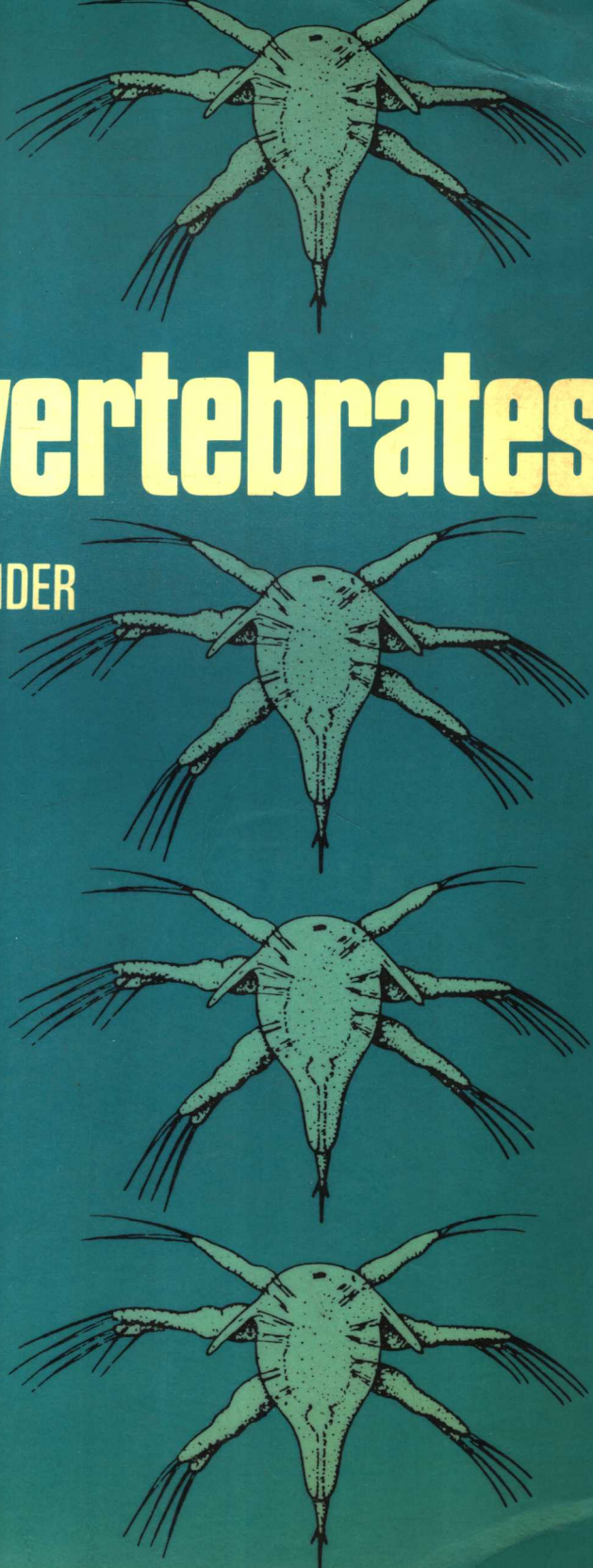

The Invertebrates

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CAMBRIDGE UNIVERSITY PRESS

CAMBRIDGE

LONDON · NEW YORK · MELBOURNE

Published by the Syndics of the Cambridge University Press
The Pitt Building, Trumpington Street, Cambridge CB2 1RP
Bentley House, 200 Euston Road, London NW1 2DB
32 East 57th Street, New York, NY 10022, USA
296 Beaconsfield Parade, Middle Park, Melbourne 3206, Australia

First published 1979

Printed in Great Britain
at the University Press, Cambridge

Library of Congress Cataloging in Publication Data
Alexander, R. McNeill.
The invertebrates.
Includes bibliographies and index.
1. Invertebrates. I. Title.
QL362.A43 592 78-6275
ISBN 0 521 22120 X hard covers
ISBN 0 521 29361 8 paperback

THE INVERTEBRATES

Preface

This book is designed for undergraduates but I hope it will also interest other people. It is about the major groups of invertebrate animals, about their structure, physiology and ways of life. It is intended to complement my book on *The chordates* but the two books overlap slightly in subject matter, as their titles imply. Sea squirts and amphioxus are both invertebrate and chordate and appear in both books so that they can be discussed in relation both to the vertebrates and to other invertebrates.

