GENERAL PATHOLOGY

J. B. WALTER
M.D., M.R.C.P.

M. S. ISRAEL
M.B., M.R.C.P., D.C.P.

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J. B. WALTER
M.D., M.R.C.P.

and

M. S. ISRAEL
M.B., M.R.C.P., D.C.P.

Both of the
Institute of Basic Medical Sciences,
Royal College of Surgeons of England.

WITH A FOREWORD BY

Professor G. J. CUNNINGHAM, M.B.E., M.D.
Conservator of the Pathological Collection and Sir William Collins Professor of Pathology in the University of London, at the Royal College of Surgeons of England.

WITH 264 ILLUSTRATIONS

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This book is dedicated
to
OUR STUDENTS,
from whom we have learned much, and
who have been a constant source of
inspiration to our further efforts.

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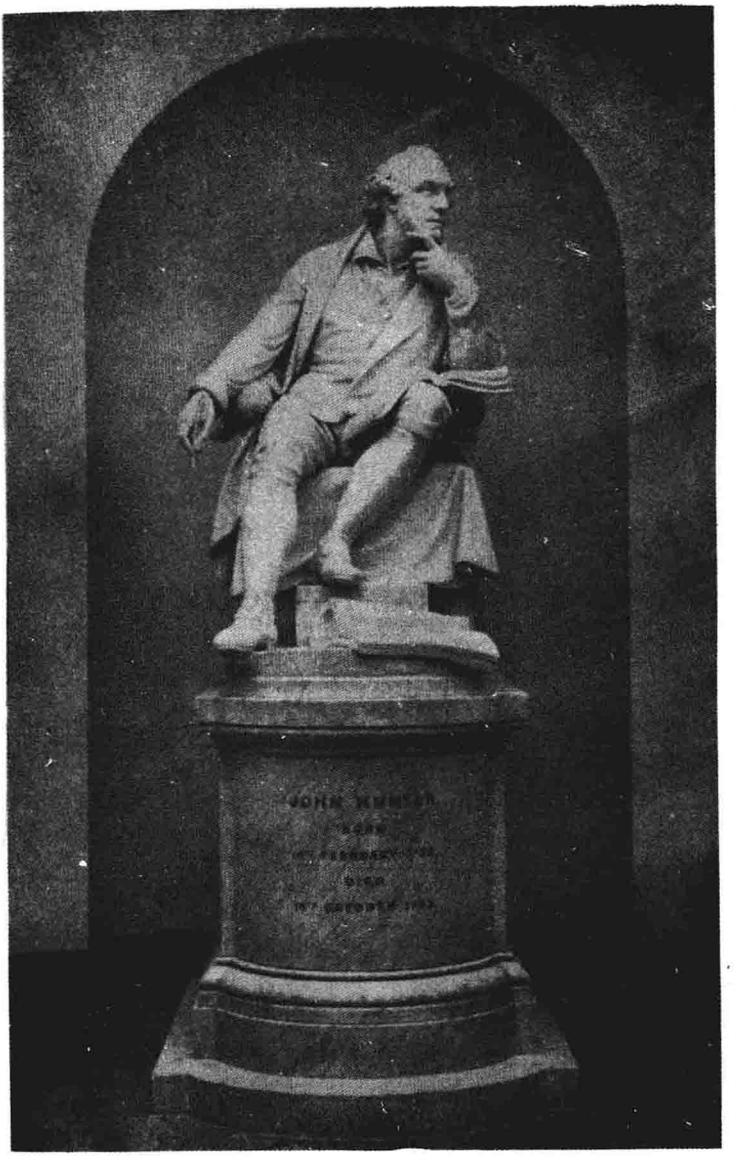


John Hunter's statue is a familiar sight to all who visit the Royal College of Surgeons of England. Admired as a surgeon, a teacher and a thinker, his methods were frequently unusual and unorthodox. His boundless energy and enthusiasm were fortunately coupled with an intense love of truth, which, it has been written,* "never suffered him even to escape from a dilemma by its slightest sacrifice". This scientific integrity has been a source of inspiration not only to his friends, like Edward Jenner, but also to the many who have since followed in his path.

