water	The biological significance of water.		1	
3. Enzymes				
(*a*) Enzymes as organic catalysts	The lowering of activation energy; the concept of the active site and specificity.	The effects of the following on enzyme activities should be investigated: 1. temperature (*6*) or pH (*7*) 2. enzyme (*8*) or substrate (*9*) concentration Suitable enzymes include amylase (*6*), urease (*9*), catalase (*8*), pepsin, invertase (*7*) [where possible, at least some of the enzymes used should be obtained from living tissues and/or commercial products e.g. 'biological' washing powders (*10*) and meat tenderizers (*11*)].	2	4
(*b*) Factors affecting enzyme activity	Cofactors and inhibitors; the effects of temperature, pH, enzyme and substrate concentration on the rate of enzyme activities.		2	

Section II Energetics

This section only requires an understanding of the overall metabolic processes. Details of the metabolic pathways, names of intermediates and individual enzymes are NOT required, unless otherwise stated.

Syllabus Topics	Explanatory Notes	Practical Work & Other Activities	Periods T	Periods P
1. The ultimate sources of energy available to living organisms	Energy derived from the sun (photosynthesis). Energy derived from chemical sources (chemosynthesis).			
2. Photosynthesis	Its significance in converting light energy to chemical energy.			
(*a*) Site of photosynthesis	The structure of a leaf in relation to photosynthesis.	The microscopic examination of leaf structure in relation to photosynthesis.	2	
	Chloroplast pigments: absorption spectrum.	The extraction and separation of leaf pigments by paper chromatography (*12*).		2
(*b*) Photochemical reactions	An outline to show photoactivation of chlorophyll (NO distinction into photosystems I and II), splitting of water, release of oxygen and production of reduced coenzyme and ATP.		1	
(*c*) Carbon fixation	An outline to show the (i) combination of carbon dioxide with a 5-C compound to form 3-C compounds; (ii) reduction of the 3-C compound by reduced coenzyme to triose phosphate, some of which condenses to yield hexose phosphate which is subsequently metabolised to sucrose and starch; and (iii) further metabolism of the remaining triose phosphate to provide a continuous supply of the 5-C carbon dioxide acceptor.		1	

Syllabus Topics	Explanatory Notes	Practical Work & Other Activities	Periods T	Periods P
(*d*) Factors affecting rate of photosynthesis	The effects of environmental factors on the rate of photosynthesis. The concept of limiting factors and its application in greenhouse management.	Oxygen bubbling experiments to investigate the factors affecting the rate of photosynthesis (*13*). Factors to be investigated may include light quality, light intensity, carbon dioxide concentration and temperature. Light intensity and carbon dioxide concentration should be shown as limiting factors.	2	4
3. Chemosynthesis	A brief consideration of one of the following: iron bacteria, colourless sulphur bacteria and nitrifying bacteria. (Names of individual bacteria are NOT required.)		1	
4. Organic compounds in plants as initial energy source for heterotrophs	Dependence of heterotrophs on autotrophs. Holozoic, parasitic and saprophytic nutrition. (Details of the processes of digestion and absorption are NOT required.)		1	
5. Respiration	The release of energy in the form of ATP should be treated as a series of controlled chemical reactions: Glycolysis Tricarboxylic acid (TCA) cycle Oxidative phosphorylation (The names of enzymes and intermediates in these processes are NOT required.) Sites of these reactions within the cell should be noted. Fate of pyruvic acid under anaerobic conditions: formation of lactic acid; fermentation to ethanol. A comparison of the energy yield of aerobic and anaerobic respiration.		3	

Syllabus Topics	Explanatory Notes	Practical Work & Other Activities	Periods T	Periods P
6. Energy flow through the ecosystem	The sun as the ultimate source of energy. The unidirectional flow of energy and its subsequent loss. Trophic levels; pyramid of energy. The efficiency of energy flow and its significance in relation to human food production.		1	

Section III Genetic Control and Inheritance

Students should understand the principal genetic terms. They should be able to relate their genetic studies to simple problems involving crosses between animals or plants, and to arrive at the answers in a logical deductive manner. Wherever possible, the social relevance of the application of the genetic principles involved should be mentioned.

Syllabus Topics	Explanatory Notes	Practical Work & Other Activities	Periods T	Periods P
1. The nature of the gene				
(*a*) DNA	The molecular structure of DNA. [Refer to Section I.2 (*d*).] The semiconservative nature of DNA replication: mechanism and evidence.	The use of models to illustrate the double helical structure of DNA and its semiconservative mechanism of replication.	3	
(*b*) Action of the gene	A brief description of the genetic code. The role of DNA and RNAs in protein synthesis. (Details of the structure of tRNA and ribosomes are NOT required.)	The use of models to demonstrate the role of DNA and RNAs in protein synthesis.	3	
(*c*) Genes and chromosomes	The concept of the chromosome as a collection of genes. [Refer to Section III.3 (*b*).] The constancy of chromosome number.	The observation of squash preparation of giant chromosomes (*14*), e.g. the salivary glands of *Chironomus* larvae.		2
2. Cell division				
(*a*) Mitosis and meiosis	A review of the processes of mitosis and meiosis to appreciate the significance of the exact duplication of chromosomes and the differences in their behaviour in the two processes.	The observation of chromosome preparations (by root tip squashes) to study the process of mitosis (*15*). Prepared slides and photomicrographs may also be used.		3
	The significance of mitosis in growth, cell replacement and asexual reproduction. The significance of meiosis in sexual reproduction and as a process to increase genetic variation through an independent assortment of chromosomes and crossing over.	The use of photomicrographs/slides to show the process of meiosis.	3	

Syllabus Topics	Explanatory Notes	Practical Work & Other Activities	Periods T	Periods P
(*b*) Cytoplasmic division	A simple treatment of cytoplasmic division in animal and plant cells.			
3. Heredity				
(*a*) Inheritance of discrete characters	Monohybrid and dihybrid crosses. Backcross and test cross.	The result of monohybrid and dihybrid crosses should be studied to illustrate the patterns of monohybrid and dihybrid inheritance (e.g. maize ears).		1
	Genes and alleles, genotype and phenotype. Homozygotes and heterozygotes. Dominance and recessiveness.			
	Incomplete dominance (e.g. petal colour in snapdragon) and codominance (e.g. blood group AB in humans).		6	
	Multiple alleles (e.g. human ABO blood groups).			
(*b*) Linkage and crossing over	Linkage: The linear arrangement of genes on a chromosome. The parallel behaviour of genes and chromosomes in inheritance. [Refer to Section III.1 (*c*).]			
	Crossing over: the exchange of genetic materials between homologous chromosomes and its role in causing variation. (Calculation of crossover values is NOT required.)	The use of photomicrographs to show the formation of chiasmata.	3	
	Sex determination in man.			
	Sex linkage (e.g. haemophilia and colour blindness). [Refer to Section III.5 (*a*).]			
(*c*) Discontinuous and continuous variations of characters	Discontinuous variations (e.g. tongue rolling, ABO blood groups in man) and continuous variations (e.g height and weight in man). The normal distribution curve. A simple treatment of polygenic inheritance and the effect of environment on it.		2	

Syllabus Topics	Explanatory Notes	Practical Work & Other Activities	Periods T	Periods P
4. Mutation				
(*a*) Gene mutation	The effect of gene mutation on amino acid sequences. (Different types of point mutation are NOT required.)			
(*b*) Chromosome mutation	A simple treatment of chromosome mutation: changes in chromosome structure and number. (Different types of chromosome mutation and their genetic consequences are NOT required.)		2	
(*c*) Causes of mutation	Spontaneous and induced mutations. (Ionizing radiation, ultraviolet light and chemicals as mutagens should be mentioned.) (Mechanisms are NOT required.)			
5. Genetics and society				
(*a*) Human genetics	Pedigree analysis (e.g. colour blindness). [Refer to Section III.3 (*b*).]			
	Genetic abnormalities (e.g. Down's syndrome).		2	
	A simple treatment of genetic screening and counselling (*16*) (e.g. early detection of Down's syndrome).			
(*b*) Genetic engineering	A simple treatment of the principles of recombinant DNA technology: insertion of DNA fragments into a new cell via a vector (bacteriophage or plasmid); synthesis of a new product by selected host cells (e.g. in the production of human insulin (*17*)). (Technical details are NOT required).		3	
	Consideration of potential benefits (*18*), hazards and ethical issues (*19*) associated with genetic engineering technology.			

Section IV Regulation and Control

It is hoped that through a study of this section, students should understand the concept of homeostasis. They should also establish the idea that every organism functions as an integral whole, and that life processes within an organism occur in a coordinated way through various regulatory control mechanisms. It should also be pointed out that examples covered in this section only represent a very small portion of the kind of regulation and control occurring in both plants and animals.

Syllabus Topics	Explanatory Notes	Practical Work & Other Activities	Periods T	Periods P
1. The need to maintain a constant internal environment in organisms	The concept of homeostasis.		1	
2. The movement of substances in and out of cells	The concept of selective permeability of cell membrane. Factors affecting permeability. Diffusion and osmosis; active transport.	Experiments to demonstrate some of the properties of cell membranes: destruction of membranes by temperature and some chemicals (e.g. alcohol) can be shown using beetroot tissue (*20*).	1	2
	An understanding of turgor and plasmolysis including water potential, osmotic potential and pressure potential. The movement of water between plant cells and the environment should be understood in terms of water potential.	One or more of the following materials should be used to show plasmolysis (*21*); to determine osmotic potential at incipient plasmolysis (*22*); and water potential by changes in mass, length (*23*), etc. : the red lower epidermis of some ornamental plants, epidermal tissue from onion scales and potato tuber tissue.	2	6
3. The regulation of water loss in flowering plants	The regulatory role of stomata in transpiration. (Stomatal mechanism is NOT required) The effect of environmental factors on the rate of transpiration.	Simple experiments on transpiration using potometer (*24*).	2	2

Syllabus Topics	Explanatory Notes	Practical Work & Other Activities	Periods T	Periods P
4. The blood system as a means of achieving a constant internal environment in mammals	The role of blood in the transport of oxygen, carbon dioxide, glucose, hormones and heat in the body. A brief treatment of the cardiac cycle. Hormonal and nervous control of the heart rate.		4	
5. Control and coordination in mammals involving				
(*a*) the nervous system	The nature of nerve impulse. (Details are NOT required.) The importance of synapse in nervous coordination. (Details of synaptic transmission are NOT required.) The control of involuntary body activities by sympathetic and parasympathetic nervous systems with reference to their antagonistic actions.		2	
(*b*) the endocrine system	The nature of an endocrine gland. The characteristics of a hormone. The differences between nervous and endocrine coordination. The examples listed should be studied in order to understand (i) the principles of endocrine coordination —the control of the thyroid gland by the pituitary gland; —the control of blood sugar by insulin and glucagon; (ii) the relationships between endocrine and nervous coordination —the secretion of adrenaline; —thermoregulation.	The use of microscopic sections/ photomicrographs to show the ductless and vascularised nature of endocrine glands.	5	

Syllabus Topics	Explanatory Notes	Practical Work & Other Activities	Periods T	Periods P
6. Control in plants	A simple treatment of the functions of auxins and gibberellins in germination and growth. The use of phytohormones in horticulture and agriculture.		2	
7. The homeostatic role of the liver	Its role in: —carbohydrate and fat metabolism. —the storage of vitamins and blood. —the synthesis of plasma proteins. —the breakdown of red blood cells. —the detoxification and deamination of amino acids. (Details of biochemical pathways are NOT required.)	The use of microscopic sections/ photomicrographs of injected liver to show its highly vascularised nature.	2	
8. The homeostatic role of the kidney	The importance of removing nitrogenous waste materials. The role of ADH in the maintenance of water balance. (The counter-current multiplier system is NOT required.) The biological principle of the dialysis (kidney) machine.	The use of microscopic sections/ photomicrographs of kidney to show the following: 1. the large number of Malpighian bodies – the filtering units; 2. the bulk of uriniferous tubules; and 3. the highly vascularised nature.	3	

Section V Variety of Life and Relations of Organisms with Their Environment

Students should be encouraged to investigate for themselves the external features of as wide a range as possible of organisms found in local habitats in relation to their ecological niches. Distribution of plants and animals within a habitat and the influence of abiotic factors on their distribution should be studied by means of appropriate field work. Students are encouraged to take part in the programme offered by the Field Studies Centre in Sai Kung or by other centres.

Syllabus Topics	Explanatory Notes	Practical Work & Other Activities	Periods T	Periods P
1. Diversity of organisms				
(*a*) The variety of life	The relation between the diversity of organisms and the variety of their ways of life (*25*). Use of a range of specimens/photographs/field observations to illustrate this concept. (Scientific names of individual organisms are NOT required.)	Part of this can be accomplished during the field studies referred to in Section V.2 (*a*).	3	
(*b*) Classification	Introducing the binomial system of naming organisms and the concept of the taxonomic hierarchy. (Scientific names of individual organisms are NOT required.) Construction and use of simple dichotomous keys based on external features.	Construction and use of simple keys.		2
2. The ecosystem				
(*a*) Concept of the ecosystem	Understanding of the terms biosphere, biome and ecosystem. The concept of habitat and niche of an organism. An outline of biotic and abiotic factors in the ecosystem and their effects on the distribution of organisms in the ecosystem.	Field studies (*26*) in one selected habitat of the distribution of plants and animals and investigations of the influence of abiotic factors on them. A range of organisms normally found in the habitat should also be studied to illustrate the relationship of the external features of these organisms to the physical characteristics of the environment and to their modes of life. (Details of internal anatomy, physiology, life cycles and behaviour are NOT required.)	4	8
(*b*) Energy flow and nutrient cycling in the ecosystem	Energy flow in ecosystems. The importance of producers, consumers and decomposers in the cycling of nutrients. (Refer to Section II.1, II.3 and II.6.)			

Syllabus Topics	Explanatory Notes	Practical Work & Other Activities	Periods T	Periods P
3. Populations	The 'S'-shaped growth curve. The exponential growth of the human population. The interaction between the biotic potential of a population and environmental factors in maintaining stable populations.		2	
4. Evolution				
(*a*) Evidence of evolution	A simple treatment of the evidence for evolution as illustrated by the study of fossils or homologous structures in pentadactyl limbs.		2	
(*b*) Possible mechanisms of evolution	The role of variations, natural selection and isolation in the development of new species.		3	
5. Artificial selection	Breeding for selected traits in domestic animals (breeding in pigs) and plants (hybrid vigour in maize and polyploidy in wheat). (Technical details are NOT required.)	Visit to a farm, e.g. Kadoorie Farm.	1	3

Section VI Man and the Environment

In this section, time should be allowed for the discussion of topical environmental issues. Wherever possible, examples or issues studied should be relevant to Hong Kong.

Syllabus Topics	Explanatory Notes	Practical Work & Other Activities	Periods T	Periods P
1. Man's impact on the environment		Discussion/Project work on human population explosion and its impact on the environment.		2
(*a*) Resource exploitation	The variety of resources exploited by man: renewable and non-renewable resources. An understanding of the ways in which man's exploitation of natural resources has modified the environment.		2	
(*b*) The effects of intensive agriculture	Soil erosion as a consequence of agricultural practice. The undesirable effects of the chemical control of pests and weeds. The excessive use of chemical fertilizers.		2	
(*c*) Pollution (i) Atmospheric Pollution	Atmospheric pollutants (e.g. sulphur dioxide and particulates) and their effects. Global issues: ozone depletion; greenhouse effect; and acid rain.	A small project/Investigation (e.g. acid rain; greenhouse effect; the lichen distribution as an indication of air pollution by sulphur dioxide; a survey of streams for water pollutants).	3	2
(ii) Water pollution	Sewage: inadequate treatment leading to the contamination of water and depletion of oxygen. Eutrophication and algal blooms. Pollution by oil and detergent.		2	

Syllabus Topics	Explanatory Notes	Practical Work & Other Activities	Periods T	Periods P
2. Man's responsibility for environmental protection and conservation	Pollution control measures: sewage treatment, the siting of power stations and industries; the control of agricultural and industrial effluents. The need for conservation of natural resources. The recycling of natural resources. Conservation of wildlife and their habitats: ecological, aesthetic and moral considerations. Countryside conservation: the Country Park Programme. The conflicts between economics and the protection of the environment. The importance of environmental education and legislation.	Discussion/Debate/Visit.	4	7

Section VII Man and Microorganisms

This section covers both the useful and harmful roles of microorganisms. The application of biological principles should be stressed. Scientific names of individual microorganisms are NOT required.

