Blacklock and Southwell

A GUIDE TO HUMAN PARASITOLOGY

TENTH EDITION

REVISED BY

W. CREWE

LONDON

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FOR MEDICAL PRACTITIONERS

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PREFACE TO THE TENTH EDITION

In recent years there have been notable advances in our knowledge of the biology of some of the parasites of man, and accordingly certain sections of the Guide have been considerably revised. In addition, relatively minor corrections and amendments have been made throughout the text.

I am grateful to several of my colleagues for valuable comments and suggestions for the improvement of this book.

January 1977 W. C.

EXTRACTS FROM PREFACE TO THE FIRST EDITION

THIS book is intended for the practitioner who, from time to time, will certainly require to make a diagnosis in diseases caused by animal parasites; it is also intended for those who are taking courses of instruction for the Diplomas of Tropical Medicine, Tropical Hygiene and Public Health. In it we have endeavoured more particularly to meet the requirements of those who have no laboratory near them at which parasitological diagnosis is undertaken.

A long experience of teaching post-graduate men and women has brought home to us the fact that, during a brief course in parasitology, the practitioner tends to become overwhelmed by the mass of new names and anatomical details, and, as a result, often fails to grasp the few essential characters by which the parasites can be diagnosed.

For this reason we have presented our subject in a manner which will doubtless appear somewhat unorthodox; our aim throughout has been to lay emphasis only on the pathogenic organisms, and to restrict our descriptions to those characters in them which are of immediate diagnostic value.

Such definitions as we have given for classes, superfamilies, and genera, are often curtailed so that they serve only for the parasites described in this book, and they will obviously break down if any attempt is made to apply them to the animal parasites in general.

We do not feel that any apology is required for the publication of this manual; there exists, in our experience, a demand on the part of practitioners, for a book in which the salient characters of the common pathogenic parasites of man, in any climate, are set out in a simple and concise way and in which the methods of diagnosis are rapid and easily learned.

Our thanks are due to Professor J. W. W. Stephens, M.D., F.R.S., and to Professor Warrington Yorke, M.D., who have been kind enough to read through our manuscript and to make various helpful suggestions. We wish also to express our indebtedness to Mr. David Dagnall of the laboratory staff of the Liverpool School of Tropical Medicine, who is responsible for the great majority of the illustrations.

D. B. B.
T. S.

November 1931

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A GUIDE TO HUMAN PARASITOLOGY

CHAPTER I

INTRODUCTION

A KNOWLEDGE of parasitology and its techniques is of great value to the medical practitioner concerned with patients suffering from conditions caused by parasites, such as malaria, amoebic dysentery, helminth infections and many others. Lacking this knowledge he is compelled to make a diagnosis on purely clinical grounds, whereas the practitioner who has been trained in parasitology is not thus handicapped, and may be able either to make a diagnosis on definite parasitic findings or to exclude a parasitic causation of the condition. Where a parasite is incriminated as the cause, treatment can be given confidently, in contrast to the uncertainty accompanying tentative treatment given in ignorance of the actual cause of the condition.

Even in circumstances where a technician is available to make parasitological investigations, it may sometimes be necessary for the practitioner to confirm the findings and check the correctness of the technique used. More importantly, an immediate diagnosis is often demanded when a technician is not available, say in the case of a patient suffering from suspected cerebral malaria during the night. The doctor who has learned the relevant techniques can take the necessary specimens, prepare them, and make an immediate examination for the possible parasitic cause. If the causal agent is identified treatment can be instituted at once, possibly saving the life of the patient.

Today the emphasis in the medical approach to disease is increasingly on its prevention rather than its treatment, and a knowledge of the epidemiology of parasitic disease is essential to understanding how it is caused and to developing soundly-based preventive measures.

In preparing this Guide we have assumed that the reader has no experience of the subject. We shall show how an individual can attain a considerable degree of proficiency in the identification of parasites without having to wait for an infected patient; and in various sections indicate where living material suitable for examination can readily be procured. It must be emphasised that the making of good films of blood and faeces is of great importance. The necessary skill can be acquired by anyone willing to take sufficient time and trouble, and those learning medical parasitology are urged to practise the techniques until they have mastered them.