* Palmer, J. F. (1835). Edr., *The Works of John Hunter, F.R.S.*, Vol. 2; London: Longmans.

[*Frontispiece*

FOREWORD

In most medical schools in this country the general principles of pathology are taught to the student during the early days of his clinical curriculum. During the remainder of his course he concentrates on special pathology and its correlation with clinical medicine and surgery. It has been my experience that most recently qualified doctors have only a very hazy idea of general pathology. This is partly because of their inability to obtain a good grasp of its principles in their early clinical days and partly because they have forgotten much of which they had previously learned. The latter seems almost inevitable as the mass of modern medical material to be studied increases. In recent years a knowledge of general pathology has become essential for certain postgraduate students embarking on a specialist career, and I have had considerable experience in providing courses for some of them. Many of these students have found it difficult to obtain a suitable source of information to supplement their lectures. It is on this account that I welcome this book, written by two of my assistants, who have taken active part in the teaching of general pathology and who, I know, have given considerable thought to the difficulties of presenting a complete and yet readable account. They are to be congratulated on having presented a modern approach to general pathology on a very broad basis. In writing such a work references to special pathology are inevitable and these have been very skilfully included in the text without overloading it. Postgraduate students will be grateful to these authors for the large amount of labour that they have put into this book and I wish it the success that it undoubtedly deserves.

G. J. CUNNINGHAM

PREFACE

Writing a text-book of pathology is not only an arduous undertaking, but also a distinctly presumptuous one in view of the excellent works already at the student's disposal. Nevertheless, the experience of eight years' intensive teaching on the course in Basic Medical Sciences for postgraduates has convinced us that there is indeed need for a comprehensive book on general pathology that should embrace the disciplines of morbid anatomy, microbiology, biochemistry and hæmatology.

Our present age is one of intensive specialisation, so much so that it is scarcely possible for the postgraduate to gain understanding of the many subdivisions that together constitute modern pathology. During the last two decades the application of new technical methods to biology has had a momentous impact upon the practice of medicine, and no branch has been affected more radically than pathology. This is to be expected, since the pathologist restricts himself to the objective study of disease, whether naturally occurring or artificially induced in the experimental animal. The discoveries of the chemist, physicist and electron microscopist with the new equipment at their disposal are now being integrated with those of their predecessors. It has not been our aim to give an account of recent advances—many excellent books and reviews are available. Instead, we have set out to present a reappraisal of the fundamentals of pathology in the light of recent findings. Our first concern has been to give a careful description of basic pathological processes in an attempt to dispel the difficulties that surround both these processes and the terms that are commonly used to define them.

This book is designed for students embarking on a specialist career in medicine, surgery or anæsthesiology. As originally planned it was intended as a guide for Primary F.R.C.S. and F.F.A. examinees. However, the course of pathology at the Institute of Basic Medical Sciences has become extended due to the ever-widening scope of the subject, and this is reflected in the comprehensiveness of the book, which we now believe will be of general interest to all students of medicine. We are well aware that our audience is a clinical one, and have tried to amalgamate the theoretical with the practical aspects of pathology.

We owe special gratitude to Professor G. J. Cunningham for initiating us into the modern concepts of dynamic pathology. It has been under his influence that we have come to appreciate the significance of general pathology as a subject in its own right. Professor Gilbert Causey has been a source of great encouragement, and has in addition provided help in respect of cellular anatomy and electron microscopy. We are greatly indebted to Dr. Cyril Long for his invaluable advice in all the biochemical matters treated in this book. Others to whom we owe much are Dr. G. C. R. Morris, who has guided us in the