Syllabus Topics	Explanatory Notes	Practical Work & Other Activities	Periods T	Periods P
1. Microorganisms and food				
(*a*) Microorganisms as food	Algae and fungi as food.			
(*b*) The use of microorganisms in food production	The concept of food production through the action of microorganisms (e.g. vinegar, soya sauce, fermented bean curd, alcoholic beverages, dairy products).	A visit to a food production plant.	3	3
(*c*) Food spoilage	Microbial degradation of food and beverages. Food poisoning and its prevention. The biological principles of food preservation using physical and chemical methods.			
2. Microorganisms and biotechnology				
(*a*) Genetic engineering involving microorganisms	The basic concept of genetic engineering involving microorganisms. [Refer to Section III 5(*b*).]		2	
(*b*) The importance of microbial biotechnology	The application of microbial biotechnology in agriculture, medicine, industry and pollution control (e.g. pig-on-litter system (*27*), insulin production, ethanol/enzyme/hepatitis B surface antigen (*28*) production, waste treatment).	Survey/Project work/Discussion/Debate/ Visit.		5

Syllabus Topics	Explanatory Notes	Practical Work & Other Activities	Periods T	Periods P
3. Microorganisms and diseases		Discussion on the social aspects of AIDS.	2	2
(a) Microorganisms as pathogens	A review of common human diseases and their causative microorganisms. Modes of transmission: droplets, wounds, infected food and water, and contact (including sexual contact).			
(b) Defence against diseases			2	
(i) External and internal protection	The integrity of the body surface, secretions and the local commensal microorganisms. Phagocytosis. A brief treatment of the inflammatory response.			
(ii) The immune response	Humoral and cell-mediated immune responses. An understanding of the action of antibodies and interferon. (Mechanisms are NOT required.) Primary and secondary responses. Immunization and vaccination.		4	
(iii) Antibiotics	The production of antibiotics by microorganisms. The action of antibiotics (e.g. penicillin). The use and misuse of antibiotics.		2	

6. NOTES FOR TEACHERS

The following notes provide hand-on information for teachers' reference. They are not intended to indicate the depth of treatment of the relevant parts of the syllabus. In fact, some notes, particularly those that are marked with asterisks (*), may be beyond the requirement of the syllabus. Teachers are advised to make discretionary use of these notes in planning their courses to meet the abilities and needs of their students.

(1) Preparation of temporary slide

Temporary preparation of materials for light microscopy can be made rapidly for quick preliminary investigation. The stages may involve sectioning, staining and mounting. Fresh material may be hand-sectioned with a razor blade. A number of stains may be used for staining purpose, and some examples are shown below:

Stain	*Suitable for*	*Final colour*
eosin	cytoplasm	pink
	cellulose	red
Leishman's stain*	blood cells	red-pink
	white blood cell nuclei	blue
methylene blue	nuclei	blue
safranine	nuclei, lignin	red
aniline sulphate or aniline hydrochloride	lignin	yellow or brown
iodine	starch	blue-black
	cuticle, xylem elements, sclerenchymatous cells	yellow/straw
phloroglucinol + conc. HCl *	lignin	red
Schultze's solution	lignin, cutin	yellow
	starch	blue
	cellulose	blue or violet

* Special staining procedure is required for these types of stains.

The sections are placed in a watch glass containing stain and left there until they are stained to the required depth. Sections can also be placed directly on a drop of stain on a slide.

Irrigation can also be used to introduce stain into the tissue of a temporary slide. A drop of the stain is placed on the slide so that it just touches the edge of the cover-slip. Fluid is then withdrawn from the opposite side of the cover-slip by means of a piece of filter paper or blotting paper. The stain then flows in to replace the fluid taken out.

The section should be mounted in a drop of water/stain/saline solution/ glycerine on a clean slide and a cover-slip applied.

A. Plant leaves (e.g. *Rhoeo discolor*)

Procedure

1. Insert the piece of leaf into a vertical slit made down the centre of a piece of moistened carrot.
2. Hold the carrot in one hand and cut the sections rapidly and smoothly with a sharp razor blade held in the other hand.
3. Place the sections in a dish of water.
4. Select one or two thin sections, including at least one that is cut through the midrib, and mount them in water on a slide.

B. Stem (e.g. *Hydrilla, Coleus, Wedelia* and *Helianthus*)

Procedure

1. Soak a stem segment in water.
2. Cut thin transverse sections with a razor blade: hold the piece of stem in one hand and the blade in the other and cut smoothly and rapidly, constantly wetting the blade and surface of the stem with water. Transfer the stem sections into a dish of water.
3. Select the thinnest sections for staining (e.g. using safranine) and mount in dilute glycerine.

C. Leaf epidermis (e.g. *Zebrina, Rhoeo discolor* and *onion*)

Procedure

1. Tear a leaf (e.g. *Zebrina*) diagonally and from the torn end of the lower epidermis, strip away a small piece of it by means of forceps.
2. Mount the epidermis on a microscope slide with a drop of water.
3. Place a cover-slip over the slide.
4. Examine the slide under the microscope.

An alternative method

Procedure

1. Place a small drop of colourless nail varnish on a clean microscope slide.

2. Place a leaf on the drop. Hold flat until the nail varnish dries.
3. Peel the leaf from the microscope slide. (A negative replica will be obtained.)
4. Examine the replica using the bright field illumination.

Notes: This method is suitable for observing the distribution of stomata for leaves (e.g. *Coleus*) whose epidermis are difficult to peel off.

D. Animal epithelial cells (e.g. Ox corneal cells)

Procedure

1. With a clean microscope slide, gently touch the surface of the cornea of a fresh ox eye or of an ox eye that had been refrigerated.
2. Add a drop of methylene blue stain on the microscope slide.
3. Place a cover-slip over the slide.
4. Examine the slide under the microscope.

Notes: Large squamous epithelial cells with prominent nuclei can be easily observed.

(2) Preparation of macerated plant material

Macerated plant material is excellent for the study of plant cell forms. A small quantity of the macerated tissue may be mounted in glycerine and observed under a microscope.

Procedure

1. Cut the plant tissue (stem or root) into small pieces of not more than 1 mm thick.
2. Put the tissue into freshly prepared macerating fluid. The fluid is prepared by mixing equal volumes of 10 % chromic acid with 10 % nitric acid.
3. Leave the tissue in the macerating fluid for about three days. (The exact number of days required depends on the type of plant material being used.)
4. Tease the tissue with dissecting needles. If the cells do not separate readily, leave the tissue in the macerating fluid for another day. If the cells separate easily, they are ready for the next step.
5. Filter off the macerating fluid and wash away the acids from the macerated material with tap water.

6. The macerated plant material may be stored in 70% alcohol.
7. The macerated material is ready for temporary mounting.

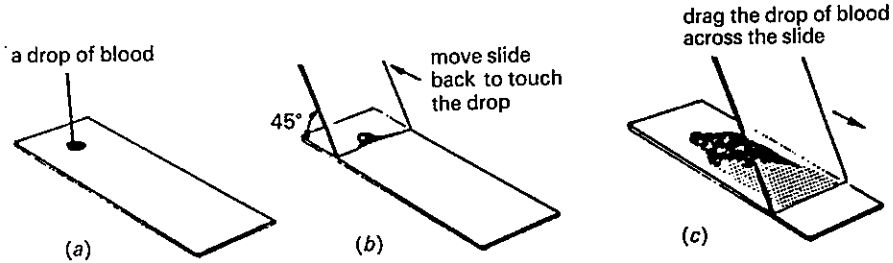
Notes: (1) *Chromic acid and nitric acid are corrosive. Avoid contact with skin.*

(2) Flowering Chinese cabbage, Chinese kale and *Zebrina* are suitable plant materials.

(3) Preparation of a blood smear using chicken's blood

Procedure

1. Collect some blood of a freshly killed chicken with a bottle containing some anticoagulant (e.g. sodium citrate).
2. Place one drop of the chicken's blood onto a microscope slide towards one end of the slide.
3. Make a blood smear by using a second microscope slide as shown in the following figures:



4. Leave the blood smear for three to four minutes until it is dry.
5. Add one or two drops of Leishman's stain and one to two drops of distilled water.
6. Leave the slide for five minutes and wash off the surplus stain under a gently-running tap.
7. Shake off the surplus water and leave the microscope slide to dry.
8. Examine the slide under a microscope.

Notes: *Taking human blood and cell samples in school laboratories carries a risk of transmitting blood-borne diseases such as AIDS (Acquired Immune Deficiency Syndrome) and Viral Hepatitis B. Experiments like mounting of human cheek cells, preparation of human blood smear and investigation of the osmotic tolerance of human red blood cells are at such risk. Such practices should be discontinued in school laboratories.*

Reagent

Anticoagulant

A 0.106M solution of sodium citrate can be prepared by dissolving 3.13 g of the dihydrate salt in 100 cm^3 of water. One volume of the anticoagulant is recommended for 9 volumes of blood.

(4) Food tests

A. Benedict's test for reducing sugar

Reducing sugars include all monosaccharides, such as glucose and fructose, and some disaccharides, such as maltose.

Benedict's solution contains copper sulphate. Reducing sugars reduce copper(II) ions present in the blue copper sulphate solution to insoluble red-brown copper oxide containing copper(I). The latter is seen as a precipitate.

Procedure

1. Add 2 cm^3 of a 0.1% glucose solution to a test-tube.
2. Add an equal volume of Benedict's solution.
3. Shake and boil the mixture.
   (The mixture is likely to bump violently during heating and extra care should therefore be taken. *It is safer to use a water bath to heat up the mixture.*)

Notes: The initial blue colouration of the mixture turns green, then yellowish and may finally form a brick-red precipitate in the presence of reducing sugar. The amount of precipitate gives a rough indication of the amount of reducing sugar present.

B. Iodine test for starch

Starch is only slightly soluble in water, in which it forms a colloidal solution.

Procedure

1. Add 2 cm^3 1% starch solution to a test-tube.
2. Add a few drops of iodine solution.

Notes: A blue-black colouration indicates the presence of starch due to the formation of a complex.

C. Sudan III test for lipids

Lipids include oils , fats and waxes.

Procedure

1. Add 2 cm^3 oil to 2 cm^3 of water in a test-tube.
2. Add a few drops of Sudan III and shake.

Notes: Oils are stained red with Sudan III. Since they are less dense than water, they separate out as a red layer on the surface of the water layer.

D. Biuret test for proteins

A suitable protein for this test is egg albumen. This is a test for the presence of peptide bonds. When mixed with dilute alkaline copper sulphate solution, nitrogen atoms in the peptide bonds form a purple complex with copper(II) ions (Cu^{2+}).

Procedure

1. Add 2 cm^3 protein solution to a test-tube.
2. Add an equal volume of 5% sodium hydroxide solution and mix.
3. Add 2 drops of 1% copper sulphate solution and mix. (No heating is required.)

Notes: (1) Purple colouration is developed in the presence of protein.

(2) Biuret reagent can be used directly for detecting the presence of protein.

(5) Identification of biochemicals in tissues

The chemical composition of cells can be investigated in two main ways. One is to grind up the tissue, or extract the juices from it, and perform chemical analyses on the materials so obtained. Alternatively the tissue can be sectioned, stained with a reagent specific to a certain constituent, and examined under the microscope.

Food test using thin sections of tissue

A. Reducing sugar in apple tissue

Procedure

1. Cut a thin section of apple tissue with a razor blade and mount it on a slide in a few drops of Benedict's reagent.
2. Examine the section under the microscope.
3. Heat the slide gently over a flame until the tissue turns brown. (It is safer to use a water bath for heating.)

4. Add water if necessary to prevent drying.
5. Allow the slide to cool and re-examine the section for any colour change.

B. Oils in castor oil seed/peanut

Procedure

1. Cut a thin section of well soaked castor oil seed/peanut with a razor blade and mount it on a slide.
2. Stain the tissue with Sudan III and wash with water and/or 70% ethanol.
3. Examine the section under the microscope.

C. Starch in potato/maize/banana

Procedure

1. Mount a section of potato tuber in dilute iodine solution.
2. Observe it under microscope.

Notes: Darkly stained starch granules can be seen in the thin section.

Food test using ground materials

D. Protein and reducing sugars in tissue

Procedure

1. Grind small pieces of solid material with a small quantity of water using pestle and mortar.
2. Squeeze the ground material through several layers of premoistened fine muslin or centrifuge it to remove solid debris. (This may be unnecessary if a fairly colourless, fine suspension is obtained.)
3. Test the clear solution as usual, with further dilution if necessary. The solid residue may also be tested if appropriate.

Notes: The following are suitable materials for this experiment:

Fruits such as apple, pear, banana, grape	(reducing sugars)
Peas, egg, soya bean	(protein)
Potato, banana	(starch)

E. Lipid in castor oil seeds/peanut

Procedure

1. Grind the tissue in a mortar.

2. Transfer the ground material to a test-tube containing water. Bring to boiling. Lipid will escape as oil droplets.
3. Perform Sudan III test.

(6) Investigation of the effect of temperature on enzyme activities

Starch is a mixture of two polysaccharides, amylose and amylopectin, the relative amounts of which can vary widely according to the source of the starch. Amylose molecules consist of long straight chains of glucose units. Amylopectin has a complicated branched structure. The two polysaccharides react with iodine to give the characteristic blue-black colour of the standard starch test.

Amylase can degrade starch by hydrolysis. Amylase degrades starch by splitting the long glucose chains into smaller and smaller intermediates, finally producing a complex mixture containing predominantly maltose, but with some glucose and some small branched oligosaccharides. This mixture does not affect the colour of iodine solution.

Amylases are found in almost all plants, animals and micro-organisms. Large amounts of amylase occur in germinating cereals and in the pancreas and saliva of higher animals.

Animal amylases usually have a pH optimum of between 6.9 to 7.1. pH 7.0 is most suitable for their activities. They are activated by chloride ions. However, most amylases extracted from plants and microorganisms have a pH optimum of about 4.5 to 5.0. They are not activated by chloride ions, but can be inhibited by heavy metal ions, iodoacetamide and urea.

In this investigation, amylase extracted from germinating mung beans is used to act on a given quantity of starch at different temperatures. When nearly all of the starch is hydrolysed, the reaction mixture will give a negative result to iodine test. The reciprocal of the time required for the disappearance of starch will give an indication of the relative rate of enzymatic reaction.

Procedure

A. *Extraction of amylase from mung bean*

Amylase can be obtained from 20 mung bean seeds which have been germinated for 72 hours between moist paper towels. Remove the testa. Grind the softened beans in a mortar with 2 cm^3 of a stabilising peptone solution into a fine suspension. Dilute with another 8 cm^3 peptone solution. After standing in the mortar for about 5 minutes with gentle stirring, the mixture is centrifuged for 5 minutes. The supernatant contains the amylase. (Or filter the mixture through four layers of cheesecloth and use the filtrate as the source of amylase.)

B. *Initial measurement of enzyme activity*

1. Put 2 cm^3 of 1% starch and 2 cm^3 of pH 4.7 acetate buffer into a test-tube.
2. Using a dropping pipette, fill each of the cavities in a spot plate with one drop of iodine solution.
3. Using a graduated pipette, add 1 cm^3 of the enzyme extract to the test-tube, and start the stopwatch at the same time. Shake the tube vigorously, and quickly transfer one drop of the reaction mixture onto one of the iodine samples and stir with a glass rod.
4. At 2-minute intervals, shake the tube, transfer a further drop of mixture onto a fresh sample of iodine. Continue until no positive results are obtained.
5. If the above reaction time is too short, dilute the enzyme extract with pH 4.7 acetate buffer so that 1 cm^3 of the diluted enzyme extract can hydrolyse most of the starch in about 10 minutes.