Each chapter except the first and last deals with a different taxonomic group, usually a phylum or class. It consists of brief descriptions of a few examples of the group, followed by more detailed discussion of selected topics. Some of these topics are peculiarities of the groups (for instance, the shells of molluscs and the flight of insects). Others are more widespread features or properties of animals which can be illustrated particularly well by reference to the group (for instance, I have found it convenient to discuss reflexes in the chapter on crustaceans). Most chapters include descriptions of many experiments because I think it as important and interesting to know how zoological information is obtained, as to know the information itself. The first chapter explains some techniques which are referred to repeatedly in later chapters, and also explains how animals are classified. The final chapter is a brief discussion of the evolution of the invertebrates.

The diversity of the invertebrates is a major problem in writing about them. Any attempt at encyclopaedic coverage results in an enormous quantity of indigestible morphological and taxonomic information. This sort of information is available in existing textbooks and indeed dominates some of them so that they fail to reflect current trends in zoology. I have tried to overcome this problem by being selective. I have described only a few examples of each major group and I have limited description to points I consider interesting and important. I have hesitated to include any phylum which has fewer than a thousand known species, but I have described some small groups because knowledge of them improves our understanding of larger groups. I would rather give students a good understanding of a few familiar animals than tell them about obscure groups they may seldom or never see.

Though I have described rather few species, I am keen that students should appreciate the extraordinary diversity of animals. They will need to study many

more animals than I have described, and it will be far better if they see them for themselves, than if they merely read about them in books. The selection of species they are able to study, in the laboratory and in the field, will depend on local opportunities.

One of the main aims of zoology is to explain the structure and physiology of animals in terms of physical science. For instance, explanations of nerve conduction, swimming and the composition of skeletons depend on physical chemistry, hydrodynamics and materials science, respectively. I have used many branches of physics and physical chemistry, but I have assumed that many readers will have little prior knowledge of them and have tried to explain them in simple terms. I have frequently used simple calculations to demonstrate the plausibility of explanations. There is little value in suggesting, for instance, that flatworms are less than a millimetre thick because oxygen could not diffuse into them fast enough if they were thicker, unless it can be shown by calculation that a flatworm a centimetre thick could not survive.

Professor M. Sleigh read the first draft of this book and made a very large number of helpful suggestions. Other experts read one or two chapters each, and saved me from numerous errors. I am extremely grateful to them all. I hope that readers who find further errors will tell me about them, so that I can correct them if demand justifies a second edition.

January 1978 R. McNeill Alexander

Contents

1
Introduction

Subsequent chapters describe a great many observations on animals, some of them anatomical and some physiological. This chapter explains some of the techniques used by zoologists to make these observations. It also explains how zoologists classify animals.

Many people seem still to think of a zoologist as a man with a microscope, a scalpel and a butterfly net. These are still important tools but a modern zoological laboratory requires a far more varied (and far more expensive) range of equipment. Some of the most important tools which will be mentioned repeatedly in later chapters are described in this one.

MICROSCOPY

It is convenient to start with the conventional light microscope (Fig. 1.1*a*). Light from a lamp passes through a condenser lens which brings it to a focus on the specimen S which is to be examined. The light travels on through the objective lens which forms an enlarged image of the specimen at I_1. This is viewed through an eyepiece used as a magnifying glass so that a greatly enlarged virtual image is seen at I_2. Each of the lenses shown in this simple diagram is multiple in real microscopes, especially the objective which may consist of as many as 14 lenses. This complexity is necessary in good microscopes to reduce to an acceptable level the distortions and other image faults which are known as aberrations.

Microscope technology has long been at the stage at which the capacity of the best microscopes to reveal fine detail is limited by the properties of light rather than by any imperfections of design. It would be easy to build microscopes with greater magnification than is generally used but this would not make finer detail visible any more than magnification will reveal finer detail in a newspaper photograph. If light of wavelength λ and glass lenses of refractive index 1.5 are used, objects less than $\lambda/3$ apart cannot be seen separately, however great the magnification and however perfect the lenses. Since visible light has wavelengths around 0.5 μm, details less than about 0.2 μm apart cannot be distinguished. This is expressed by saying that light microscopes are incapable of resolutions better than about 0.2 μm.