physiological aspects of electrolyte balance, renal failure and œdema, Dr. N. Ambache, who has assisted with the pharmacology of the chemical mediators of acute inflammation, and Dr. L. W. Proger, who has given us the benefit of his wide experience in morbid anatomy. We are also indebted to Professor W. G. Spector of St. Bartholomew's Hospital Medical School for his assistance with the chapter on acute inflammation. Both Mr. D. E. A. Jones of Mount Vernon Hospital and Dr. H. J. G. Bloom of the Royal Marsden Hospital have helped in the sections on ionising radiations, and Dr. Walter Somerville of the Middlesex Hospital has provided valuable criticism of the chapter on heart failure. We thank Dr. W. M. Christopherson of Louisville, Kentucky, for his guidance on the cytological aspects of early cancer diagnosis. Our colleagues, Professor G. V. R. Born, Mr. G. R. N. Jones, Dr. A. J. M. Reese, Dr. S. J. Surtees and Dr. B. D. Wyke have made many helpful suggestions.

We are grateful to our Dean, Mr. E. G. Muir, and to Sir Arthur Porritt for their encouragement. We thank the President and the Council of the Royal College of Surgeons of England for permission to reproduce the photographs of specimens in the Wellcome Museum of Pathology. The great majority of the specimens illustrated are from this museum, and may be identified by the catalogue number which appears at the end of each caption. In the case of published work we wish to thank the authors, editors and publishers who have allowed us to reproduce their material. The references are given after each figure. We owe special gratitude to those who have given us unpublished material or who have allowed us to modify their original published work: Bamforth, J., Fig. 30.6; Bearcroft, W. G. C., Fig. 4.4; Blair, G. H., Fig. 9.2; Brewer, D. B., Fig. 4.6; Causey, G. W., Figs. 2.5 and 5.2; Chesterman, F. C., Fig. 15.3; Crick, F., Fig. 2.6; Crowle, A. J., Fig. 1.3; Dawson, I. M. P., Fig. 11.10; Director, Archæological Museum, Florence, Fig. 15.1; Dixon, F. J., Fig. 12.2; Epstein, M. A., Figs. 2.2, 2.4, 2.10, 2.11, 2.12 and 22.4; Gahan, P. B., Fig. 1.6; Hadfield, G., Figs. 37.3, 37.4, 37.5, 37.6 and 37.7; Hale, A. J., Fig. 1.1; Horne, R. W., Figs. 22.1, 22.2 and 22.3; Kawerau, E., Fig. 1.2; Kemp, N. H., Figs. 2.8, 2.9, 3.6 and 3.7; Levene, C. I., Fig. 5.1; Najarian, J. S., Fig. 15.2; Proger, L. W., Figs. 4.5, 4.8, 11.4, 28.7, 29.2 and 29.3; Reese, A. J. M., Fig. 1.5; Steward, S. P., Fig. 9.5; Thomson, A. D., Figs. 21.3, 27.28 and 31.4; Waldenström, J., Figs. 45.1 and 45.2; and Wintrobe, M. M., Fig. 49.1.

Our thanks are due to Mr. C. H. Redman for photographing the museum specimens, and to Mrs. J. B. Walter and Mr. A. L. E. Barron for help with the photomicrography. It is also a pleasure to acknowledge the help provided by the Librarian of the College, Mr. W. R. Le Fanu, and his assistants in tracing references.

A problem facing any author is that of furnishing references. Our policy has been to provide a guide to further reading rather than a comprehensive bibliography. Our references are those that we have read and found useful. We have made extensive use of recent reviews, annotations and leading articles; these, by being liberally annotated, at

once direct the reader to the previous literature. We have also received considerable help from other text-books, and these too are included in the further reading.

We are grateful to our publishers, in particular Mr. J. Rivers, who played such an important role in initiating the venture, and Mr. A. S. Knightley, who has guided us in matters of production detail.

Finally, we have much pleasure in expressing our gratitude to Miss Linda Chiaramonte of Greenfield, Massachusetts, but for whose good humour, good organisation and good typing this book would never have been completed.