C. *The effect of temperature on amylase activity*

1. Use a dropping pipette to place 1 drop of iodine solution to each cavity of seven spot plates.
2. Label 7 test-tubes from 1A to 7A.
3. Using graduated pipettes, add 2 cm^3 of pH 4.7 acetate buffer and 1 cm^3 of the diluted enzyme extract to each tube.
4. Label 7 additional test-tubes from 1B to 7B. Using a graduated pipette, add 2 cm^3 of 1% starch solution to each tube.
5. Place the above tubes in water baths maintained at different temperatures as shown below for 5 minutes.

Water bath	*Temperature maintained*	*Tubes*
1	0°C	1A & 1B
2	10°C	2A & 2B
3	20°C	3A & 3B
4	30°C	4A & 4B
5	40°C	5A & 5B
6	60°C	6A & 6B
7	100°C	7A & 7B

6. Add the starch solution in each tube B to the correspondingly numbered A tube. Start the stopwatch immediately. Mix well and place the A tubes in their respective water baths described in step 5 for 5 minutes.
7. At 2-minute intervals, shake each tube and transfer one drop of reaction mixture onto an iodine spot. Note for the disappearance of dark blue colour and record the time. (The test may be done at 1-minute intervals if the students can manage to do the test for all seven tubes within this time interval.)

Notes: (1) In carrying out the iodine test for starch in the assay of amylase activity, it is advisable to use a very dilute iodine solution. Do not fill up the cavities of the spot plates with the dilute iodine solution all at the same time because the brown colour gradually fades.

(2) A spot colour made up by 0.01% starch may be used as an arbitrary standard for the absence of starch.

Reagents

1. *Peptone solution*
   A peptone solution is made from 20 g peptone dissolved in 1 000 cm^3 hot distilled water. It must be fresh and should not be more than 24 hours old.
2. *pH 4.7 acetate buffer*
   The buffer solution is prepared by using 8 g anhydrous sodium acetate and 6 cm^3 glacial acetic acid made up to 1 000 cm^3 with distilled water. It should be freshly prepared and should not be more than 24 hours old.
3. *Iodine solution*
   A stock iodine solution is prepared by dissolving 1 g iodine and 2 g potassium iodide in 100 cm^3 distilled water. 0.5 cm^3 of the stock solution is added to 10 cm^3 distilled water prior to use.

(7) Investigation of the effect of pH on enzyme activities

Invertase (sucrase) catalyses the hydrolysis of sucrose to glucose and fructose. Its activity has been demonstrated in bacteria, moulds, higher plants and in intestine of animals.

The pH optimum of invertase is between 4 and 6. The invertase from yeast has a pH optimum of between 4.7 and 4.9 while the invertase from honey has a pH optimum of between 5.5 and 6.2. With yeast invertase a maximum rate of hydrolysis is obtained with sucrose concentrations of 5–10%, and then with higher concentrations, the rate decreases. Heavy metals such as Cu^{2+}, Hg^{2+} and Ag^{+} will also inhibit its activity.

In this investigation, the invertase extracted from grasshopper is allowed to act on a given quantity of sucrose at different pHs. The amount of end-products (reducing sugars) formed can be measured by the amount of red/orange precipitate obtained on boiling with Benedict's' solution.

Procedure

A. *Extraction of invertase from grasshopper*

1. Kill the grasshopper by decapitation. Remove its wings and limbs. Affix it with pins in a wax tray and cover it up with water. Dissection is done in water if it can be carried out quickly or in Insect Ringer if slowly.
2. Slit the grasshopper open dorsally and isolate the intestine. Remove the thoracic muscles, fats and reproductive organs which cover the intestine.
3. Transfer the intestine onto a watch-glass. Slit open the intestine to remove any food or faecal content. Rinse with chilled distilled water.
4. Place the intestine in a mortar. Add 2 cm^3 of chilled distilled water and grind it with a pestle. Allow the extract to settle.

B. *The effect of pH on invertase activity*

1. Set up six test-tubes each containing 1 cm^3 of 10% sucrose solution and 1 cm^3 of citrate-phosphate buffer solution as shown below.

Tube	*1A*	*2A*	*3A*	*4A*	*5A*	*6A*
pH of buffer solution	3.2	4.0	5.2	6.0	7.0	8.0

2. Label 6 additional test-tubes from 1B to 6B. Using a graduated pipette, add 3 cm^3 of Benedict's solution to each tube.
3. Add 20 drops of enzyme extract to each tube A.
4. Allow the tubes to stand at room temperature for about 5 to 10 minutes.
5. Add the Benedict's solution in each tube B to the correspondingly numbered A tube. Agitate gently and heat in a beaker of boiling water for 5 minutes. Measure the amount of orange/red precipitate formed. (Students could be asked to design their own methods to assess the relative or absolute amount of orange/red precipitate formed.)

Notes: (1) The reaction time should be kept constant for all tubes.

(2) Excess amounts of Benedict's solution should be used to ensure that all the reducing sugars formed will be oxidised.

(3) The amount of orange/red precipitate formed can be estimated/compared by one of the following methods:

(*a*) Use an arbitrary system of '+' to denote the relative amount of precipitate.

(*b*) Measure the depth of precipitate sedimented in the test tubes.

(*c*) Weigh the precipitate formed. (The filter paper with the precipitate should be dried and cooled in a desiccator before weighing.)

Reagent

Citrate-Phosphate buffer

Buffers of different pH values are prepared by mixing solutions A (0.1M citric acid) and B (0.2M disodium hydrogenphosphate) in the proportions shown below.

pH	*Solution A (cm³)*	*Solution B (cm³)*
3.2	37.6	12.4
4.0	30.7	19.3
5.2	23.2	26.8
6.0	18.4	31.6
7.0	8.8	41.2
8.0	1.4	48.6

(8) Investigation of the effect of enzyme concentration on enzyme activities

Catalase is an enzyme present in nearly all living cells. It catalyses the decomposition of hydrogen peroxide to water and oxygen.

$$2H_2O_2 \longrightarrow 2H_2O + O_2$$

Hydrogen peroxide is produced in cells as a by-product in a number of chemical reactions involving oxidative enzymes. It is chemically very reactive, and toxic to cells. Catalase located in cell organelles is believed to protect the cells by destroying hydrogen peroxide.

The activity of catalase on the decomposition of hydrogen peroxide to water and oxygen can be determined by:

(1) measurement of the heat production of the catalase reaction. The maximum rise in temperature after the start of the reaction can serve as an approximate measure of the enzymatic activity;

(2) direct measurement of the amount of end-product (oxygen); or

(3) indirect measurement of the amount of substrate (hydrogen peroxide) not acted upon.

In this investigation, the third method is used to study the effect of enzyme concentration on the action of catalase on hydrogen peroxide.

Assay of catalase activity by iodometric method

The activity of catalase can be determined by measuring the amount of hydrogen peroxide that has not undergone degradation by adding them to excess potassium iodide and then titrating the liberated iodine with sodium thiosulphate.

Hydrogen peroxide reacts with iodide in an acid medium. Ammonium molybdate is added as a catalyst.

$$H_2O_2 + 2H^+ + 2I^- \longrightarrow I_2 + 2H_2O$$

The iodine liberated is then titrated with standard sodium thiosulphate solution using starch as the indicator.

$$I_2 + 2S_2O_3^{2-} \longrightarrow S_4O_6^{2-} + 2I^-$$

Procedure

1. Label six 250 cm³ conical flasks from 1A to 6A and six test-tubes from 1B to 6B.
2. Use a cork borer to cut out 6 potato cylinders with a length of about 5 cm and diameter 0.8 cm. Trim one end of the cylinders with a sharp razor blade and slice off 15 discs, each about 2 mm thick, from each cylinder.
3. Add 4 cm³ of 2M sulphuric acid, 3 cm³ of 10% potassium iodide to each conical flask. Mix the contents well.
4. Add 10 cm³ of hydrogen peroxide (1 vol or about 0.1M) to each of the 6 test-tubes.
5. Place the potato discs into the six test-tubes as shown below and start timing instantaneously.

Tube	*1B*	*2B*	*3B*	*4B*	*5B*	*6B*
Number of potato discs	4	8	12	16	20	25

6. After exactly 10 minutes, transfer a 5 cm³ sample from each test-tube to the correspondingly labelled conical flask.

7. Add 1 drop of 3% ammonium molybdate solution to the mixture. Shake to mix the contents well. Allow the contents to stand for 2 minutes.
8. Titrate the iodine that has been set free with standard (0.1M) sodium thiosulphate solution until a faint straw colour develops.
9. Add 2 cm^3 of 1% starch solution and continue the titration until the blue colour is just discharged.
10. The volume of sodium thiosulphate required for titration is used as an indication of the relative rate of catalase activity.

(9) Investigation of the effect of substrate concentration on enzyme activities

Urease occurs in bacteria, moulds, higher plants, particularly in the Cucurbitaceae and Leguminosae and in some lower animals. Urease is virtually substrate-specific. It acts on urea and converts it into ammonia and carbon dioxide by hydrolysis of the C-N bonds. Thiourea [$CS(NH_2)_2$], though having the same C-N bonds, is not hydrolysed at all by urease.

$$H_2N\text{–}CO\text{–}NH_2 + H_2O \longrightarrow 2NH_3 + CO_2$$

The ammonia liberated can be detected by using the indicator bromothymol blue which turns yellow/green in acid solution, but is blue in alkaline solution. As urease can retain its optimal activity over a wide pH range (between pH 5 and 10), the experiment can be started on the acid side of pH 7 and that increase in pH due to the liberation of ammonia in the course of reaction has little effect on the rate of enzymatic reaction.

In this investigation, the effect of different concentrations of urea on the rate of hydrolysis of urea by urease to carbon dioxide and ammonia is studied.

Procedure

A. *Extraction of urease from soya beans*

1. Weigh 1 g of soya beans.
2. Soak the beans in 5 cm^3 of distilled water in a test-tube for 1 day.
3. Pour the softened beans into a mortar and grind it into a milky paste.
4. Add 25 cm^3 of distilled water to dilute the paste.
5. Filter the suspension through 4 layers of muslin cloth to remove cell debris and centrifuge for 5 minutes. Use the supernatant liquid as a source of urease.

B. *Effect of urea concentrations on urease activity*

1. Label 6 test-tubes from 1 to 6.

2. Using graduated pipettes, place the following volumes of bromothymol blue solution, 2% urea solution and distilled water in tubes 1 to 6 as follows:

Tube	*1*	*2*	*3*	*4*	*5*	*6*
Bromothymol blue (cm^3)	1	1	1	1	1	1
2% urea solution (cm^3)	2	1.5	1	0.8	0.5	0.2
Distilled water (cm^3)	0	0.5	1	1.2	1.5	1.8

3. Mix the contents of each of the 6 test-tubes well. Place them in a water bath at 35°C for 5 minutes.
4. Add 0.5 cm^3 of the enzyme extract into each of the six tubes. Start the stopwatch immediately. Shake thoroughly to mix the contents of the tubes and quickly place them back in the water bath.
5. Record the time taken for the bromothymol blue to change from yellow/green to blue.

(10) Investigation of the enzymatic activities of biological washing powders

Biological washing powders contain amylase and proteases. Their presence can be demonstrated by the action of these enzymes on starch and milk protein (casein).

The enzyme amylase is allowed to digest starch in agar. The disappearance of starch is indicated by a clear zone surrounding the solution of the biological washing powder when the starch-agar is flooded with iodine solution.

The white colour of milk-agar is due to casein. If protease is present, casein will be digested by the enzyme giving a clear zone in the milk-agar plate.

Procedure

1. Prepare solutions of 3 different brands of biological washing powders.
2. Take one Petri dish containing milk agar and another containing starch agar.
3. Flame the end of a cork borer in a Bunsen flame and allow it to cool.
4. Lift the lid off a Petri dish. Gently press the borer down into the agar to make four 'wells' in the agar. Replace the lid of the Petri dish as quickly as possible.

5. Repeat steps (3) and (4) for the other Petri dish.
6. Take the lid off one of the Petri dishes. Use a dropper to place drops of a solution of biological washing powder into one of the four wells until it is full. Care should be taken not to flood the well or allow the drops to fall elsewhere on the agar. Replace the lid as quickly as possible.
7. Repeat step (6) for the other Petri dish. Rinse the dropper thoroughly.
8. Repeat steps (6) and (7) for each of the other two samples of biological washing powder.
9. Place distilled water into the last well of each Petri dish.
10. Incubate the plates for 24 to 48 hours at 35°C.
11. Measure and compare the diameter of the clear zones around the wells in the milk-agar plate by placing the Petri dish on a graph paper and examine the plate against light.
12. Take the lid off the starch-agar Petri dish. Add enough iodine solution to cover the agar completely. After 1 to 2 minutes, discard the iodine solution. Wash off any remaining iodine solution under slow-running water.
13. Measure and compare the diameter of the clear zones around the wells in this plate as in step (11).

Preparation of milk-agar plate

1. Dissolve 2 g of dried milk powder in 20 cm^3 of distilled water.
2. Suspend 1 g of agar powder in 80 cm^3 of distilled water. Bring to boiling with constant stirring so as to dissolve the agar completely.
3. Transfer the milk solution to the hot agar solution. Stir to mix.
4. When the milk-agar solution is cooled to 45-50°C, pour it into clean Petri dishes. Replace the lids and allow the plates to cool and set.

Preparation of starch-agar plate

1. Suspend 2 g of agar powder in 50 cm^3 of distilled water. Bring to boiling with constant stirring so as to dissolve the agar completely.
2. Heat 1 g of soluble starch in 50 cm^3 of distilled water to form a colloidal solution.
3. Allow to cool and then mix with the agar solution.
4. When the starch-agar solution is cooled to 45-50°C, pour it into clean Petri dishes. Replace the lids and allow the plates to cool and set.

Preparation of washing powder solution

Add about 3 g of the washing powder to a test-tube. Add 10 cm^3 of distilled water to the test-tube with a measuring cylinder. Mix and allow to settle.

(11) Investigation of the enzymatic activity of meat tenderizers

Emulsion layers of photographic negative (B/W) contain silver granules impregnated in a layer of gelatin (a protein). If a protease solution is dropped onto it, the gelatin is digested, and the black silver granules are released, thus upon washing, a clear spot will be left behind at the digested region.

Procedure

1. Dissolve 1 g of a sample of meat tenderizer to be tested in 2 cm^3 of distilled water.
2. Cut 2 strips of film (0.5 × 3.5 cm) from an exposed and developed photographic negative.
3. Mount the two strips onto a microscope slide with adhesive tape and put it in a moist chamber made of Petri dish containing a piece of moist filter paper.
4. Add 2 drops of the meat tenderizer solution onto the emulsion surface of one strip and 2 drops of distilled water onto the emulsion surface of the other strip.
5. Incubate the Petri dish at 37°C for 2 hours. After 2 hours, wash the two strips and note for any clear spot formed.

Notes: (1) The incubation time depends on the quality and source of photographic negatives. Some may take a longer time to obtain good results. It is advisable to try out the material first and determine the appropriate incubation time required.

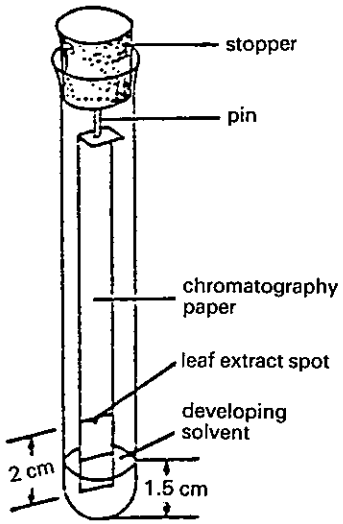
(2) B/W photographic negatives which have been exposed, developed and fixed (without hardener) are good materials for this experiment. B/W and colour photographic negatives, which have been exposed, developed and fixed in the presence of hardener, may also be used. However, longer incubation times and/or higher temperatures are needed for the reaction.