The methods of diagnosis referred to in the pages that follow assume that routine parasitological laboratory services are available to the medical practitioner. The greatest emphasis is, however, laid upon the methods which he himself learns to practise and upon which he will rely in the first instance, and which require only stains and some simple reagents, a hand lens and a microscope, and common laboratory equipment. In the event of these methods yielding negative results, more elaborate cultural, serological and other techniques may be needed, demanding the special skills and equipment of a complete laboratory. These techniques are not described, but some of the positive or negative diagnostic findings obtained by their use are discussed.

For the identification of parasites, the sizes of protozoa and protozoal cysts, as well as of helminths and their ova, are frequently given an importance which is far greater than is justified by the value of size as a diagnostic criterion. Naturally the practitioner requires to know the relative sizes of the various parasites he may encounter, but when he is considering the actual sizes of individual parasites he should bear in mind that he is dealing with living animals, and therefore that even within a single species considerable variation in size must be expected to occur normally. Protozoa and their cysts, or worms and their eggs, show as much relative variation in size as, say, hens and their eggs. The fixation of living tissues for preservation often accentuates differences in size, and may result, for example, in a segment of a tapeworm being relatively long or broad according to its state of expansion or contraction at the time of fixing. In practice the size of protozoal cysts is of considerable diagnostic value, although specific identification depends on other morphological characters; on the other hand, helminth eggs are identified primarily on definite and striking morphological characters, and with few exceptions size is of little importance except as corroborative evidence of the identification.

Parasites

Parasitology is the science which deals with parasites. These are organisms which have adapted themselves to existence in, or on, another organism, the latter, which harbours the parasite, being termed the host. Ectoparasites are those which live outside the body, endoparasites those which occur inside the body, of the host. A parasite may be of animal nature, for example a helminth (such as the large round worm *Ascaris lumbricoides*) or a protozoon (such as *Plasmodium vivax*, one of the malaria parasites), and it is chiefly with such animal parasites that we are here concerned.* It may, further, be temporary or permanent. The larva of *Cordylobia anthropophaga*—a muscid fly—lives through its several stages

* This Guide deals mainly with those parasites of man which can be seen with simple staining or an ordinary lens or microscope. Viruses, rickettsiae, bacteria and fungi are excluded.

in the skin of man, and only leaves the skin in order to continue its development into a pupa and adult outside man's body; the parasitism is thus temporary and limited strictly to the larval stages. On the other hand, all the pathogenic protozoa and helminths which we discuss here may be regarded, broadly speaking, as permanent parasites; they tend to remain in the body until either age or some immunizing process or treatment removes them.

A parasite of man may be quite non-pathogenic, as, for example, *Entamoeba coli*, or, on the other hand, it may be definitely pathogenic and live at the expense of the tissues and fluids of the host, as does *Entamoeba histolytica* which is the cause of amoebic dysentery. The damage produced in the host's tissues by pathogenic species of animal parasites may be effected mechanically, or be due to the deleterious action of metabolic products of the parasite, or result from the combined action of both these causes. *Plasmodium vivax*, a malaria parasite, not only destroys the red cells of human blood in a mechanical manner during its growth inside them, but also generates products which give rise to symptoms such as rigors. Similarly *Entamoeba histolytica*, the pathogenic agent in amoebic dysentery, causes damage to the large intestine of man, producing ulcers there partly by its mechanical activities and partly by its histolytic products. Extreme variations in the degree of pathogenicity may occur within those species of parasite which are usually pathogenic to man. Thus malaria parasites, which are usually very pathogenic to newcomers, may be present in considerable numbers in the inhabitants of endemic areas without giving rise to any obvious symptoms. On the other hand, the presence of a single larva of *Echinococcus granulosus* (hydatid) or of *Taenia solium* (Cysticercus cellulosae) may be sufficient to produce fatal results if present in a vital situation.