J. B. W.
M. S. I.

London

CONTENTS

CHAPTER 1

INTRODUCTION

PATHOLOGY, the scientific study of disease processes, has its roots deeply implanted in medical history. The earliest observations, ranging from Celsus (about 30 B.C.–A.D. 38) to Morgagni in the eighteenth century, were based upon the naked-eye appearances of diseased individuals and organs. Only as the technique of microscopy improved, was the Germanic School of Pathology, headed by Virchow (1821–1905), able to investigate changes at a cellular level.

In France, Pasteur, using the microscope, laid the foundation of the science of bacteriology, while later on the German dye industry enabled Koch, Ehrlich and Domagk to extend this knowledge and open the era of chemotherapy. Advances in pathology were thus closely related to advances in technology. This in no way belittles the efforts of inspired experimenters like Jenner, who in England pioneered the way to active immunisation.

Nowadays technological advances are occurring so rapidly that no student or practitioner can hope to have a working knowledge of all the techniques that are available. Nevertheless, it is only by utilising these complex techniques that problems can be fully investigated. The student of medicine should therefore have some knowledge of the techniques which are available, and the type of problems which they may be able to solve. In this way investigators conversant with many different disciplines are brought together to their mutual benefit.

TECHNIQUES AVAILABLE IN PATHOLOGY

Microdissection

Apparatus is available for dissecting cells. In this way the nucleus can be removed, and the effect of this on the deprived cell observed. Micropipettes can be inserted into the capillaries, and microelectrodes placed in single nerve cells or fibres.

Microscopy

The Light Microscope

The ordinary light microscope has two disadvantages:

(*a*) Its *resolution* is limited by the wavelength of light. The ability to distinguish between two adjacent points is called the resolving power. In theory resolution should be possible up to a distance of half the wavelength of the light used, but in practice using green light the resolving power of the microscope is about 250 mμ.* Thus only the larger viruses like those of the psittacosis-lymphogranuloma inguinale

* 1 mm = 1,000 μ, 1μ = 1,000 mμ, 1 mμ = 10 Å.

group can be studied with a good optical microscope. The shorter the wavelength of the light used, the better is the resolution, and with ultraviolet light it can be improved 2–3 times. It is important to distinguish between *visibility* and *resolution*. Particles as small as 75 mμ may be visible, but no detailed structure can be resolved. A simple analogy illustrates this difference. If an open book is placed at a distance from the eyes, the writing can easily be distinguished as such (i.e. it is visible), but it may be quite impossible to recognise individual letters because their details cannot be resolved.

(*b*) Living tissue is transparent, and the homogeneity in optical density of its components hides its detailed structure. Staining techniques must therefore be used to see cellular details, but these must almost invariably be performed on dead fixed tissue. It is possible to stain cells by supravital techniques, e.g. the mitochondria of living leucocytes can be stained by Janus green, but even this causes rapid damage to them so that the cells soon lose their motility and begin to die.

Three techniques have been developed to overcome these difficulties in examining living cells.

Dark-ground illumination relies upon the fact that objects placed in a beam of light may be seen by the rays which they reflect in much the same way that dust particles are rendered visible by a shaft of sunlight. The method finds particular application in the demonstration of organisms which cannot be readily stained, e.g. *Tr. pallidum*.

Phase Contrast microscopy takes advantage of the different refractive indices of various parts of the cell. These differences are converted into differences in optical density. In this way living cells can be examined; mitochondria, at one time thought to be artefacts, have been shown to occur in the normal cell.

Interference microscopy works on a different principle. It produces a picture of the cell in which its different components appear in different colours. The interference microscope has one further advantage: the actual amount of a chemical substance in a cell can be measured.

Staining Techniques. A variety of staining techniques has been developed as an aid to light microscopy. Hæmatoxylin and eosin ("H and E") serve to distinguish acidic from basic substances; hæmatoxylin being basic combines with acids, while eosin reacts with bases. Since the nucleus is invariably acidic, and the cytoplasm usually basic, this combination of stains is of great use in routine microscopy, when a general examination of structure is required. Another method of particular value in demonstrating structures which will not readily take stains, is to outline them with metallic silver. This technique is used to show reticulin fibres, *Treponema pallidum*, etc.