(12) Extraction and separation of leaf pigments by paper chromatography

Plant material : *Allium* leaves or *Zebrina* leaves or spinach leaves
Extraction solvent : acetone/petroleum ether (1:1)
Developing solvent : acetone/petroleum ether (1:9)

Procedure:

1. Prepare strips of chromatography paper of the right width so that each can be hung freely in a boiling tube without touching the wall. The length of the paper should be trimmed according to the level of solvent to be put into each boiling tube.
2. Mark an origin for spotting on the paper about 2 cm from the lower edge of the paper.
3. Transfer appropriate amount of the developing solvent into the boiling tube (a depth of not more than 1.5 cm). Stopper them for equilibration.
4. Preparation of pigment extract:
   (*a*) Wash leaves and blot dry.
   (*b*) Cut leaves into small segments and put into a mortar.
   (*c*) Grind the tissue in the extraction solvent with a pestle to extract the pigments.
5. By means of a capillary tube spot the extract onto the origin marked on the paper strip and allow it to dry.
6. Repeat the spotting and drying processes to obtain a small area of concentrated leaf extract.
7. Pin the paper onto the lower surface of the cork and insert the paper into the boiling tube. The solvent should cover the lower end of the paper but without touching the spot.



8. Allow the chromatogram to develop until the solvent front has moved up for 10 cm or more.
9. Take out the chromatogram, mark the solvent front and allow the chromatogram to dry in air.
10. Use a pencil to circle each spot.
11. Calculate the R_f value of the various pigments.
12. Identify the pigments by their colours and their relative positions on the chromatogram.

(13) Investigation of the effects of environmental factors on the rate of photosynthesis

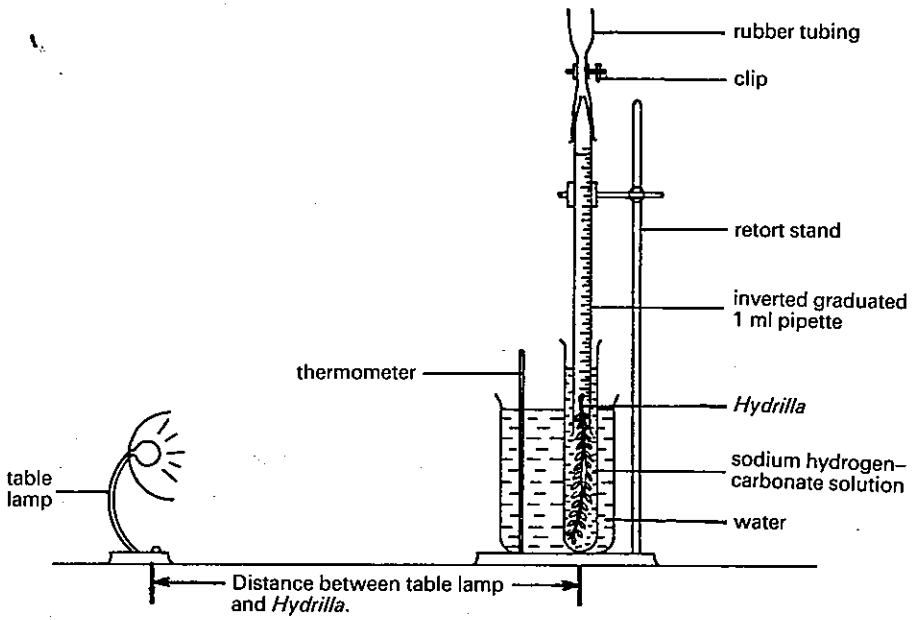
The rate of photosynthesis of a green plant is affected by factors such as light quality, light intensity, carbon dioxide concentration and temperature.

A convenient way of investigating the effects of these factors on the rate of photosynthesis is to measure the volume of oxygen given off by a plant in a given time.

When a particular factor is being investigated, it is essential that other factors are kept constant and, if possible, at optimum levels so that no other factor is limiting.

Pondweeds, like *Hydrilla* and *Ceratophyllum*, are suitable plant materials for this experiment. It is advisable to use the plant materials that have been well illuminated for three or four hours before experiment.

The following diagram shows an experimental set-up for the investigation of the effects of environmental factors on the rate of photosynthesis.



A. To investigate the effect of light quality on the rate of photosynthesis

Procedure

1. Cut the stem of a well illuminated piece of *Hydrilla* to about 10 cm long and place it, cut surface upwards, in a test-tube filled with 0.25% solution of sodium hydrogencarbonate.
2. Wrap the test-tube with a red cellophane.
3. Set up the apparatus as shown in the above diagram.
4. Stand the test-tube in a beaker of water at room temperature. Record the temperature of the water, which acts as a heat shield, and check it at intervals throughout the experiment. It should remain constant and the water should be renewed if necessary.
5. Put a 100 watt table lamp at 20 cm from *Hydrilla*. Turn on the lamp.
6. Wait for about 5 minutes for *Hydrilla* to equilibrate.
7. Suck the sodium hydrogencarbonate solution from the test-tube into the pipette. Close the clip and record the initial reading. Make sure that the clip is tightened to prevent the solution in the pipette from falling due to gravity.

8. After a fixed period of time (e.g. 5 minutes), record the final reading in the pipette and calculate the volume of gas collected.
9. Repeat the experiment using white and green cellophane respectively.

B. To investigate the effect of light intensity on the rate of photosynthesis

Procedure

1. Cut the stem of a well illuminated piece of *Hydrilla* to about 10 cm long and place it, cut surface upwards, in a test-tube filled with 0.25% solution of sodium hydrogencarbonate.
2. Set up the apparatus as shown in the above diagram.
3. Stand the test-tube in a beaker of water at room temperature. Record the temperature of the water, which acts as a heat shield, and check it at intervals throughout the experiment. It should remain constant and the water should be renewed if necessary.
4. Put a 100 watt table lamp at 20 cm from *Hydrilla*. Turn on the lamp.
5. Wait for about 5 minutes for *Hydrilla* to equilibrate.
6. Suck the sodium hydrogencarbonate solution from the test-tube into the pipette. Close the clip and record the initial reading. Make sure that the clip is tightened to prevent the solution in the pipette from falling due to gravity.
7. After a fixed period of time (e.g. 5 minutes), record the final reading in the pipette and calculate the volume of gas collected.
8. Repeat steps (5) to (7) respectively at different distance (d) of the table lamp from *Hydrilla*: 10 cm, 30 cm, 40 cm and 50 cm.
9. Plot a graph of the photosynthetic rate (volume of gas collected per unit time) against light intensity ($1/d^2$).

C. To investigate the effect of carbon dioxide concentration on the rate of photosynthesis

Procedure

1. Repeat the above B(1) to B(7) steps respectively at different concentrations of sodium hydrocarbonate solution: 0.1%, 0.15%, 0.2%, 0.25%, 0.3% and 0.4%.
2. Plot a graph of the photosynthetic rate (volume of gas collected per unit time) against concentration of sodium hydrogencarbonate solution.
3. From the graph, determine the concentration of the sodium hydrogencarbonate solution at which the volume of gas collected begins to level off.

4. Repeat the above B(1) to B(7) steps at the concentration determined in (3) using a higher light intensity by putting the table lamp at a closer distance.

D. To investigate the effect of temperature on the rate of photosynthesis

Procedure

Repeat the above B(1) to B(7) steps respectively at different temperatures: 5°C, 10°C, 15°C, 25°C, 30°C, 35°C and 40°C.

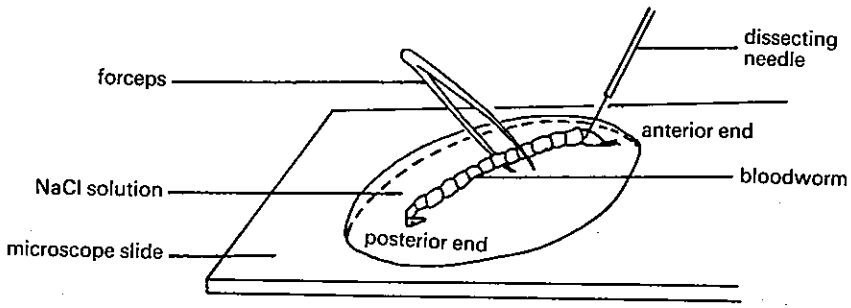
Notes: At each change of experimental conditions in the above investigations, allow the plant to equilibrate before readings are taken.

(14) Preparation of squash of giant chromosomes

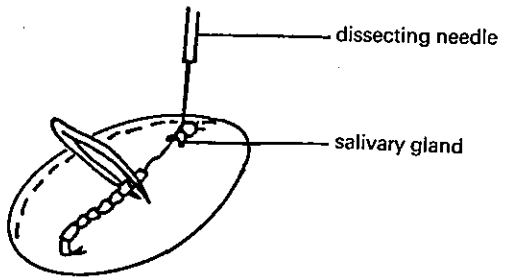
Certain insect chromosomes, notably those in the salivary glands of Chironomid/*Drosophila* larvae, are unusually large and if appropriately stained can be seen to be banded. Correlations between cross-over data and the positions of the bands suggest that the bands correspond to specific gene loci.

Procedure

Place a chironomid larva (bloodworm) on a slide with a drop of water/NaCl solution. Hold the larva firmly with forceps. Then, with a downward and forward movement of the dissecting needle, pull the head end and separate it from the rest of the body.



The salivary glands will generally be drawn out cleanly with the head and can easily be recognised as two flattish structures, attached to the anterior of the gut, with large cells and prominent nuclei.



Add a drop of aceto-orcein stain. Leave the gland in the aceto-orcein stain without covering for about 20 minutes. If the gland begins to dry up, add a second drop of stain. Place a cove-slip over the gland in the drop of stain. Lay a paper towel on the desk, turn the slide over, and lay it face down on the towel. Press firmly on the back of the slide with the tip of the index finger, so as to blot thoroughly. The gland can then be squashed as follows: Place the slide on the desk, cover-slip up; and holding the edge of the cover-slip steady with the left hand, press hard and intermittently on the cover-slip with the end of the wooden handle of a dissecting needle.

(15) Preparation of root tip squash for the study of mitosis

Onion (*Allium cepa*) is very useful for root-tip preparation in the study of different stages in mitosis.

Preparation of onion root tip

1. Select a large onion bulb with undamaged modified disc-like stem.
2. Under a running tap, gently rub the stem with finger tips to remove old, withered roots and adhering soil particles.
3. Place the bulb on a glass jar of suitable size filled with tap water so that the stem is completely immersed in water.
4. Change the water in the jar twice daily.
5. When the adventitious roots have grown to 2–3 cm, cut and fix the distal 1–2 cm of the roots in freshly prepared fixative (acetic alcohol). This should be collected at about noon because the peak of mitotic activity for onion are from 1.00 p.m. to 2.00 p.m. and 11.00 p.m. to

midnight. [*Remember*: it is the terminal 1–3 mm part of the root which will be used for preparing slides and, therefore, do not discard the tip portion of the root.] Shake the contents of the vial. Pour off the used up fixative, and add more of the same. Allow it to stand at room temperature for 24–48 hours.

6. After 24–48 hours pour off the fixative, and add 70% alcohol to the vial and shake. Pour off the alcohol, repeat twice, and finally fill the vial with 70% alcohol. The roots are now ready for making squash preparations but, if you do not intend to use them in the next week or two, store them in a refrigerator. Properly fixed roots can be stored for 2–3 months at low temperature without any impairment of the quality of squashes made from them.

Notes: Onion bulbs may not sprout if the temperatures are too warm, e.g. during summer.

Procedures for making squash

1. Near one edge of a clean slide, place a root tip and excise the terminal 2–3 mm portion. Discard the proximal part of the root.
2. Place a drop of aceto-orcein stain in the middle of the slide and transfer the terminal portion of the root to it with forceps.
3. With a spear needle and a narrow blade scalpel tease the root tip into a number of small pieces.
4. With the aid of a 'masher', crush the root tip tissue into a thorough pulp so that no large pieces are observable on the slide. If necessary, add another drop of stain but avoid excess.
5. Drain off the 'masher' of all the stain sticking to it to minimise the loss of cellular material from the slide.
6. Place a cover-slip over the suspension of cells. Gently tap the cover-slip with the rubber end of a pencil or the wooden end of a dissecting needle to spread the material. Care must be taken not to move the cover-slip.
7. Place the slide over a steam bath or warm it over a spirit lamp until the stain at the cover-slip edges begins to show signs of drying off.
8. Remove the slide from the steam bath and further flatten the cells by applying firm pressure from above while the slide is being held between the folds of a mat made from sheets of toilet tissue paper. This will also expel the excess stain from under the cover-slip, as well as spread the chromosome content of dividing cells in an even layer.

Precautions:

(*a*) Avoid too much pressure as this will result in breaking the cover-slip.

(*b*) The cover-slip, while flattening the cells, must retain its original position on the slide and should not be displaced, even slightly. If this happens, the preparation will be marred.

9. This slide may be used for observation for about 30 minutes without sealing. This is a temporary preparation and of limited useful life. Its usefulness can be extended to 2–3 days if the stain and cells under the cover-slip are sealed off by nail varnish. The initial stain may intensify upon storage.
10. When examining the preparation, correct lighting is important and green light is most beneficial. Very good squash preparations can be examined with an oil-immersion lens.
11. The above method uses fresh materials and does not require fixation. Alternatively, the plant material can be fixed for 24 hours in Carnoy's fixative. The fixed material is then stained by warming it in aceto-lacmoid + HCl stain for five minutes. The HCl is important in aiding the penetration of the stain by macerating the tissues. Lack of fixation sometimes results in rather bubbly chromosomes, which occasionally appear double. In such case care is required in interpreting such preparations.

Reagents

1. *Acetic-alcohol*

Glacial acetic acid	1 part
Absolute ethanol	3 parts

(Mix just before using.)

2. *Aceto-orcein stain (1% solution in 45% acetic acid)*

(*a*) Introduce 1 g of orcein into a flask.

(*b*) Add about 55 cm^3 of boiling water into the flask to dissolve the stain.

(*c*) Add about 45 cm^3 of glacial acetic acid into the flask and shake for thorough mixing.

(*d*) Filter and store in a glass stoppered dark bottle in a refrigerator.

3. *Carnoy's fixative*

Glacial acetic acid	1 part
Chloroform	1 part
Absolute ethanol	1 part

4. *Aceto-lacmoid stain*

Lacmoid	0.2 g
Orcein	3.3 g
Glacial acetic acid	100 cm^3

(Add two drops of 1M HCl to a watch-glassful of the stain before use.)

(16) Genetic screening and counselling

Congenital defect is a structural or chemical imperfection that develops during pregnancy. It may or may not be inherited. Some congenital abnormalities are produced by environmental factors that affect the developmental process. For example, if a woman contracts the viral disease, rubella (commonly known as German measles) during the first 3 months of pregnancy, there is a substantial risk that her offspring will show congenital malformations. Many other congenital abnormalities, however, are the result of mutations involving a single locus (e.g sickle cell anaemia, glucose-6-phosphate dehydrogenase (G6PD) deficiency and phenylketonuria), or resulting from an abnormal number of chromosomes (e.g. Down's syndrome).

Down's Syndrome

Down's syndrome is a genetic abnormality caused by the presence of an extra small autosome. Individuals with this syndrome have characteristic physical features, and varying degrees of mental retardation. It is usually caused by meiotic non-disjunction of the chromosome pair No. 21 during the production of ova. The fusion of an ovum containing 24 chromosomes with a normal sperm will result in an offspring having 47 chromosomes. The technical name for Down's syndrome is trisomy 21.

The occurrence of Down's syndrome is not very uncommon. It occurs in about 0.1–0.2% in all births. However, there is a marked increase in the incidence of Down's syndromes with increasing maternal age.