Even this resolution is only possible with an oil-immersion lens of high

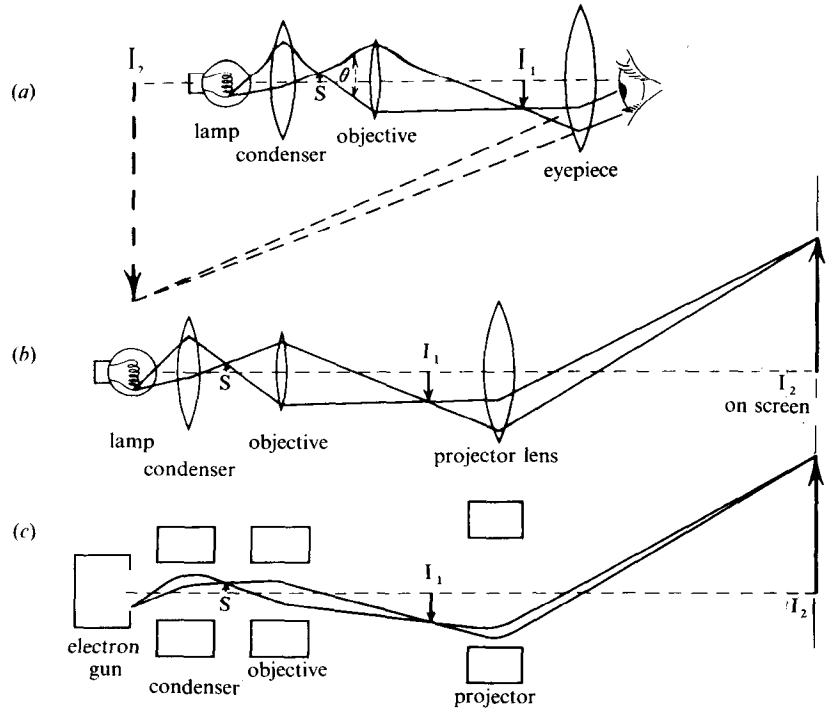

Fig. 1.1. Diagrams of (*a*) a conventional light microscope, (*b*) a projecting light microscope, and (*c*) a transmission electron microscope. The paths of a few rays (of light or of electrons) are indicated.

numerical aperture. An oil-immersion lens is one designed to have the space between it and the specimen filled by a drop of oil of high refractive index. High numerical aperture implies that light from a single point on the specimen may enter the objective at a wide range of angles, i.e. that the angle θ (Fig. 1.1*a*) is large.

Fig. 1.1(*b*) shows a projection microscope. The image I_1 is just outside the focal length of the projector lens whereas in a conventional microscope it is just inside the focal length of the eyepiece. Consequently the final image I_2 is real instead of virtual and can be projected onto a screen. The projection microscope has little use in zoology except in teaching, and is illustrated solely for comparison with the transmission electron microscope (Fig. 1.1*c*). This uses a beam of electrons instead of a beam of light. Magnetic fields set up by electric currents in coils of wire serve as lenses, refracting the rays of electrons in the same way as convex glass lenses refract rays of light. Electrons from the electron gun are focussed on the specimen by the condenser, and other magnetic lenses produce a greatly magnified final image in similar fashion to

the lenses of a light projection microscope. The image is thrown onto a fluorescent screen which can be viewed directly, or onto photographic film.

The electron beam has a wavelength which depends on the potential difference used to accelerate it. Electron microscopes use large potential differences which make the wavelength exceedingly small, so that extremely fine resolution is possible in principle. The resolution actually achieved is much less good because even the best magnetic lenses are far less free from aberrations than the lenses used in light microscopes. Very small numerical apertures have to be used to reduce the aberrations to an acceptable level, making the resolution less good than is theoretically possible. It is still far better than for the light microscope. Resolutions around 1 nm (0.001 μm) are achieved in biological work.

The difference in resolution between the light and the electron microscope is illustrated by Fig. 14.12, which shows sections of the digestive gland of a cockle. Fig. 14.12(*a*) shows as much detail as can be seen by light microscopy, while (*b*), (*c*) and (*d*) have been drawn from electron micrographs which show much more detail than has been drawn. The flagella *f* have diameter 0.25 μm, so though they are visible by light microscopy, no detail can be seen within them. Fig. 14.12(*b*) includes the base of a flagellum and shows many strands within it and Fig. 2.10(*a*) shows some of the finer detail which can be resolved in flagella. Some large molecules such as the haemocyanin of snail blood (a protein: diameter about 30 nm) are large enough to be studied individually by electron microscopy.