Other staining methods are available for demonstrating specific chemicals within cells. This technique is called *histochemistry*, and today has very wide applications. Two very good examples are:

1. *Perls's Prussian blue reaction for hæmosiderin.* Acid is applied to the tissue to release Fe^{+++} from hæmosiderin. Next, potassium ferro-

cyanide is applied: this reacts with the Fe^{+++} to form blue ferric ferrocyanide.

2. *Periodic-Acid Schiff Reaction* (*P.A.S.*). This stain is less specific, but nevertheless useful. When periodic acid is applied to a section, many carbohydrate components are oxidised to aldehydes. Aldehydes will produce a red colour with Schiff's reagent (a solution of basic fuchsin decolorised by sulphurous acid). Therefore, if Schiff's reagent is applied to the treated section, the parts containing carbohydrate will be stained red. The periodic-acid Schiff reaction is useful for the demonstration of glycogen, ground substance and epithelial mucus.

It will be appreciated that most staining techniques are employed on tissue which has been fixed, dehydrated and embedded in paraffin wax. This procedure removes certain chemicals (like fat) and alters others (like enzymes). To meet this difficulty the *cryostat* has been developed: in essence this is a microtome with which sections are cut at −30°C. This is achieved by having the microtome enclosed in a refrigerator. The sections of frozen fresh material so obtained are so little damaged, that they are very suitable for histochemical study.

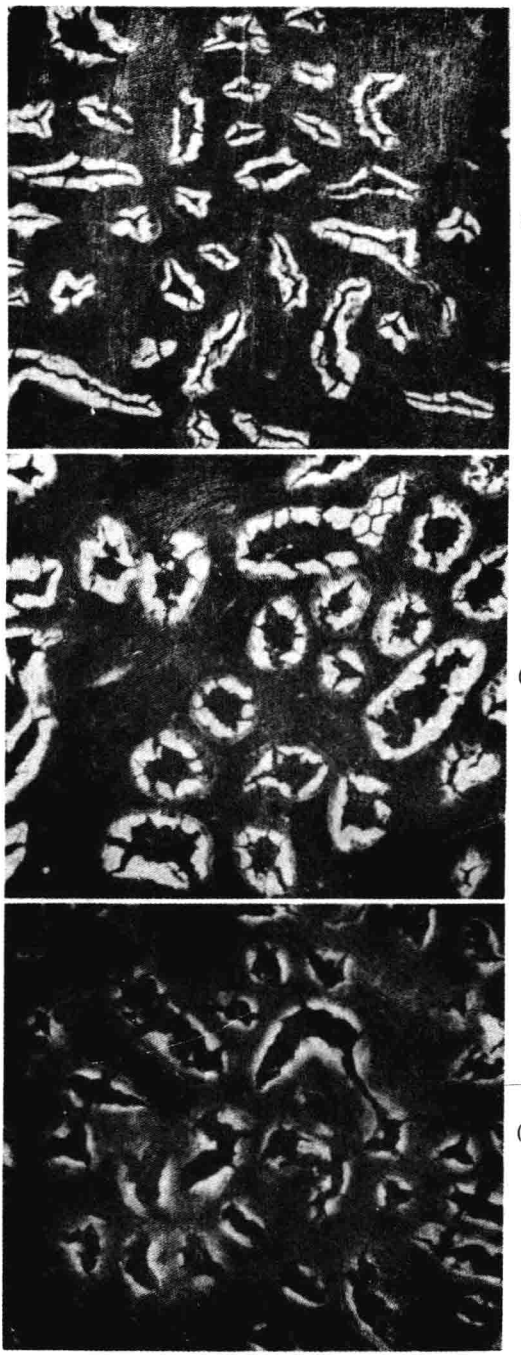
The demonstration of an enzyme is well illustrated by the method used for alkaline phosphatase. The section is placed in a solution of the substrate, β-glycerophosphate in the presence of calcium. The enzyme splits off phosphate which forms calcium phosphate. The latter is invisible. Addition of cobalt nitrate converts it into cobalt phosphate, and ammonium sulphide changes this to black cobalt sulphide.