Maternal Age	*Estimated Rate (No. of Down's syndrome patients/birth)*
20	1/2000
25	1/1200
30	1/900
40	1/360
45	1/30
50	1/12

Early detection of whether or not a foetus will develop into a child with Down's syndrome can be achieved by the following intrauterine diagnostic techniques. Prenatal diagnosis of this chromosome abnormality is mostly performed on pregnant women over 35 years of age, as they have a high risk of having children with Down's syndrome.

(1) *Amniocentesis*

In this diagnostic technique, a needle is inserted through the lower abdomen of the pregnant woman and through the wall of the uterus into the uterine cavity, and a sample of the fluid surrounding the foetus (the amniotic fluid) is obtained by drawing it into a syringe. The position of the foetus and the needle can be determined through ultrasound scanning. The living foetal skin cells suspended in the amniotic fluid are grown in cultures in the laboratory. After 2 to 3 weeks, the dividing cells from the culture can be studied for any evidence of the genetic abnormality, Down's syndrome.

(2) *Chorionic villus sampling (CVS)*

In this diagnostic technique, a narrow tube is inserted into the womb via the vagina, and a sample of embryonic tissue is taken from the chorionic villi by suction. Ultrasound scanning is used to help to guide the tip of the tube to the chorionic villi. As the chorionic villi cells are undergoing active cell division, they can be studied directly, and the result of the test can be available within 24 hours.

Although CVS may be associated with a higher risk of infection or miscarriage than amniocentesis (1–2%), it has the advantage of being able to be carried out at 10–12 weeks of pregnancy (sometimes as early as the eighth week of pregnancy) compared with 16–18 weeks for amniocentesis.

With the aid of the above diagnostic techniques, it is now possible to counsel parents about the incidence of Down's syndrome in their unborn child. The parents are then able to decide whether or not to have the pregnancy terminated. This is always a hard decision to make. Some people believe it ethically or morally wrong to prevent any foetus from reaching maturity. Others are of the opinion that children with very severe abnormalities should not be borne because of the suffering they, and their parents alike, may have to endure.

Phenylketonuria (PKU)

Phenylketonuria is an example of hereditary diseases known as inborn errors of metabolism. Individuals with phenylketonuria are homozygous recessive.

They cannot convert phenylalanine (an amino acid abundant in some dairy products) to another amino acid (tyrosine), because their liver cells cannot synthesise the enzyme specific for this chemical conversion. Instead the phenylalanine accumulates in the blood, causing irreversible damage to the brain. Other organs and tissues such as muscles and cartilage also fail to grow and develop normally.

A PKU infant is usually healthy at time of birth because the mother produces enough enzymes to prevent accumulation of phenylalanine. However, if PKU is not detected soon after birth, the child usually becomes mentally retarded and cannot walk properly.

Infants with phenylketonuria can now be diagnosed easily by the presence of high levels of phenylalanine in their blood or by the presence of an abnormal compound, phenylpyruvic acid, in their urine. Infants, identified to have PKU at birth, can be raised from birth on a special diet low in or free of phenylalanine. This special diet can be stopped at about nine years of age when their brains will have been fully developed. Females with PKU must return to the special diet when they become pregnant. If they do not, the high concentration of phenylalanine in their blood will damage the brain of the developing foetus. It is therefore important that all PKU females are to be carefully monitored, not only during their infancy and childhood, but throughout their reproductive life as well.

PKU is a rare genetic abnormality. Its incidence is about 1 in 25 000 (0.004%) in the general population. However, it is relatively more common in Europe and the USA (about 1 in 10 000 births). Because PKU can be successfully treated if diagnosed early, most states in the USA have extensive PKU screening programmes in which the blood of newborn babies is routinely checked for excessively high level of phenylalanine.

Glucose-6-phosphate dehydrogenase (G6PD) deficiency

G6PD deficiency is one of the most common inborn errors of metabolism, affecting over 100 million people. The G6PD gene is located on the X-chromosome and is highly polymorphic, with over 300 alleles known. Individuals with G6PD deficiency will have varying degrees of haemolytic anaemia. When they are treated with drugs of certain groups (such as antimalarials, sulphonamides, nitrofurans, antipyretics, analgesics, and sulphones), their red blood cells, due to the deficiency of the enzyme, G6PD, will undergo haemolysis. G6PD deficiency is now known to be responsible for an acute anaemia called favism which develops in some people after eating broad beans, *Vicia faba* or inhalation of the pollens of this plant.

G6PD deficiency is quite common in Hong Kong. The prevalence of G6PD deficiency in local male babies is about 4.5%. Because of the high incidence of G6PD deficiency in boys, and the suspected effect of naphthalene in causing haemolytic anaemia in pupils with G6PD deficiency, the use of naphthalene (previously for melting point experiments) in secondary schools has been discontinued.

A neonatal screening programme for glucose-6-phosphate dehydrogenase (G6PD) deficiency has been introduced in 1983 by the then Medical and Health Department. This screening programme now covers all babies born in Hong Kong, including those born in the private hospitals.

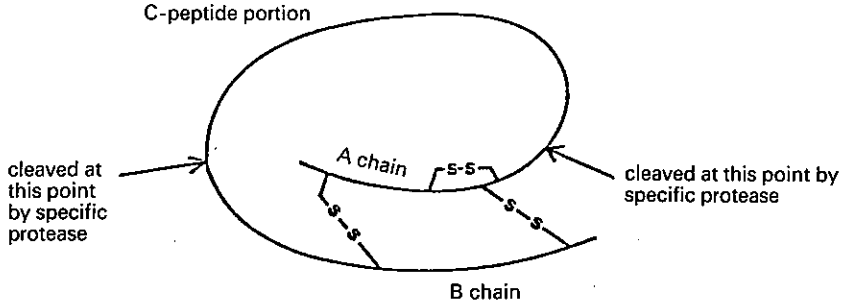
(17) Application of recombinant DNA technology in the production of human insulin

Diabetes mellitus is a disease of abnormal carbohydrate metabolism in which less glucose can enter the body's cells and be utilised, and therefore remains in the blood in high concentrations. There are two forms of diabetes mellitus. One form [the non-insulin dependent diabetes (NIDD)] is more common (about 80%) and less serious. It usually starts in middle to late life, and is frequently associated with obesity. It is not due to the failure of insulin secretion. In fact, the insulin levels are the same as or even higher than those found in normal individuals. Cells of individuals with this form of diabetes do not have sufficient insulin-recognition receptors on their surface membranes; and hence do not respond to insulin by taking in and utilising blood glucose as normal cells do. Its treatment does not require insulin injection. It is treated either by restricting carbohydrates in the diet or by pills, orally active anti-diabetic agents, which cause a lowering of blood sugar levels.

The second form of diabetes is known as insulin-dependent diabetes (IDD). It occurs less frequently (20%), but is much more serious. It often, though by no means always starts before the age of 20. It is due to the destruction of the beta cells in the pancreas which are specialised for insulin secretion. If untreated, it is usually fatal. However, it can be treated by insulin injections.

Before the 1980s, patients with IDD are treated by injections of insulin extracted from the pancreas of cattle and pigs. These insulins are very similar to the human insulin. The pig insulin differs from that of human by only one amino acid. However, such slight difference is sufficient for the body's defense mechanisms to recognise the pig insulin as a foreign substance, and produced antibodies against it. This has caused some undesirable side effects. In the 1980s, human insulin became increasingly available through the use of recombinant DNA technology.

Insulin consists of two separate polypeptide chains which are held together by two disulphide bonds. One, the A chain, contains 21 amino acids; and the other, the B chain, is 30 amino acid residues long. However, these two polypeptide chains are not synthesised separately. In fact, insulin is formed from a single precursor polypeptide chain (the proinsulin). Human proinsulin consists of 86 amino acids. The first 30 amino acids will later form the B chain and the last 21 amino acids the A chain. Between them is a stretch of 35 amino acids called the C-peptide portion. When the whole



Human Proinsulin

proinsulin molecule folds up, the C-peptide portion will bring the A and the B chains together in correct alignment so that the specific disulphide bonds are formed between them. Specific proteases then cleave the proinsulin at 2 points, and free the biologically active insulin molecule from the C-peptide portion.

Production of human insulin using recombinant DNA technology

A. *Obtaining the human insulin gene*

There are two ways that this is commonly done:

(*a*) *Making a DNA complementary copy (cDNA) of the messenger RNA (mRNA) for human insulin*

Messenger RNAs from human pancreas beta cells are isolated and purified. Special enzyme (reverse transcriptase) is used to make cDNA from the insulin mRNA. The single-stranded DNA is then used to make double-stranded DNA molecules. By this method, DNA coding for the synthesis of proinsulin is obtained.

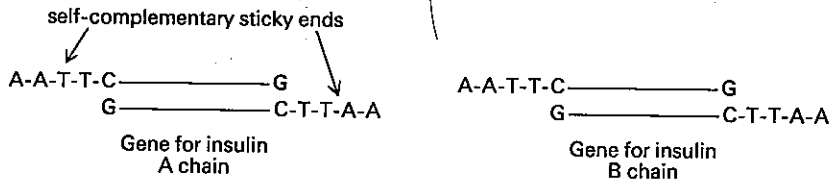
(*b*) *Making the required gene by joining nucleotides together in vitro chemically*

Since the amino acid sequences of insulin A chain and B chain are known, synthetic genes for these two polypeptides are constructed by joining the nucleotides, one at a time, into the predetermined sequences.

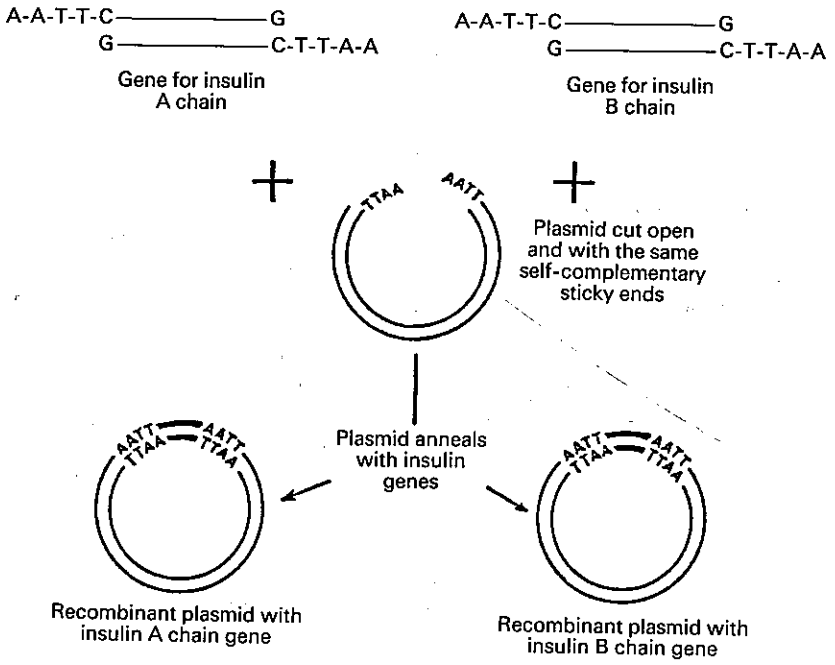
The human insulin gene obtained in either way can be used for the production of insulin. However, the technical details of the production are quite different for DNA obtained in different ways. The production of human insulin using DNA obtained in (*b*) is described below to illustrate the principles of recombinant DNA technology.

B. *Joining the human insulin genes to a vector*

Each of the two synthetic insulin genes from (*b*) is first attached to a bacterial control system (the *lac* operon), and single-stranded 'sticky ends' of self-complementary base sequences are added to each end of the DNA fragments.

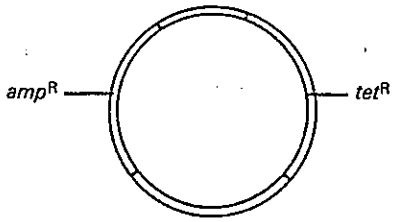


A suitable plasmid is cut open by a special enzyme (restriction endonuclease) that leaves the plasmid with the same self-complementary sticky ends as the synthetic human insulin genes.



The plasmid and the synthetic insulin genes are mixed together under conditions that favour annealing of complementary strands. The human insulin gene is inserted into the plasmid through base pairing between their complementary sticky ends. Special enzyme (DNA ligase) is used to join the ends together to reseal the DNA, thereby creating a recombinant plasmid with a human gene in it. Some recombinant DNA plasmids will have the gene for human insulin A chain inserted in them, while some will have the gene for B chain inserted in them.

Notes: Plasmids are small double stranded circular DNA molecules present in many bacteria. They can leave some bacterial cells and enter other bacterial cells freely. One group of plasmids, the R-plasmids (e.g. pBR322), confer antibiotic resistance to the bacterial cells that host them. Under selective conditions, these R-plasmids can replicate much faster than the main bacterial chromosome, so that hundreds of copies may be present in a single bacterial cell. Consequently, any foreign gene artificially inserted into such a plasmid will also simultaneously be replicated.

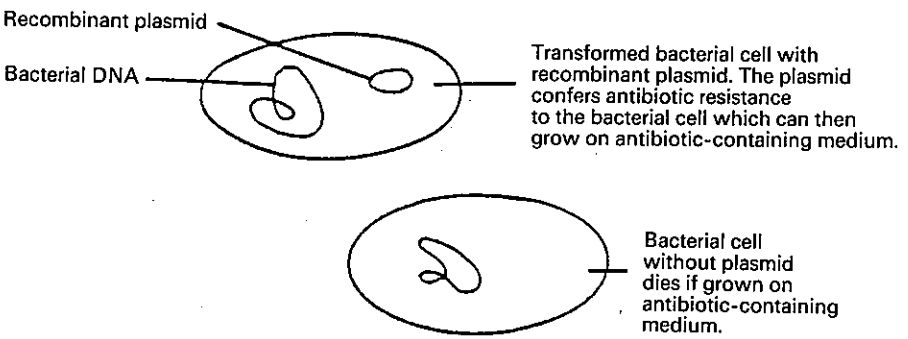


Plasmid pBR322

Key: amp^R = gene for resistance for the antibiotic ampicillin
tet^R = gene for resistance for the antibiotic tetracycline

C. *Introducing recombinant DNA plasmids into bacteria*

The gut bacteria *Escherichia coli* is used as the host cell. If *E. coli* and plasmids are mixed together in a test-tube, some of the bacteria will take up the recombinant DNA plasmid and those that do are said to be transformed.



D. *Finding the bacteria which have taken up the correct piece of DNA*

The bacteria are spread onto nutrient agar in a Petri dish. The agar also contains substances such as an antibiotic which allows growth of only the transformed bacteria. By repeated cell division, each transformed bacterium gives rise to a visible spot called a colony. Each colony is a clone of millions of identical cells.

Special methods are used to identify the clone that contains the gene for insulin A chain and the clone for B chain. The two clones are isolated and purified. Cultures of these clones are grown in liquid medium. The bacteria's protein synthesis machinery is turned on and human insulin A chains and B chains are synthesised by the bacterial cells.

Insulin A chains and B chains are extracted and purified. The two polypeptide chains are then mixed under conditions that favour the formation of the correct disulphide bonds, and the biologically active human insulin is manufactured. (Disulphide bonds between A chain and B chain can form in more than one way. Only one of the arrangements yields a product identical to human insulin.)

Notes: There is now a simple and cheap method of modifying the amino acid sequence of pig insulin to make it indistinguishable from the human insulin. This chemically modified pig insulin has been used satisfactorily for treatment of the insulin-dependent diabetes patients.

(18) Application of genetic engineering

A. *Production of precious human proteins*

The large-scale production of commercially important human proteins by genetically engineered bacteria is now very common. Human proteins, which normally may be only produced at a barely detectable level, can now be synthesised in *E. coli* at a level in excess of 10% of the total cellular protein. However as bacteria often leave methionine on one end of the protein chain, mammalian and yeast cells have now been increasingly used to host the human genes and to direct the production of their products. The table below shows some of the proteins produced by genetically engineered organisms and their potential uses.