The term commensal is sometimes applied to parasites which live on the superfluous fluids or solids in the host, and are non-pathogenic; *Entamoeba coli* is such a commensal.

Organisms which have been swallowed, and have merely passed through the alimentary canal in a passive condition, but have not been living in the host as true parasites, are frequently found in faeces; the term coprozoic is applied to them and also to those organisms which invade faeces after they have been passed.

Notes on the Nomenclature of Parasites

In the classification of animal parasites very numerous subdivisions of the main groups are necessary, and the names applied follow certain laws, laid down in the International Rules of Zoological Nomenclature. It is not necessary here to do more than indicate the general principles. The parasites with which we deal belong to three phyla of the animal kingdom, namely Protozoa, Platyhelminthes and Nemathelminthes, and a group, of which the exact classification is undecided, called the Spirochaetales; these

last are probably more nearly akin to the bacteria than to the protozoa. Each phylum is divided into classes, and these in turn are divided into orders, families, genera, and species. Occasionally other subdivisions are included, such as superfamilies and subfamilies. For the medical man, however, the most important of the names are those of the genus and the species. The generic name of an animal parasite consists of a single word, as in the genus *Plasmodium*, and the specific name of two words, as in the species *Plasmodium vivax*, the generic name with a capital, the specific name with a small initial letter. A variety is expressed by three words, *e.g. Pediculus humanus corporis*. The name of the author of the scientific name is written immediately after it, without any punctuation; after the author's name a comma is inserted, and then the date, followed by a full stop, *e.g. Onchocerca volvulus* Leuckart, 1893. The author of a generic name, and the date, are placed in brackets, if the name applied by him is altered; the new author's name, with date, being outside the brackets. The names of genera and species are underlined in writing them, or printed in italics. An example of the divisions is as follows:

DIVISION	TERMINATION	EXAMPLE
Phylum	—	Platyhelminthes
Class	—	Cestoidea
Order	-idea	Cyclophyllidea
Superfamily	-oidea	Taenioidea
Family	-idae	Taeniidae
Genus	—	*Taenia*
Species	—	*Taenia solium*

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The following list is intended only to suggest various starting-points for the interested reader who wishes to find further or more detailed information; it is not exhaustive, nor are the lists at the ends of other chapters. The publications mentioned in these lists are mainly more recently published textbooks and review articles, and although these are unlikely to contain all the information required they will give the reader further references to the relevant literature.

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CHAPTER II

THE MICROSCOPE

BEFORE he attempts to study the parasites, it is absolutely essential that the beginner should learn to use the microscope. Modern microscopes vary considerably in form and complexity, but all work on the same basic principles as the conventional microscope (Fig. 1). This type of microscope is the simplest compound microscope in general use, and has the great advantage that it can be used with almost any form of lighting. This, together with its relative cheapness and robustness, make it of great value under primitive conditions, as in field stations in the tropics. The conventional microscope consists of a base from which arises a short upright pillar, and, hinged to this pillar, a body which carries the illuminating apparatus, the stage, the tube with its milled screws for coarse and fine adjustments, and the oculars and objectives.

The illuminating apparatus comprises a mirror having plane and concave sides, a condenser below the stage (substage condenser), and an iris diaphragm by which to regulate the amount of light passing up from the mirror.

The *stage* is fixed to the body, and on it are placed the objects, usually mounted on slides, which are to be examined. The stage, which may be round or rectangular in shape and has a central aperture, can be surmounted by an apparatus, called the "mechanical stage," for moving the slides horizontally.

The *tube* is attached to the body above the stage, and is fitted, at its lower end, with a nosepiece into which the objectives are screwed; at its upper end, the eyepiece, or ocular, is inserted. The tube carrying the ocular and objective can be lowered and raised by the adjustments, coarse and fine, for the purpose of finding and focusing the object on the slide.* Inside the tube there is sometimes an inner tube called the draw tube which can be extended; when an inner tube is present, the ocular is fitted into the upper end of this. On the inner tube a scale is engraved for the purpose of adjusting the tube-length; the length required for the lenses varies in different microscopes.