Although the amount and situation of the black sulphide is indicative of the amount and site of the phosphatase originally present, it is evident that errors can creep in at each of the stages: Glycerophosphate → calcium phosphate → cobalt phosphate → cobalt sulphide. The *x-ray microscope* demonstrates this very well. This instrument takes radiographs of sections using x-rays generated at 5–10 kV. The rays are absorbed by elements of high atomic number and like the conventional full-size radiographs show up deposits of calcium. Fig. 1.1 shows how the original calcium phosphate is localised, whilst there has been considerable diffusion by the time the last step is reached. The x-ray microscope can, of course, be used on unstained tissues to show deposits of calcium as well as other structures.

Histochemical techniques may also be applied to the study of cells by electron microscopy (see Fig. 2.4, p. 18).

The Electron Microscope

The electron microscope resembles the ordinary microscope except that a stream of electrons is used instead of light, and electromagnetic fields instead of lenses. Its resolution is about 100 times greater than the light microscope, and objects as small as 1·0 mμ can be resolved. By its use viruses can be seen with ease, and our knowledge of detailed cell and tissue structure has increased enormously (see Chapter 2). The electron microscope has, however, four disadvantages.



(*a*)

(*b*)

(*c*)

FIG. 1.1. (*a*) Historadiograph of rat kidney after incubation of the section with β-glycerophosphate in the presence of calcium chloride. The deposits of calcium phosphate indicate the site of alkaline phosphatase activity.

(*b*) Section treated as (*a*) above, but the calcium phosphate has been converted into cobalt phosphate by the action of cobalt nitrate.

(*c*) Final treatment with ammonium sulphate converts the cobalt phosphate into cobalt sulphide. The historadiograph shows that there has been considerable diffusion, and that the distribution of the radio-opaque material no longer corresponds accurately to that of the alkaline phosphatase. Cobalt sulphide is black, and is visible under the light microscope. However, it is evident that a radiograph at stage (*a*) gives a clearer indication of the actual distribution of the enzyme. × 150.

(*From photograph supplied by Dr. A. J. Hale. Published previously Hale, A. J.* (1961), J. biophys biochem. Cytol., **11**, 488–492)

1. Only very small pieces of tissue can be examined (e.g. 2–3 mm blocks are usual).

2. The tissue must be examined in a vacuum—living cells cannot therefore be used.

3. Tissue must be fixed immediately, preferably *in vivo*, if artefacts are to be avoided. This must be borne in mind if it is proposed to examine human surgical material.

4. The best fixative so far discovered is osmium tetroxide: this shows up lipoprotein components like cell membranes very well, but details of other structures like the nucleus are not so well demonstrated.

The Ultracentrifuge

The tremendous centrifugal force which may be applied by this machine can be utilised to separate mixtures of large molecular chemicals. The present day instrument can develop a centrifugal field sufficient to spin down particles as small as 10Å. The velocity with which sedimentation occurs can be measured, and the ratio:

$$\frac{\text{Sedimentation velocity}}{\text{Centrifugal Field}}$$

is called the Sedimentation Constant. It is measured in Svedberg units (S).

The ultracentrifuge has been used to separate mixtures of proteins and it was in this way that the macroglobulins were identified (see Chapter 45).

The various components of cells can also be separated and examined by physical and chemical means. If cells are disrupted either physically or by ultrasonic vibration, the cell membranes, nuclei, mitochondria and microsomes can be isolated in a fairly pure state after centrifugation. By analysis of these fractions the results of histochemistry may be correlated with those of the well-established procedures of chemistry. The many enzyme systems of mitochondria have been investigated in this way. The granules in cloudy swelling have been isolated and identified as altered mitochondria (see p. 52).