To reveal fine internal detail, tissues must be cut into very thin slices. They cannot be cut thin enough without prior treatment. Frozen blocks of tissue can be cut thin enough for some purposes but most specimens for light microscopy are embedded in molten wax which is allowed to solidify and then sliced in a machine called a microtome. Sections only 2 μm thick can be cut. Thinner sections for electron microscopy are cut from specimens embedded in plastics such as Araldite. They can be cut 50 nm thick.

Preparation of specimens for sectioning is quite complex. Each specimen must first be treated with a fixative such as formaldehyde to give it the structural stability it needs to withstand further treatment. Formaldehyde seems to act by forming bridges between protein molecules (this is a process like vulcanization which converts liquid latex to solid rubber by inserting sulphur between the molecules). Next the water in the specimen must be removed and replaced by a liquid miscible with molten wax, so that the wax can permeate the specimen. This is usually done by transferring the specimen from water, through a series of water–ethyl alcohol mixtures, to pure alcohol and then to xylene (which is miscible both with alcohol and with wax, but not with water). This unfortunately tends to shrink the specimen. For instance, sea urchin eggs in xylene have been found to have only 48% of their initial volume. Specimens for electron microscopy have to be treated in rather similar fashion. They are

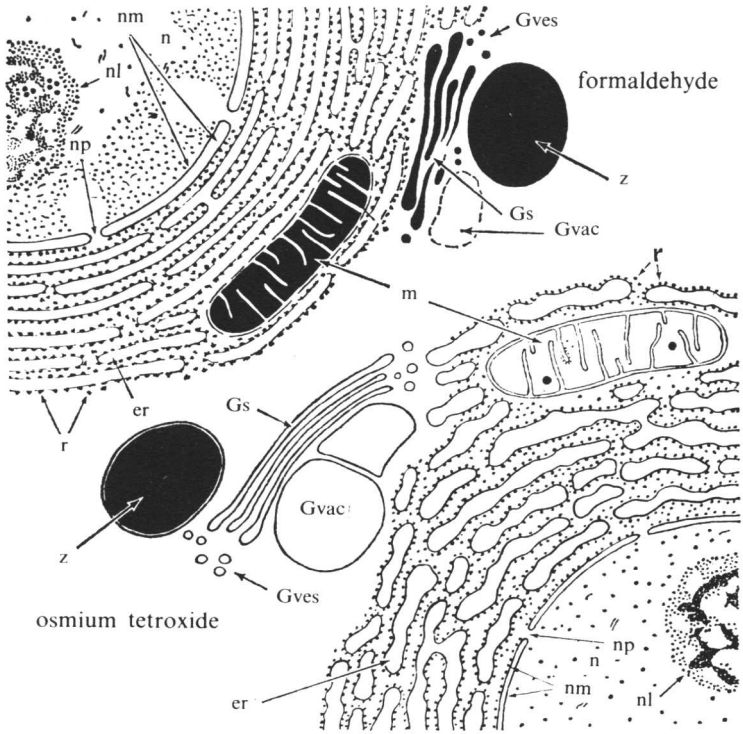

Fig. 1.2. Drawings of electron micrographs of parts of two similar cells (from a mouse pancreas). The one shown above and to the left was fixed by formaldehyde and the other (below and to the right) by osmium tetroxide. er, endoplasmic reticulum; Gs, Golgi saccule; Gvac, Golgi vacuole; Gves, Golgi vesicle; m, mitochondrion; n, nucleus; nl, nucleolus; nm, nuclear membranes; np, nuclear pore; r, ribosome; z, zymogen granule. From J. R. Baker (1966). *Cytological technique*, 5th edn. Methuen, London.

more often fixed with osmium tetroxide than with any other fixative, but they are passed through a series of concentrations of alcohol before being embedded in plastic.