* In some modern microscopes the body and the tube with its objectives and oculars are fixed and the coarse and fine adjustments are incorporated in the stage which is therefore moveable vertically.

Objectives. There are three objectives in general use, usually known as the low power, high power and oil immersion objectives. These respectively have focal lengths of 16mm, 4 mm and 2 mm, and magnifications of about $\times 10$, $\times 40$ and $\times 100$.

Oculars. Two of these are required for ordinary work, for example, a low power, $\times 6$ ocular, and a high power, $\times 10$ ocular. The magnification obtained by the microscope is the product of the magnifying power of the objective and that of the ocular. Binocular compound microscopes or attachments, owing to their complex prism equipment, often increase the magnification by a further quarter or half.

The Use of the Microscope

The microscope should not be used with direct sunlight as the source of illumination; if bright diffuse daylight cannot be obtained, it is more satisfactory to use artificial light; and if better types of artificial light are not available, good work can be done with the light of an ordinary kerosene lamp. The amount of light reflected by the mirror on to the object always requires careful adjustment in order to get clear definition of the object. Thus, in examining a film of faeces with the low power $\times 10$ objective, the faecal matter cannot be seen if too much light is admitted. The iris diaphragm must therefore be closed gradually until the best definition of the film is obtained. When the high power $\times 40$ objective is turned on to the same object, it will be found necessary to admit more light by slight opening of the diaphragm, to obtain the best result. The same principle of the use of the diaphragm applies when the $\times 100$ oil immersion is employed after the $\times 40$ objective. In the binocular microscope the amount of light reaching the eyes is reduced and brighter illumination is needed than in a monocular.

In using the microscope for examination of objects described in this book, the beginner is advised to rack up the substage condenser and to use the flat side of the mirror, except for the specified objects mentioned later. The eyepiece may be removed long enough to adjust the mirror so that the field of view through the tube appears evenly illuminated with the iris diaphragm open. Subsequently the diaphragm alone should be used in order to adjust the illumination, by opening it to increase, or closing to decrease, the amount of light. It should be a rule to examine microscopic objects first with the low power and only then to proceed to the high powers. For example, a stained blood film should be examined first with the low power $\times 10$ objective, then with the high power $\times 40$ objective, and lastly with the oil immersion $\times 100$ objective. The low power will enable one to see microfilariae, and the high power to detect trypanosomes or relapsing fever spirochaetes, if present; whereas all these parasites can easily be missed if the oil immersion lens is used at once. Wet films of blood, fresh unstained films of faeces and iodine-stained preparations of faeces should

be covered with a coverslip, and similarly examined in sequence with the low power and then the high power objective, otherwise important pathogenic parasites may not be discovered.

For dry films, say of blood, tissue smears, and puncture fluids, which have been stained and which are being examined without a coverslip, examination with the lower power and oil immersion objectives is carried out in the ordinary way, but the high power × 40 objective will only give satisfactory results if the film is cleared by spreading over it a thin layer of xylol or immersion oil. The low power is used first in order to detect the presence of such organisms as microfilariae, and the remainder of the examination is made with the higher powers.

Finding and Focusing

In using the low and high powers, the objective is first lowered to slightly less than the working distance* from the microscope slide, after having arranged the illumination to the best advantage. While looking down the microscope the tube is now gradually *raised* by rotating the coarse adjustment milled head till the object comes into view. This is then focused carefully, using the fine adjustment. The × 100 oil immersion lens requires great care, especially in trying to find individual objects in films. A small drop of immersion oil is placed on the centre of the coverslip, or directly on the film if there is no coverslip, and the × 100 objective is lowered carefully with the coarse adjustment till it enters the drop. In order to do this, if the left hand is used for rotating the milled head, the downward progress of the lens is watched from the right side with the eye held level with the front of the descending lens until its contact with the drop is seen to be effected; at the moment of contact a sudden little flash occurs as the oil spreads out on the lens. Then, looking down the tube of the microscope, the tube is moved very slowly up with both hands on the coarse adjustment heads; if the object does not come into view, the tube is now very cautiously lowered, with small downward and smaller upward movements alternately, till it is seen. It is necessary to ascertain, by early and repeated practice, what degree of closure of the diaphragm gives the best definitions for each power of objective with unstained and stained films, as the attainment of a proper degree of illumination is of the utmost importance. When a structure has been found with a low power, and it is desired to turn on a higher power, the object under examination must be arranged by movement of the mechanical stage so that it lies exactly in the centre of the field; unless it is so centred before the high power is turned on, it will