Human Proteins	*Potential Use*
Insulin	Treatment of diabetes mellitus
Growth hormone	Growth promotion
Interferons	Anti-viral, anti-tumour, anti-inflammatory
Blood clotting factors (VIII & IX)	Treatment of haemophilia

B. *Production of commercially important products*

Microorganisms have long been exploited by man to produce many commercially important products, e.g. antibiotics, food, alcohols, vitamins, amino acids and enzymes. The rate, efficiency and finally cost of production of these products can be improved by directed alteration to and/or manipulation of the genes of the organisms used.

For instance, the large-scale production of ethanol by yeast cells in fermenters can be made more efficient if recombinant DNA technology is used to make yeast cells that can:

(i) drive the ethanol content of a fermented fluid from the present maximum of 13–14% to 20% or even higher;

(ii) carry out the fermentation process and produce ethanol not at room temperature or at 37°C but at 60°C; and

(iii) ferment cheap raw materials (e.g. wood wastes, crop residues such as wheat straw or wastes from processing of sugar cane) to produce ethanol.

C. *Production of vaccines*

One of the most successful areas of application of recombinant DNA technology is in the production of vaccines, especially those against virus. With conventional vaccination, an attenuated or killed pathogen is injected into the animal where it cannot cause disease but its antigens are just as effective in stimulating antibody formation. Such vaccination is not without risk. The person or animal who receives it would have the disease should the vaccine be accidentally virulent. As vaccine production requires the growth of a large quantity of the responsible pathogen, there is a potential risk to the personnel involved. Furthermore, viral vaccines are expensive to produce because the viruses must be grown in live animal cells.

Recombinant DNA technology allows the production of cheaper and safer vaccines. One approach is to isolate the antigen gene and clone it in bacteria. Purified antigen can be extracted and used as vaccine. As the vaccine consists of antigen only, there is no risk of contamination with virulent pathogens. Another approach is to render a pathogen harmless by site-directed mutagenesis. This involves isolating and mutating specific genes, and then reinserting them into the pathogen. By selecting appropriate genes for mutation, an organism may lose its pathogenic actions, but still able to trigger the immune response, and so lead to antibody production.

Some examples of vaccines commercially manufactured are: vaccines against foot-and-mouth disease of cattle; hepatitis B (refer to Teachers' Notes (28) for details), herpes, rabies and cholera in humans.

D. *Diagnosis and therapy of genetic disorders*

The purpose of the majority of the recombinant DNA work at the present time is to obtain the desired pieces of DNA in sufficient quantities for use in future investigations. One of the major clinical applications has been in the diagnosis of inherited diseases such as sickle-cell anaemia and thalassaemia. DNA isolated from the patients' white blood cells is digested and separated by gel electrophoresis. Radioactively labelled copies of the gene specific for a genetic disorder, prepared by recombinant DNA technology and act as DNA probes, are added. If strong binding is observed between the DNA probes and few of the patients' DNA fragments, then the patient can be said to have that genetic disorder.

The identification of the affected gene in many inherited disorders and the availability of cloned copies of their normal counterparts has created the possibility of correcting a genetic defect by supplying the patient with a normal functional gene. The most obvious candidate diseases for this approach are those which involve the haemopoietic cells of the bone marrow. These cells could be removed, treated *in vitro*, and transplanted back into the patients. Introduction of a normal β-globin gene, for example, can correct β-thalassaemia.

E. *Agriculture*

(*a*) The availability of nitrogen in the form of nitrates and ammonia is one major factor limiting the productivity of agricultural land. Only few microorganisms, bacteria and blue-green algae, have the ability to convert atmospheric nitrogen gas into forms which plants can use. Attempts are now being made to incorporate the nitrogen-fixing (*nif*) genes into cereals such as wheat, corn, rice and barley. If successful, this could have a dramatic impact on the world food production.

(*b*) Genes for resistance to aridity, waterlogging, temperature excess or saline soil may also be transferred to economically important plants so enabling more wastelands to be utilised.

F. *Mining industry*

The use of conventional mining technology to extract metals from the low-grade ores is too expensive. However, there are microorganisms that can leach metals from the low-grade deposits and mining wastes. Uranium and copper have been successfully and economically recovered through such bacterial actions. Genetically engineered microorganisms can be made and they are better than their naturally occurring counterparts in the leaching of metals.

G. *Oil pollution*

Various species of microorganisms such as *Pseudomonas* can consume the hydrocarbons in oils. Since each species only consumes a very limited range of hydrocarbons, two approaches have been adopted:

(i) Use a mixture of strains. This method has been successfully used to clear up oil-contaminated water in abandoned ships and in cleaning up water supplies.

(ii) Genetically engineer a 'superbug' so that all the oil-consuming genes are in one strain. This has been accomplished, but its usefulness under field conditions has yet to be tested.

(19) Safety and ethics in relation to recombinant DNA technology

Escherichia coli was and still is the commonest host bacterium for recombinant DNA work. As *E. coli* is a natural symbiont in the human intestine, there has always been a concern among scientists and a worry among laymen that there is a possibility that a clone of highly pathogenic recombinant *E. coli* were made by accident, then escaped from the laboratory and caused an epidemic for which no drugs were available.

In the early days of recombinant DNA work, scientists knew they had the power to create organisms which had never existed before. They were so concerned that in 1975 they called a representative international meeting to discuss the regulation of their own research work. As a result of this meeting the Recombinant Advisory Group (RAG) in the United States and the Genetic Manipulation Advisory Group (GMAG) in the United Kingdom (UK) were instituted to draw up guidelines for future experiments involving recombinant DNA technology.

The scientists agreed to two kinds of control, called physical containment and biological containment. The front line was the physical containment. Experiments were to be carried out under secure physical conditions as to prevent the escape of microbes from the laboratory where the research was being performed. Special laboratory facilities designated P1, P2, P3 and P4 were required for experiments considered of minimum risk, low risk, moderate risk and high risk respectively.

The lowest-risk experiments involved DNA from organisms that normally swapped genes with *E. coli.* They could be done using normal microbiological practices. The highest risk, where DNA from adult primates or animal viruses known to contain harmful genes was involved, would have to be done in specially designed laboratories with facilities similar to those in the laboratories where nerve gases and biological weapons were developed.

In addition to physical containment, recombinant DNA work would use only those strains of host bacteria and vectors which had been 'disabled' in such a way as to make them impossible to survive in anything other than the special laboratory environment. [***Notes***: Reference can be made to the *National Institute of Health (NIH) Guidelines for Research Involving Recombinant DNA Molecules* issued by the Department of Health and Human Services of the United States of America for further details on physical and biological containments.]

As research had progressed and experience with the recombinant DNA technology had gained, fear for the new technology receded. There has been some relaxation of the controls on laboratory experiments but scientists must still seek approval for recombinant DNA work from safety committees. In 1984, GMAG was replaced by ACGM (Advisory Committee on Genetic Manipulation), whose main function is to report to the UK Government on the potential hazards associated with genetic manipulation.

Concern remains, however, regarding the release of genetically engineered organisms into the environment. It is always possible that an antibiotic-resistant plasmid could be accidentally incorporated into a dangerous pathogen with serious medical consequences. What would happen if bacteria engineered to digest oil were released to clear an oil spill and then took up residence in oil wells or internal combustion engines? Approval has been recently given for the restricted release of a genetically engineered virus which is toxic to certain species of caterpillars and also of a bacterium capable of reducing frost damage in crops. The potential impact of these new organisms on the ecosystems is being carefully assessed.

The question of safety, however, was not the only issue. Questions which are more difficult to answer are the philosophical, social and ethical ones. For instance, is it right to create life forms in ways that nature never intended? Do we want to assume the basic responsibility for life on this planet — to develop new life forms for our own purpose? How far do we want to develop genetic engineering? Shall we take into our own hands our own future evolution? If we can use recombinant DNA technology to cure thalassaemia today, will we not be tackling social rebelliousness tomorrow? Who should decide on what is an ethical and safe experiment?

(20) Demonstration of the effects of high temperature and chemicals on the permeability of cell membranes

Living beetroot cells are suitable material for experiments to demonstrate the effects of high temperature and chemicals on the permeability of cell membranes. Beetroot contains a red pigment called anthocyanin, which is located in the large central vacuoles of the beetroot cells. As long as the cells and their membranes are intact, the anthocyanin will remain inside the vacuoles. However, if the membranes are damaged, anthocyanin will leak out and produce a red colour in the water surrounding the beetroot. The intensity of red colour in the water allows students to assess damage to living membranes by different factors easily.

High temperature and organic solvents e.g. alcohols, denature membrane proteins and increase fluidity of membrane lipids. Organic solvents at high concentrations can also dissolve lipids. Acetone, alcohol and chloroform are organic solvents that severely destroy membranes.

A. By high temperature

Procedure

1. Use a cork borer to cut cylinders of tissue from a beetroot.
2. Cut the cylinders of beetroot into thin discs of about 3 mm thick.
3. Rinse the beetroot discs in running water to wash off pigment that leaked out as a result of cutting.
4. Pipette 5 cm^3 of water into six test-tubes and labelled as 30, 40, 50, 60, 70 and 80.
5. Heat a boiling tube containing water up to 80°C using water bath.
6. Gently lower 5 pieces of beetroot discs into the hot water and leave it immersed for exactly 1 minute.
7. Carefully remove the discs and place in the prepared test-tube labelled as 80.
8. Repeat steps 5 to 7 for temperatures at 70°C, 60°C, 50°C, 40°C, and 30°C.
9. Leave the discs in each test-tubes for 20 minutes. Shake the tubes occasionally for the pigment to leak out of the cells.
10. Remove the beetroot discs from each test-tube after 20 minutes.
11. Compare the intensity of red colour in each tube. Use a one to ten "+" signs to indicate the relative intensity of red colour.

B. By chemicals

Procedure

1. Use a cork borer to cut cylinders of tissue from a beetroot.
2. Cut the cylinders of beetroot into thin discs of about 3 mm thick.
3. Rinse the beetroot discs in running water to wash off pigment that leaked out as a result of cutting.
4. Place 5 cm^3 of chloroform, alcohol, paraffin oil and water separately into 4 test-tubes. Cover each tube with parafilm to prevent evaporation of the chemicals. Label the test-tubes.
5. Put 5 pieces of beetroot discs into each of the 4 test-tubes. Allow the tubes to stand for 1 hour with occasional stirring and observe the result.

Notes: (1) Chloroform and alcohol can destroy the structure of cell membranes of beetroot, and the red pigment will diffuse out. The pigment is soluble in alcohol. As the pigment is not soluble in chloroform, the red pigment will form a separate layer and float on top of chloroform.

(2) Paraffin oil and water do not destroy the cell membranes.

(3) *Acetone and chloroform are volatile and harmful. Teachers should advise students not to inhale the vapour from these chemicals when handling them.*

(4) *Acetone and alcohol are inflammable. Avoid using naked flames near such solvents.*

(21) Demonstration of plasmolysis

When a cell is in contact with a solution of lower water potential than its own content, water leaves the cell by osmosis through the plasma membrane. The protoplast shrinks and eventually pulls away from the cell wall. This process is called plasmolysis and the cell is said to be plasmolysed. The process of plasmolysis is usually reversible by placing the plasmolysed cell in water.

Procedure

1. Peel a small strip of lower epidermis from a leaf of *Rhoeo discolor*/ *Zebrina.*
2. Mount this strip of epidermis on a slide with water, and examine it under the microscope. Look for the intact pigmented cells.
3. Replace the water by 0.4M sucrose solution, using the irrigation method.
4. Observe the process of plasmolysis in the same pigmented cells during irrigation.
5. Replace the sucrose solution by water, using the same irrigation method, to observe the process of de-plasmolysis.

(22) Determination of osmotic potential at incipient plasmolysis

Samples of leaf epidermis (e.g. *Zebrina*) are allowed to come to equilibrium in a range of solutions of different concentrations and the aim is to find which solution causes incipient plasmolysis, i.e. shrinkage of the protoplasts to the point where they just begin to pull away from the cell walls.

At incipient plasmolysis, the pressure potential of the cell is equal to zero. The osmotic potential of the cell is equal to that of the surrounding solution with which it has come into equilibrium. In practice, incipient plasmolysis cannot be observed under the microscope. The water potential varies between cells in the same tissue and some cells plasmolyse in more dilute solutions than others. Therefore, it is assumed that incipient plasmolysis occurs in the tissue when about half (50%) of the cells are plasmolysed. If this assumption is true, the osmotic potential of the tissue is equivalent to the osmotic potential of the solution that produces 50% plasmolysis in the tissue.

Procedure

1. Using the 0.5M sucrose solution and distilled water, prepare a series of solutions of different molarities of 0.5, 0.4, 0.35, 0.3, 0.25, 0.2, 0.15, 0.1 and 0.0 according to the dilution table below. Make sure that the solutions are mixed thoroughly by shaking.

Molarities of sucrose solution (M)	0.5	0.4	0.35	0.3	0.25	0.2	0.15	0.1	0.0
Volume of 0.5M sucrose solution (cm^3)	10	8	7	6	5	4	3	2	0
Volume of distilled water (cm^3)	0	2	3	4	5	6	7	8	10

2. Add the solutions to the appropriate labelled petri dishes.
3. Place a piece of leaf epidermis in each of the sucrose solutions. Leave for about 30 minutes.
4. Remove the leaf epidermis from the 0.5M solution and mount it on a slide in sucrose solution of the same concentration. Add a cover-slip and examine with a microscope.
5. Select a suitable area of cells using low power. Switch to a medium or high power objective and move the slide through the selected area, recording the state (plasmolysed or unplasmolysed) of the first 100 cells viewed. Cells in which there is any sign of the protoplast pulling away from the cell wall should be counted as plasmolysed.
6. Repeat steps (4) and (5) for the leaf epidermis in sucrose solutions of molarities of 0.4, 0.35, 0.3, 0.25, 0.2, 0.15, 0.1 and 0.0.
7. From the total number of cells counted and number plasmolysed, determine the percentage of plasmolysed cells for each solution.
8. Plot a graph of percentage of plasmolysed cells (vertical axis) against molarity of sucrose solution (horizontal axis).
9. Read off from the graph the molarity of the sucrose solution which causes 50% of the cells to plasmolyse.

Notes: Plant materials like *Rhoeo discolor* and onion are also suitable for this experiment. The teacher may need to adjust the concentration of the initial sucrose solution for different plant materials.

(23) Determination of water potential of potato tissue by changes in length/mass

The water potential of a plant tissue can be determined by the following principle: When the tissue shows no net gain or loss of water when immersed in a solution of known molarity, its water potential is equal to that of the external solution.

Samples of the tissue are allowed to come into equilibrium in a range of solutions of different concentrations. When the tissue shows neither an increase nor a decrease in mass or length, the water potential of the potato tissue is the same as that of the external solution.

A. By length

Procedure

1. Using a 1M sucrose solution and distilled water, prepare a series of 10 cm^3 sucrose solutions in boiling tubes of concentrations: 1M, 0.8M, 0.6M, 0.4M, 0.2M, and 0.0M. Label the boiling tubes.
2. Use a cork borer to obtain cylinders of potato tissue having the same diameter. Cut them all to the same length of 5 cm. It is important to work quickly to avoid loss of water through evaporation as this would lower the water potential of the tissue.
3. Immerse two cylinders of potato in each tube and cover the tubes with parafilm.
4. Leave the set up for one hour.
5. Remove the cylinders from each tube. Measure the length of the cylinders, and calculate the percentage change in length using the formula:

$$\% \text{ change in length} = \frac{\text{final length} - \text{initial length}}{\text{initial length}} \times 100\%$$

6. Find the mean percentage change in length of the cylinders at each concentration.
7. Plot a graph of the mean percentage change in length (vertical axis) against the molarity of the sucrose solution (horizontal axis).
8. From this graph, determine the molarity of the sucrose solution which causes no change in length of the tissue.
9. The water potential of the potato tissue can be expressed in terms of the molarity of sucrose solution that causes no change in length of the tissue.