Chemical and Physical Analysis of Substances of Biological Interest

It is beyond the scope of this book to deal with this subject. It is sufficient to note that methods are available which have resulted in the complete analysis of substances as complex as the insulin molecule (51 amino acid residues) and the enzyme ribonuclease (124 amino acid residues). Modern methods of rapid analysis have been introduced into clinical practice in many instances (see Fig. 1.2 and legend). Two other methods find particular application in pathology.

Electrophoresis

If a mixture of proteins is placed in an electric field at a known pH, individual proteins move at particular rates dependent to a great

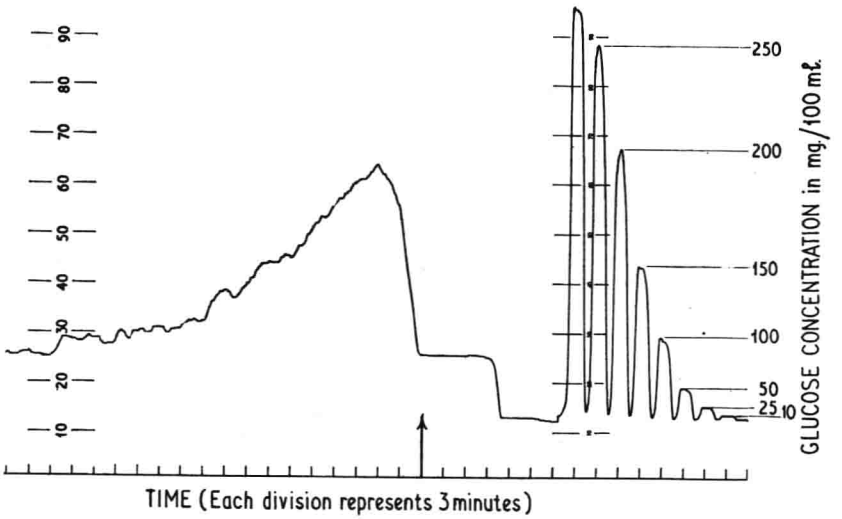


FIG. 1.2. Continuous blood sugar recording obtained by the use of an auto-analyser. The graph reads from right to left. Standard solutions of glucose (10, 25, 50, 100, 150, 200 mg/100 ml, etc.) were first fed into the instrument to obtain the calibration spikes shown on the right. Next, continuous sampling of blood was obtained by means of a small catheter inserted into an arm vein. At the point indicated by the arrow 20 g of glucose was administered intravenously, and the blood sugar level rose from 84 mg/100 ml to 195 mg/100 ml. The form of the curve showing the subsequent return to normal is within normal limits. The instruments can be adapted to measure other blood constituents either as a continuous recording or for a large number of separate specimens.

(*Photograph of a recording provided by Dr. E. Kaverau*)

extent upon their size and charge. The test is conveniently performed on blotting paper, and after passing an electric current for a suitable time the paper is dried, and the separated proteins are stained with a simple dye, e.g. light green. Fig. 45.1 shows a typical electrophoretic separation of the plasma proteins. Combined with the agar diffusion technique electrophoresis has proved invaluable in separating protein mixtures (see Fig. 1.3 and legend).

Paper Chromatography

This is useful for separating a mixture of substances dissolved in a fluid. A drop of it is placed on a filter paper and dried, and then a solvent is allowed to flow across the paper. The various components are carried along at different speeds. The ratio:

$$\frac{\text{distance travelled by component}}{\text{distance travelled by solvent}}$$

or R_f, is a constant for each component under the particular conditions of the test. The solvent (often itself a mixture) must be carefully chosen according to the nature of the mixture which is to be separated. It is made to travel across the paper in one of two ways:

Capillary Action. This is used in ascending and radial chromatography (see Fig. 1.4).