Even all this processing produces sections in which little detail can be seen because most tissue constituents are transparent, especially in thin sections. Dyes are usually used in light microscopy, to colour different constituents different colours. The ways in which most of them work are not fully understood, but some of them are marvellously effective. For instance the Mallory technique colours muscle red, collagen blue and nerves lilac. Contrast in electron microscopy depends on different parts of the specimen scattering electrons to different extents. The greater the mass per unit area of section, the more electrons are scattered and the darker that part of the section appears in the image. Dyeing would be ineffective but contrast can be enhanced by treatment with compounds of heavy metals, such as phosphotungstic acid and

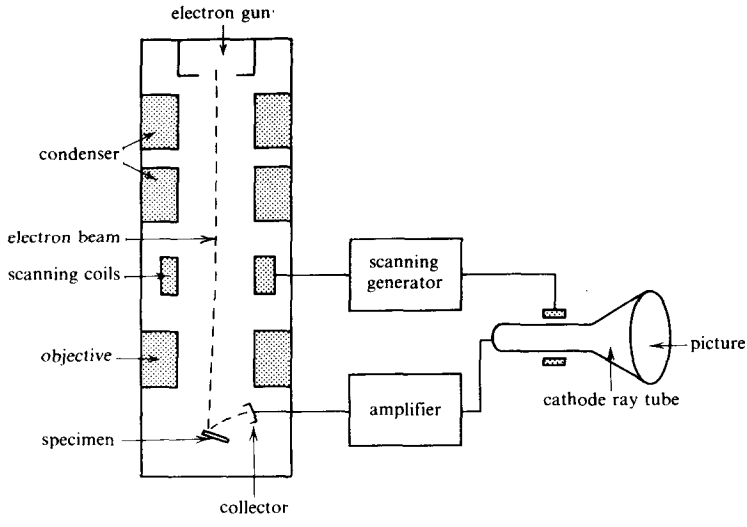

Fig. 1.3. A diagram of a scanning electron microscope.

uranyl acetate. These 'electron stains' attach preferentially to certain cell constituents.

How does all this treatment alter the structure of the specimens? Is the structure seen through the microscope more or less as in the living animal, or is it largely new structure produced by chemical treatment? Fig. 1.2 shows that the same material prepared by different but accepted techniques may look a little different. However, fairly similar appearances are obtained by grossly different techniques and it seems more likely that the structure which is seen was there initially than that it is formed independently by several different treatments.

The technique of serial sectioning is often used. A specimen is cut into sections all of which are examined, to discover the three-dimensional structure of the original specimen.

Protozoans and isolated cells can be examined alive and intact at high magnification, but little detail can be seen in them by ordinary light microscopy. Most of their parts are more or less equally colourless and transparent so details in them are as hard to see as glass beads in a tumbler of water. Fortunately there are differences of refractive index, and these are used to reveal more detail in the techniques of phase contrast and interference microscopy. The swallowing movements of the protozoan illustrated in Fig. 3.13 were revealed by phase contrast microscopy.

The polarizing microscope uses polarized light to show which parts of a specimen have the property of birefringence, due to alignment of molecules or larger structures parallel to each other. It also indicates the direction of alignment. It shows for instance that the molecules are more accurately aligned

in the radial threads of spiders' webs than in the threads which form the sticky spiral (chapter 20).

Ordinary (transmission) electron microscopes are used for examining thin sections. Scanning electron microscopes are used for examining solid objects. They have been found particularly convenient for studying the hard parts of animals and have been used, for instance, to examine broken pieces of mollusc shell and find out how the crystals in them are arranged (Fig. 13.5). Fig. 1.3 shows how they work. The beam of electrons is focussed to a tiny spot on the specimen. One of its effects on the specimen is to release other electrons which are drawn to the positively-charged collector, where they are detected. The beam is deflected by the scanning coils which are used to move the spot systematically backwards and forwards over the specimen. The signal from the collector is used to control the brightness of a spot on a cathode ray tube (like the tube of a television set). This spot is moved backwards and forwards over the screen in precisely the same way as the spot is moving over the specimen, so it builds up a picture of the specimen in the same way as a television picture is built up by a bright, rapidly scanning spot. The pictures give an excellent three-dimensional effect: hollows look dark and projections throw shadows.

The scanning electron microscope cannot resolve detail finer than the diameter of the spot, which is typically 10 nm. Its resolution is therefore much less good than that of the transmission electron microscope, though very much better than that of the light microscope. It is sometimes used at very low magnifications to observe details which are easily visible by light microscopy, because it has much greater depth of field than the light microscope. When a thick specimen is examined by light microscopy it is necessary to focus up and down to see the detail at different levels. When a scanning electron microscope is used to view the same specimen, often everything can be seen simultaneously, all crisply in focus.