B. By mass

Procedure

1. Repeat steps (1) and (2) in (A).
2. Slice up each cylinder into six discs of approximately equal thickness. Place each group of discs on a separate piece of filter paper.
3. Weigh each group of discs and record the results.
4. Put the groups of discs in each of the labelled tubes. Cover the tubes with parafilm.
5. Leave the set up for one hour.

6. Remove the discs from each tube. Blot off any surplus fluid quickly and gently with filter paper and re-weigh them. Record the new weight of each group of discs.
7. Calculate the percentage change in weight using the formula:

$$\% \text{ change in weight} = \frac{\text{final weight} - \text{initial weight}}{\text{initial weight}} \times 100\%$$

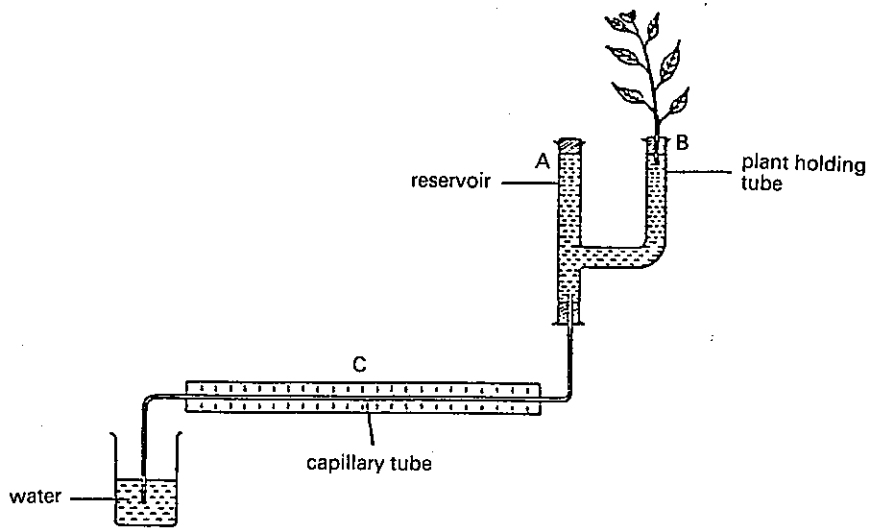
8. Plot a graph of the percentage change in weight (vertical axis) against the molarity of the sucrose solution (horizontal axis).
9. From this graph determine the molarity of the sucrose solution which causes no change in weight of the tissue.
10. The water potential of the potato tissue can be expressed in terms of the molarity of the sucrose solution that causes no change in weight of the tissue.

(24) The use of a simple potometer to measure the rate of transpiration

A simple experiment is described below to demonstrate how the rate of transpiration of common plants found in Hong Kong can be determined with the use of a Darwin's potometer. Moreover, students can also be given the opportunity to estimate the stomatal density of leaves from different plants to evaluate the relationship between different transpiration rates and this morphological feature.

A. Estimation of transpiration rate using a simple potometer

Procedure



1. Immerse a leaf with petiole or a branch of a plant with several leaves in water. With a sharp razor blade, make a slanted cut at the lower end of the petiole or the branch under water. Leave the cut end in water.

Carry Out Steps 2–4 Under Water

2. Insert the petiole or branch through the hole of the stopper provided with the potometer, keeping the leaf/leaves well above the stopper and the water. With a sharp razor blade, cut away another 1 cm from the cut end again.
3. Immerse the potometer in a basin of coloured water until the capillary tube, the reservoir and the plant holding tube are completely filled.
4. Transfer the stopper with the leaf/branch to the plant holding tube and insert the stopper securely into the tube. Ensure no bubbles are trapped within the system.
5. Lift the entire set-up out of the water and immerse the capillary end into a beaker filled with water, ensuring no air bubbles are trapped during the transfer.
6. Lift the capillary end out of the water surface long enough to allow air to enter the capillary tube as transpiration takes place. Reimmerse the capillary tube into the water so that an air bubble can be trapped within the tube.
7. Estimate the rate of transpiration by measuring the distance moved by the air bubble per unit time. Continue to take measurements until three successive readings obtained show close agreement.
8. If time permits, estimate the rate under different environmental conditions, e.g. under direct incandescent light illumination, under low light condition, in rapid air movement (provided by a fan) or in still air.
9. Calculate the leaf area of the experimental plant by using a graph paper.
10. Express the transpiration rate in terms of volume of water transpired per unit time per unit area of leaf surface.

Notes: (1) Do not forget to include the areas of both upper and lower surfaces if there are stomata on both sides.

(2) Ganong potometer can also be used for this investigation.

B. Estimation of stomatal density on leaf surface

Procedure

1. On a microscope slide, prepare a small drop of water to be used as mountant.
2. With a needle or the sharp point of a pair of fine forceps, make a shallow cut on the leaf surface.

3. Use a pair of fine forceps to try to peel off the epidermis.
4. Mount the epidermis in water and try to find a portion which occupies the entire microscope field of vision at 100X magnification. Count the number of stomata within the area occupied.
5. Repeat this procedure for 3–5 times to obtain a good average value.
6. Repeat the same procedure for both the upper and lower epidermis.
7. Given a copper wire of known diameter, estimate the area covered by the microscope field of view at 100X magnifications.
8. Calculate the stomatal density of the upper and lower epidermis of the experimental plant.

Notes: (1) If it is difficult to obtain the epidermis by the method described above, then try the nail varnish method as mentioned in Teachers' Note No. 1 (*C*).

(2) Plant materials suitable for this experiment are: *Hibiscus rosa-sinensis, Ligustrum sinense, Bauhinia blakeana and Ficus microcarpa.*

(25) Diversity of organisms

A. *The variety of life*

An appreciation of the diversity of organisms should be encouraged by reference to as wide a range of specimens/photographs/field observations as possible. It should be clearly understood that, however many examples the students see, they are but *a few* examples of the 1.4 million species of organisms so far described, and further that the estimate for the number of species of organisms so far not described is some 3 to 20 times this number.

Emphasis should be placed on relating this diversity of forms to the variety of ways in which organisms live, i.e. their ecological niches and thus the importance of diversity in the biosphere.

The effects of destroying the environments which have supported this diversity of life forms for more than a billion years should be covered — wild species as a critical sustainable economic resource in underdeveloped countries; loss of species which could be of importance to man for food, medicine, etc.; and a loss of part of the natural world under our stewardship, which is just as important a part of our heritage as art, language and achievement.

B. *Classification*

Taxonomic hierarchy means the way in which all organisms are organized into a series of successive levels for biological classification: Phylum/Division, Class, Order, Family, Genus, Species.

(26) Safety precautions in biological field work

A. *General*

1. The teacher should pay attention to weather forecast in the evening before and in the morning of the scheduled date. A field trip must be cancelled whenever severe cold front or thunderstorm or typhoon is forecasted.
2. Clothing and footwear of each participant should be suitable for the work and the time of the year.
3. Suitable amount of food and drink should be carried individually by all participants when on a whole day trip, unless easily available elsewhere.
4. A map, a compass and a whistle should be carried when a trip is made in remote area. The school should inform the police and each participant's family about the route of the trip and the due time of return. Please refer to the General Schools Miscellaneous Circular captioned 'Organised visits by School Children: Safety Precautions', which is issued by Education Department at the beginning of each school year.
5. A field trip must not be extended to hours of darkness.
6. The group must carry a first aid kit, which should include plasters, bandages, sunburn cream, iodine tincture and anti-histamine cream. It is much better to have someone with formal first aid training accompanying the group.
7. If glassware (test-tubes, beakers, bottles and Petri dishes) is to be carried it should be protected from accidental breakage. Care should be taken not to leave it on the ground, where it could become a hazard.
8. Keep to the established footpaths and avoid making new short-cuts. Snakes and venomous insects may hide among bushes and grass. Moreover, uncontrolled formation of new paths often starts soil erosion which is difficult to control.
9. Students who are not physically fit enough or exempted from Physical Education lessons must not participate in a field trip.

B. *Terrestrial habitats*

1. To avoid cuts and wounds due to spines and thorns of plants, the participants should put on long-sleeved shirts and jeans. A hat with wide brim should be put on to avoid snake bite at the neck and head during a trip among trees.

2. Quite a number of plants in the wild are poisonous. Teachers should warn students not to ingest fruits, seeds or leaves of wild plants. Students should be warned not to touch those plants which are known to contain substances that induce allergic reaction or dermatitis on skin contact. *Nerium indicum* (oleander), *Rhus hypoleuca* (sumac), *Thevetia peruvidna* (yellow oleander) and *Euphorbia milii* (crown of thorns) are the most notorious examples.
3. Students should be warned not to irritate wild animals, since quite a number of them are venomous themselves or are vectors of fatal diseases.
4. Keep to cleared foot-paths where possible.
5. Examine the surrounding area carefully before crawling under bushes to observe and collect specimens.
6. Examine rock of logs before sitting on them. Do not lift stones or other objects by hands, always use a stick to turn them over. Do not reach into holes.
7. Dead specimens of vertebrates in the field should not be collected or closely examined because of the risk of infectious diseases.
8. Plants and seeds collected from the field should be free from pests or diseases to limit the chance of infection on other plants.
9. On collecting animals and plants that bear spines or thorns, extreme care should be exercised to prevent injuring the body.
10. When collecting plants known to contain poisonous or irritating sap, avoid handling them with naked hands. Gloves should be worn.
11. When doing experiments of soil analysis, extreme care should be taken to prevent bites from organisms which may be hidden in the soil.

C. *Shore habitats*

1. The teacher should always bear in mind the tide-time, and make sure to retreat before high tide.
2. Each participants should be properly dressed in shorts and plimsolls with adequate tread. Sandals and leather shoes must be avoided.
3. Never attempt rock climbing, caving, swimming and diving.
4. On rocky shores, the surface is uneven and often covered with slippery algae. It is sensible to try footing before putting weight down. Jumping from rock to rock may be dangerous.
5. On mangrove and sandy beaches, the surface may be slippery and the substratum unstable. It is safe to try footing before putting weight down.

D. *Freshwater habitats*

1. Never work in streams after heavy rainfall, as flash flood may occur suddenly.
2. Except for very shallow ponds and ditches, work in freshwater habitats should be regarded as potentially dangerous, since current, submerged objects and slippery or muddy bottoms all pose hazards.
3. Each participant should be properly dressed in shorts and plimsolls with adequate tread.
4. Extreme care should be taken when placing the feet into stream. Always expect potholes and underwater obstacles.
5. Wading and swimming in pools and ponds should be discouraged unless where the depth of water and the nature of substratum are known with certainty.

(27) The pig-on-litter system

In the past, washing of animal wastes (particularly pig wastes) into water courses led to severe water pollution problems and legislation has now been introduced to control this. Various methods of control are possible – collection and then disposal centrally; *in situ* treatment of the wastes in some form of digestor; and prevention of waste accumulation by the pig-on-litter system.

Thus, the pig-on-litter system is a waste management method for controlling pig wastes in Hong Kong. It involves the use of a special bedding material comprising a mixture of sawdust and bacterial enzymes (or cells) which rapidly decompose and stabilize the waste within the pen as and when it is produced.

The method has been used in Japan and Taiwan for several years and it is now being tested in Hong Kong. It is particularly useful here, because the large number of small pig farms (over 80% of local pig farms have less than 200 pigs each) make other disposal options economically unfavourable.

This litter bed is mixed thoroughly once a week and, every 10–15 days, additional bacterial product applied. Sawdust is added as necessary to maintain an appropriate thickness. Once established, the litter bed can be reused for many years and so successive crops of pigs can be introduced onto the beds at little extra cost for sawdust or bacterial product.

Eventually the litter can be dried and used as a composting material.

(28) Hepatitis B surface antigen

Hepatitis B is a viral infection which attacks the liver and a major cause of liver cirrhosis. About one person in 10 in Hong Kong is a chronic carrier of the disease, and infections can come from contact with the blood or body secretions from any one of these carriers.

There is no effective treatment for hepatitis B, and the only way to fight against this disease is through prevention by vaccination. Vaccines for hepatitis.B are derived from two sources: blood and genetic engineering.

Blood-derived vaccines are derived from serum drawn from infected patients and there is always a risk that they can be contaminated by the hepatitis virus or other diseases which can be transferred to those inoculated. Another disadvantage of these vaccines is that the quantities that can be manufactured were dependent on donor supplies of infected blood and, as such, can never be made cheaply enough, or in the vast amounts needed to mount a viable battle against the deadly infection worldwide.

Vaccines made from genetic engineering methods, however, will be safe since these are not dependent on contaminated blood supplies. Enormous amounts of vaccines can also be quickly and efficiently produced. To develop these vaccines, scientists had first to crack the genetic code of the virulent hepatitis B virus, and splice it to retain only the non-infective gene which was by itself enough to stimulate an immune response without any fear of infecting inoculants with the disease. Then some way had to be found to clone this gene into another organism where it could be reproduced successfully.

The hepatitis B virus has only four proteins and its DNA is composed of about 3 200 nucleotides. Researchers found that a sequence of 678 nucleotides was responsible for the formation of a protein composed of 226 amino acids. This protein, called the hepatitis B surface antigen (HBsAg), makes up the outer coat of the virus and is also the specific agent which can be used in vaccines to immunise against the infection. It is the body's ability to recognise the surface antigen as a marker of a foreign substance and to produce specific antibodies against it (and through it the whole virus) which lies at the heart of the way vaccines work against the infection. But being able to identify the specific gene responsible for producing the hepatitis B surface protein is only the first step for cloning it in the test-tube. The gene must be spliced into something else, a vector, which will enable replication when transferred into a host cell capable of reproduction. The vector is a plasmid from *E. coli*. The recombined plasmid has to be transferred to a host cell which can 'read' its genetic information and decode it to assemble the needed amino acids to produce the hepatitis B surface protein. The tests using *E. coli* bacteria as host cells were fruitless. The protein, the bacteria was genetically programmed to produce, appeared to be toxic to these organisms and they died before any positive results could be decided. Mammalian cells were similarly tried as host cells and discarded due to technical problems. In the end, ordinary baker's yeast, *Saccharomyces cerevisae*, was found to make an excellent host. The problem was that a foreign plasmid inserted would ordinarily be rejected as unnecessary to its life cycle. By special techniques, yeast cells could be tricked into accepting the plasmid and to read its genetic coding as their own. These recombinant

yeast cells were able to read and translate the genetic information to produce the hepatitis B surface protein. After the initial batch of "seed" recombinant yeast cells have been perfected, they are stored in thousands of ampoules. Each seed is capable of starting off a fermentation process to produce vast quantities of the needed antigen.

At present, a Hepatitis B Vaccination Programme is implemented by the Department of Health. This programme covers all new born babies, and health workers who are in frequent contact with blood. The vaccines used are produced by the genetically engineered yeast cells.

TEACHING RESOURCES

A. Suggested Audio-visual Resources

I. Teaching Resources Available in Schools

Teachers may find the following resource materials, which had been distributed to schools, useful in the teaching of the curriculum as well as in providing ideas for project work.