Though specimens do not need sectioning for scanning electron microscopy, they need some processing. Since a beam of electrons in air is scattered before it has travelled far, there has to be a vacuum in the microscope. In the vacuum, water would evaporate rapidly from a specimen which had not been previously dried. The specimen must be dried out without shrivelling it. This presents no problem when the specimen is a rigid one, like a piece of mollusc shell, but soft tissues need the special technique of critical point drying.

CHEMICAL ANALYSIS

Zoologists often want to know the chemical composition of a structure or fluid. If large enough samples are available ordinary analytical techniques can be used. Two instruments deserve special mention. One of them is the automatic amino acid analyser, which separates automatically the constituent amino acids of proteins and measures how much of each is present. The other is the X-ray diffraction spectrometer which measures the spacings of the repeating patterns

in crystals. These two techniques have been used, for instance, to show that the horny skeletons of sea fans consist largely of a protein extremely like the collagen of which the tendons of vertebrates are made (chapter 6). Further analysis was needed to show why they are so much stiffer than tendons.

Often zoologists have only tiny samples available. This was the case, for instance, in an investigation of the contractile vacuole of an amoeba which is described in chapter 2. The contractile vacuole is a drop of fluid of diameter about 50 μm. The contents of contractile vacuoles were analysed individually.

The first problem in an investigation like this is to obtain the sample. This is often done by sucking it into a micropipette, a glass capillary drawn out to a very fine point. The tips of the micropipettes used in the contractile vacuole investigation had diameters less than 5 μm. They were stuck into the amoeba under a microscope. This would have needed a phenomenally steady hand if the micropipette had not been held in a micromanipulator, a device in which very fine movements in three dimensions are produced by quite coarse movements of knobs.

The zoologist often wants to know the osmotic concentration of his sample. It is usually most convenient to discover this by measuring the freezing point. If the osmotic concentration of the sample is X Osmol l^{-1} its freezing point is $-1.86X$ °C. A tiny sample is sucked into a fine glass capillary and frozen. It is watched through a microscope while it is warmed again in a specially designed bath, until the last of the ice disappears. The temperature at which this happens is measured by a Beckmann thermometer, graduated in hundredths of a degree.

The most plentiful cations in animals are sodium and potassium. Their concentrations in small samples are usually measured by flame photometry. If you heat a little of a sodium salt in a hot flame it emits yellow light of wavelength 589 nm. This is because electrons in the vaporized sodium atoms are temporarily excited to a higher energy level. When one falls back to its initial level it releases just enough energy to produce a photon of light of wavelength 589 nm. Potassium is similarly affected but the energy change is smaller so the wavelength of the light is longer, 766 nm. The intensity of the emitted light is used in flame photometry to measure the concentrations of the elements.

Fig. 1.4 shows a simple flame photometer. Air and fuel (often acetylene) are blown into the spray chamber. The air, entering through the nebulizer, draws the sample in through the long tube on the left and makes it into a spray which mixes with the fuel and so gets heated in the flame. Light from the flame is made to pass through a filter (to cut out wavelengths emitted by elements other than the one being measured) before falling on a photocell. The reading of the microammeter indicates the intensity of the light reaching the cell and the concentration of the sample can be calculated from it.

The most plentiful anion in animals is chloride. It is measured by titration with silver nitrate, which produces a precipitate of silver chloride. If the sample

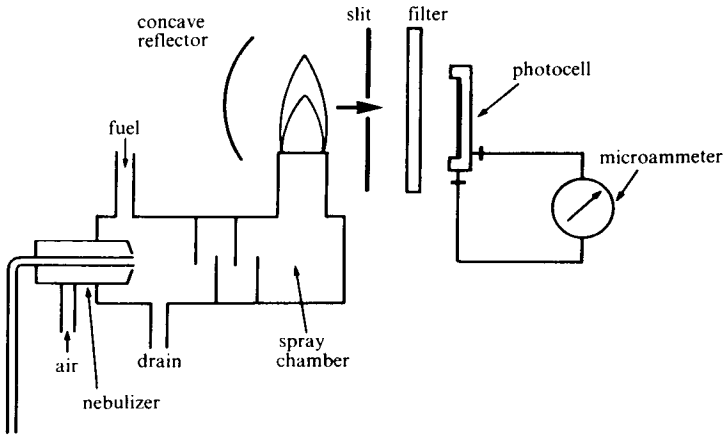

Fig. 1.4. A diagram of a simple flame photometer. From R. Ralph (1975). *Methods in experimental biology*. Blackie, Glasgow.

is large the titration can be done with a burette in the conventional way. If it is small the technique must be modified, for instance in the way shown in Fig. 1.5. The sample is a single drop which is stirred by a jet of air. Silver nitrate is added to it from a syringe operated by a micrometer. The endpoint is sensed electrically: the potential of the silver electrode changes rapidly at the endpoint as the concentration of silver ions in the drop rises.