Title	*Publisher/ Author*	*Year*	*Type*
A Freshwater Stream Visit – An Ecology Project for Upper Secondary Students	WWFHK & HKASME	1987	Teaching Pack
A Geography of the Mai Po Marshes (English Version)	WWFHK	1988	Booklet
A Geography of the Mai Po Marshes (Chinese Version)	WWFHK	1990	Booklet
A Teaching Kit on AIDS	ED	1988	Teaching Pack
Animals in Man's Home Environment	HKASME	1990	Worksheet
Anti-Pollution Pack – Educational Kit on Environmental Protection	EPD	1990	Teaching Pack
Asian Tropical Forests	WWFHK	1990	Report
Beach Pollution	WWFHK & HKGA	1990	Teaching Pack
Biological Diversity	WWFHK	1989	Pamphlet
Climate Change	WWFHK	1990	Report
Country Parks – 1	WWFHK & HKGA	1990	Teaching Pack
Country Parks – 2	WWFHK & HKGA	1990	Teaching Pack
Hill Fires	WWFHK	1989	Teaching Pack
Hong Kong's Great Environmental Debate	WWFHK	1989	Teaching Pack
Saving the African Elephant	WWFHK	1990	Brochure
The Tree Trunk Microhabitat – An Ecology Project for Upper Secondary Students	WWFHK & HKASME	1987	Teaching Pack
The Wall Habitat	WWFHK	1989	Teaching Pack
Wetlands in Danger	WWFHK	1989	Report

Key:
ED = Education Department, Hong Kong Government
EPD = Environmental Protection Department, Hong Kong Government
HKASME = Hong Kong Association for Science and Mathematics Education
HKGA = Hong Kong Geography Association
WWFHK = World Wildlife Fund For Nature, Hong Kong

II. Audio-visual Materials Available On Loan

The audio-visual materials listed below are available for loan to schools upon application to the Audio-Visual Resources Library, Visual Education Section, Advisory Inspectorate, Education Department, Room 228, Lee Gardens, 2nd Floor, Hysan Avenue, Causeway Bay, Hong Kong.

KEY: Col = In Colour
BW = In Black and White
f = Number of Single Frame (Filmstrip)
df = Number of Double Frame (Filmstrip)
(Pu) = With Putonghua Commentary
(c) = With Cantonese Commentary

1. 16 mm Films

Title	*Code*	*Duration*	*Remark*
Adaptation to Environment	F1447	18 min	Col
Beach and Sea Animals	F1292	11 min	Col
Cell Biology	F1471	17 min	Col
Cell Biology: Life Functions	F767	19 min	Col
Cell Biology: Mitosis and DNA	F768	16 min	Col
Cell Biology: Structure and Composition	F735	14 min	Col
Cell Division – Mitosis and Meiosis	F1277	20 min	Col
Conserving Our Environment: The Pollution Crisis	F1440	14 min	Col
Darwin and the Theory of Natural Selection	F769	14 min	Col
Ecology – Wanted Alive	F1428	10 min	Col
Ecology of a Swamp	F1509	8 min	Col
Ecology of the Prairie	F1510	7 min	Col
Ecology of Forests	F1508	8 min	Col
Ecosystems and Interactions	F1495	15 min	Col
Ecosystem: Inter-relationship	F1427	7 min	Col
Energy for the Future	F1189	15 min	Col
Evolution of Man	F838	14 min	BW
Exploring the Body Series: Kidney	F1321	11 min	Col
Food Chain – A Bond of Life	F1541	15 min	Col
Fundamental of Nervous System	F1140	17 min	Col
Fungi	F1560	16 min	Col
Genetics: Chromosomes and Genes (Meiosis)	F1543	16 min	Col
Genetics: Function of DNA and RNA	F1441	12 min	Col
Genetics: Human Heredity	F771	14 min	Col
Heredity	F256	11 min	BW
Human Body: Circulatory System	F1209	13 min	Col
Human Body: Excretory System	F1210	13 min	Col
Human Body: Endocrine System	F1537	16 min	Col

Title	Code	Duration	Remark
Human Body: Nervous System	F1536	22 min	Col
Human Heredity	F501	14 min	Col
Infectious Diseases and Natural Body Defenses	F1498	11 min	Col
Introducing Insects	F637	17 min	Col
Learning about Cells	F1238	16 min	Col
Life Between Tides	F1291	11 min	Col
Living Cell DNA	F1228	20 min	Col
Mitosis and Meiosis	F1554	17 min	Col
New Prospectors	F1513	17 min	Col
Plant-Animal Communities: Ecological Succession	F1274	13 min	Col
Pollution in Hong Kong	F1036	23 min	Col
Population and Pollution	F793	7 min	Col
Population Ecology	F710	19 min	Col
Populations	F1494	13 min	Col
Problems of Conservation: Our Natural Resources	F1557	11 min	Col
Rain Forest Part 1	F1528	29 min	Col
Refuse with a Future	F1514	12 min	Col
Seashore Ecology	F1448	16 min	Col
The Blood	F1222	16 min	Col
The Cell – Structural Unit of Life	F482	11 min	BW
The Cell: A Functional Structure	F1583	29 min	Col
The Changing Forest	F549	19 min	Col
The Immune Response	F1582	20 min	Col
The Isolation and Growth of Bacteria	F1385	15 min	Col
The Living Bird	F324	14 min	Col
The Meadow – An Ecosystem	F1540	13 min	Col
The Soil and Life	F1544	14 min	Col
The Story of the Blood System	F791	22 min	Col
The Work of Kidney	F1230	20 min	Col
Viruses: The Threshold of Life	F1004	13 min	Col
What is Ecology?	F757	11 min	Col
Woodland and Ecology: Flora	F1449	14 min	Col
World in a Marsh	F552	22 min	Col

2. 8 mm Loop Films

Title	Code	Duration	Remark
Cell Division	Fes 213	2 min	Col
Estimating Cell Size	Fes 569	4 min	Col
Making a Blood Smear	Fes 565	4 min	Col

Title	Code	Duration	Remark
Smear and Squash Techniques I	Fes 358	4 min	Col
Smear and Squash Techniques II	Fes 359	4 min	Col

3. Filmstrips

Title	Code	Number	Remark
Cell Division: Structure	FS1310	16f	BW
Cells and Tissues	FS869	22f	Col
Charles Darwin	FS1534	49f	Col
Circulation	FS654	22f	Col
Classification of Plants	FS936	71f	BW
Classification of Plants	FS1249	28f	Col
Digestion of Food: Functions of the Liver	FS673	24f	Col
Environmental Protection – Environment Biospher	FS3604	41f	Col
Environmental Protection – Environment Crisis	FS3605	48f	Col
Environmental Protection – Environment Protection	FS3506	46f	Col
Evolution	FS484	31f	BW
First Ideas about Plant (1) – Plant Diversity	FS3083	60f	Col
First Ideas About Plant (2) – Plant Interaction	FS3084	60f	Col
First Ideas About Plant (3) – Plants and Energy	FS3085	60f	Col
Heredity Part 1: The Mechanism of Inheritance	FS1247	19f	Col
Heredity Part 2: Mendel's Laws	FS1248	31f	Col
Man's Microbe Enemies Part 1	FS561	24f	BW
Man's Microbe Enemies Part 2	FS562	24f	BW
Photosynthesis – Chlorophyll and Light	FS2848	35f	Col
Photosynthesis – Synthesis	FS2851	37f	Col
Photosynthesis – Sugar and Starch	FS2849	41f	Col
Pollution, Part I: The Cities Air	FS3025	62f	Col
Pollution, Part II: Water & Garbage	FS3026	62f	Col
Surviving the Ecology Crisis Series—Overpopulation	FS2893	70f	Col
Surviving the Ecology Crisis Series—People in Poverty	FS2894	76f	Col
Surviving the Ecology Crisis Series—The Polluted Planet	FS2892	80f	Col
Surviving the Ecology Crisis Series—The Power Drain	FS2895	72f	Col

Title	Code	Duration	Remark
The Blood	FS664	12f	Col
The Evolution of Life, Part 1 Life To-day	FS929	32f	Col
The Evolution of Life, Part 2 Preparation for Life	FS930	23f	Col
The Evolution of Life, Part 3 First Life	FS931	36f	Col
The Evolution of Life, Part 4 Plants in the Sea	FS932	36f	Col
The Evolution of Life, Part 5 Plants on the Land	FS933	41f	Col
The Evolution of Life, Part 6 Fishes, Amphibians, Reptiles	FS1535	49f	Col
The Morphology of the Leaf	FS2114	38df	Col
Young Scientists Investigate Pollution – (1) Smog and Plant Growth	FS2584	51f	Col
Young Scientists Investigate Pollution – (2) Air Pollution and Lung Tissue	FS2585	53f	Col
Young Scientists Investigate Pollution – (3) Measuring Water Pollution	FS2586	51f	Col

4. Slides

Title	Code	Number	Remark
Biological System: Biosynthesis	S287	12 nos	Col
Biological System: Cell Respiration	S292	12 nos	Col
Biological System: Enzymes	S291	12 nos	Col
Biological System: Hormones	S289	12 nos	Col
Biological System: Photosynthesis	S293	12 nos	Col
Biological System: Replication of Genetic Material	S288	12 nos	Col
Biological System: The Immune Response	S290	12 nos	Col
Ecology of a Stream	S246	73 nos	Col
Ecology: Sampling Methods and Field Techniques Part 1	S190	80 nos	Col
Ecology: Sampling Methods and Field Techniques Part 2	S191	80 nos	Col
Environmental Pollution and Conservation	S241	24 nos	Col
Food Chains: The Balance Threatened	S201	12 nos	Col
How Animals Feed	S248	76 nos	Col
Intermediate Biology Set 1: Part 2 Gaseous Exchange and Respiration	S276	20 nos	Col

Title	Code	Number	Remark
Intermediate Biology Set 1: Part 4 Nervous and Endocrine system	S278	20 nos	Col
Intermediate Biology Set 1: Part 6 Plant Anatomy	S280	20 nos	Col
Intermediate Biology Set 2: Part 3 Cells of the Blood	S284	20 nos	Col
Intermediate Biology Set 2: Part 4 The Animal Cell	S285	20 nos	Col
Our Natural Resources: Where Are They Now? Where Will We Find Them Tomorrow?	S196	51 nos	Col
Our Natural Resources: Where Are They Now? Where Will We Find Them Tomorrow?	S197	29 nos	Col
Our Natural Resources: Where Are They Now? Where Will We Find Them Tomorrow?	S198	48 nos	Col
Our Natural Resources: Where Are They Now? Where Will We Find Them Tomorrow?	S199	32 nos	Col
Photomicrographs for Biology	S14	31 nos	Col
Plant Tissues	S26	6 nos	Col
Pollution Folio 2: Ponds and Rivers	S200	12 nos	Col
Pollution: Impact and Control	S242	24 nos	Col
Renewable Energy Resources: Wind, Water and Solar Rays Part I	S226	80 nos	Col
Renewable Energy Resources: Wind, Water and Solar Rays Part II	S227	80 nos	Col
The Mai Po Marshes	S250	80 nos	Col
Tropical Rain Forest Environment	S231	24 nos	Col
World of a Pond	S251	50 nos	Col

5. Overhead Transparencies (OHTs)

Title	Code	Number	Remark
Air Pollution	OHT218	14 nos	BW
Cells and their Organelles	OHT219	10 nos	BW
Dominant Heredity in Humans	OHT220	4 nos	Col
Immunizing Reaction of the Body	OHT223	3 nos	Col
Mitochondrion	OHT224	1 no	BW
Nucleus	OHT225	1 no	BW
Preservation of the Environment I	OHT355	29 nos	Col
Recessive Heredity in Humans	OHT221	4 nos	Col
Sex Linked Heredity	OHT222	2 nos	Col

6. Videotapes

Title	*Code*	*Duration*	*Remark*
All About Animals: Insects	VHS559	12 min	Col
Animal Classification: Echinoderms and Vertebrates	VHS284	10 min	Col
Animal Classification: Protozoa and Arthropoda	VHS283	10 min	Col
Beginning of the Food Chain: Plankton (2nd Edition)	VHS358	12 min	Col
Biological Sciences: Molecular Biology	VHS614	15 min	Col
Chemistry of Heredity I	VHS359	15 min	Col
Chemistry of Heredity II	VHS360	9 min	Col
Chromosomal Basis of Heredity	VHS361	16 min	Col
Conserving Our Environment: The Pollution Crisis	VHS63	15 min	Col
Ecology of Human Survival: Resources for Survival	VHS292	13 min	Col
Environment, People and Pollution: Land	VHS291	12 min	Col
Environment, People and Pollution: The Air	VHS289	10 min	Col
Environment, People and Pollution: Waters	VHS290	12 min	Col
Environmental Pollution and Conservation	VHS287	10 min	Col
Feeding Mechanisms in Animals (I)	VHS285	8 min	Col
Feeding Mechanisms in Animals (II)	VHS286	9 min	Col
Grouping Living Things	VHS565	18 min	Col
Human Body: Endocrine System	VHS610	16 min	Col
Human Body: Nervous System	VHS611	23 min	Col
Human Populations and Resources	VHS295	9 min	Col
Life on Earth Series: Building Bodies	VHS4	55 min	Col
Life on Earth Series: Conquest of the Waters	VHS7	55 min	Col
Life on Earth Series: Hunters and Hunted	VHS13	55 min	Col
Life on Earth Series: The First Forests	VHS5	55 min	Col
Life on Earth Series: The Invasion of Land	VHS8	55 min	Col
Life on Earth Series: The Rise of the Mammals	VHS11	55 min	Col
Life on Earth Series: The Swarming Hordes	VHS6	55 min	Col
Life on Earth Series: Theme and Variations	VHS12	55 min	Col

Title	*Code*	*Duration*	*Remark*
Life on Earth Series: Victors of the Dry Land	VHS9	55 min	Col
Life on Earth Series: The Infinite Variety	VHS3	55 min	Col
Plant-animal Communities: Ecological Succession	VHS70	14 min	Col
Plant-Animal Communities: Interrelationships	VHS71	13 min	Col
Plant-Animal Communities: Physical Environment	VHS72	11 min	Col
Plant-Animal Communities: The Changing Balance	VHS73	11 min	Col
Plants and Their Environment	VHS282	13 min	Col
Pollution Indicator Organisms	VHS288	6 min	Col
Pre-natal Diagnosis: To Be or Not To Be	VHS58	41 min	Col
Rain Forest	VHS206	32 min	Col
Simple Ecosystems: Problems of Pollution	VHS103	23 min	Col
The Living Body Series: Water	VHS212	26 min	Col
The Living Body Series: Nerves at Work	VHS214	26 min	Col
The Planets (Horizon)	VHS664	50 min	Col
The Scientist and the Baby	VHS169	50 min	Col
Water: A Clear and Present Danger	VHS219	26 min	Col

B. Suggested Reference Books

Teachers may find the following books useful in the teaching of the curriculum. In addition, articles in the *Scientific American, The School Science Review, Biologist, Journal of Biological Education, The American Biology Teacher and The Science Teacher* may also be useful.

1. *General*

Brightman, J. & Parker, S., *Biology – A Functional Approach Study Guide,* Thomas Nelson, 1983.

Education Department, *Safety in Science Laboratory,* Hong Kong Government Printer, 1990.

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2. *Section I – The Structure and Functions of Cells*

Bracegirdle, B. & Miles, P. H., *An Atlas of Plant Structure Volume 1* Heinemann, 1971.

Dodge, *An Atlas of Biological Ultrastructure,* Edward Arnold, 1968.

Freeman, W. H. & Bracegirdle, B., *An Atlas of Histology,* (*2nd ed.*), Heinemann, 1967.

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Roland, J. C. & Roland, F., *Atlas of Flowering Plant Structure,* Longman, 1980.

Shaw, A. C., Lazell, S. K. & Foster, G. N., *Photomicrographs of the Flowering Plant,* Longman, 1968.

Shaw, A. C., Lazell, S. K. & Foster, G. N., *Photomicrographs of the Non-flowering Plants,* Longman, 1968.

Wheater, P. R., Burkitt, H. G. & Lancaster, P., *Colour Atlas of Histology,* Longman, 1985.

Wynn, C. H., *The Structure and Function of Enzymes,* Edward Arnold, 1979.

3. *Section II – Energetics*

Bryant, C., *The Biology of Respiration,* (*2nd ed.*), Edward Arnold, 1980.

Hall, T. A. & Rao, K. K., *Photosynthesis,* (*4th ed.*), Edward Arnold, 1987.

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4. *Section III – Genetic Control and Inheritance*

Cherfas, J., *Man-made Life: An Overview of the Science, Technology and Commerce of Genetic Engineering,* Basil Blackwell, 1982.

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