Histochemistry is used to find out which cells in a microscope section contain a particular chemical compound. Substances are added which react with the compound to produce a coloured product which can be seen under the microscope. There is an account in chapter 8 of an investigation in which histochemical techniques were used to discover which cells produce which digestive enzymes in flatworms. Histochemical techniques have been devised for electron microscopy, as well as for light microscopy.

Another analytical technique which uses the electron microscope is electron probe microanalysis. It measures the concentrations of elements in selected parts of sections. An electron beam is focussed, in a modified electron microscope, onto a small spot on the specimen. The atoms bombarded with electrons emit X-rays of characteristic wavelengths, just as atoms in the flame photometer emit light of characteristic wavelengths (X-rays have shorter wavelengths than light so there is more energy in each quantum, and the electrons of an element have to be raised to even higher energy levels to make them emit X-rays when they fall back, than to make them emit light). X-rays of different wavelengths, characteristic of different elements, are separated by diffraction from crystals. The intensity of each characteristic wavelength is measured and used to calculate the concentration of the element in the part of the section on which the electron beam is focussed. Volumes of about 1 μm^3 can be analysed in this way. The technique is most easily applied if the

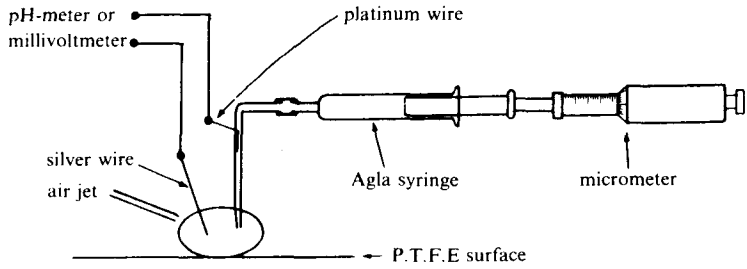

Fig. 1.5. Apparatus for titration of chloride in small samples. From R. Ralph (1975). *Methods in experimental biology*. Blackie, Glasgow.

elements under investigation are present as insoluble compounds. If they are soluble, special precautions have to be taken to make sure that they do not move from one part of a cell to another in the course of preparation. The tissue is frozen rapidly, sectioned while frozen, and examined while still frozen in a special chamber within the electron microscope. This has been done in an investigation of the concentrations of sodium and potassium in different parts of the cells of the excretory organs (Malpighian tubules) of insects (chapter 18).

There are two techniques of electron probe microanalysis, energy dispersive and wavelength dispersive. It is the latter which has been described.

USES OF RADIOACTIVITY

Many zoological experiments exploit the properties of radioactive isotopes. The atoms of an element are not all identical. For instance, carbon consists mainly of atoms containing six protons and six neutrons, giving a mass number of 6+6 = 12. However, it includes about 1% of atoms with six protons and seven neutrons (mass number 13) and a tiny proportion with six protons and eight neutrons (mass number 14). These three types of atom are described as isotopes of carbon and are represented by the symbols ^{12}C, ^{13}C and ^{14}C. Their chemical properties are identical, apart from small differences in rates of reaction.

^{12}C and ^{13}C are stable but ^{14}C is not. One of the neutrons in its nucleus disintegrates, becoming a proton and an electron. This leaves the nucleus with seven protons and seven neutrons so that it is no longer carbon but the common isotope of nitrogen.

^{14}C	$\rightarrow$	^{14}N	+	β^-
6 protons		7 protons		1 electron
8 neutrons		7 neutrons		

The electron leaves at high velocity because the change releases energy. Fast-moving electrons emitted like this by radioactive materials are known as β–rays.

Breakdown is a random process so the number of ^{14}C atoms in a sample falls