* The working distance is not the same as the focal length of the objective. It is much less; about 8 mm for the × 10 objective and about 1 mm for the × 40 objective.

not be found in the high power field. The objectives should be arranged on the nosepiece so that when they are rotated clockwise they swing into position in order of increasing magnification, *i.e.* × 10, × 40, and × 100.

In passing to the examination of specimens with the oil immersion objective, it is advisable to rack up the microscope tube a little before the × 100 objective is rotated into position. If this is not done the objective may touch the microscope slide as it is rotated (the working distance of an oil immersion objective may be as little as 0·2 mm) and the objective, the microscope slide, or both may be damaged. When it is in position, the objective may safely be lowered into the drop of oil. In general, it is inadvisable to use an oil immersion objective with wet preparations, especially films of faeces, as relative movements of the objective and the microscope slide are apt to move the coverslip, and the object sought for is thereby displaced. Wet preparations will dry out during lengthy examinations; this can be prevented by ringing the coverslip round its edges with melted paraffin wax applied with a heated wire rod.

Examination of Gross Objects

For such things as tapeworm segments or small worms which have been cleared in pure liquid carbolic acid, a small magnification only is required; and usually a good hand lens, × 6 or × 8, is all that is necessary. However, if the microscope is used for examining such large objects, the condenser should be racked down and swung out of action, and a low power objective and a low power ocular used with the concave surface of the mirror, which then serves as the condenser.

Care of the Microscope

The microscope should be kept in its box when not in use. If it is in fairly constant service it must be protected, during intervals of work, from dust and also from strong light, especially in the tropics. This can be done by wrapping around it a clean duster large enough to protect the condenser and mirror. After use, the oil should be wiped off the immersion lens which is cleaned with a drop of xylol, and dried with lens tissue, a silk cloth or soft handkerchief kept for the purpose. In cleaning oculars, objectives, or the surface of the substage condenser, it is advisable not to rub them in a circular manner but wipe across them; it is essential that the cloth should be clean and free from particles of grit. Alcohol must not be used for cleaning lenses as it dissolves the cement in which they are set.

Dirt on lenses, condensers and objectives. If, on looking down the microscope, dirt is seen, locate it as follows:

(*a*) Move the slide by means of the mechanical stage; if the dirt moves, it is on the object, or slide.

(*b*) If the dirt does not move, rotate the eyepiece while looking steadily down the microscope; if the dirt now moves, it is either on or in the eyepiece.

(*c*) If neither of these tests moves the dirt, then it is either on the condenser, or on, or in, the objective; wipe the condenser and objective clean; if this fails, unscrew the objective from the nosepiece, and wipe the upper surface of the lens gently with a soft handkerchief; breathing into the objective will, as a rule, only make the dust particles stick more firmly and should be avoided. The component parts of the objective lenses should not be unscrewed except as a last resort, and only in cases where sending it to a maker is impossible. In order to prevent dust passing into the tube, an ocular should always be kept in it.

Oiling. The friction surfaces of the moving parts of the microscope and mechanical stage should be oiled very sparingly with a little thin machine oil from time to time.

Measurement of Microscopic Objects

The unit of microscopic measurement is the one-millionth part of a metre, *i.e.* 0·001 mm, and it is called one *micron* and written 1μm. The measurement is most easily carried out by the use of a micrometer placed in the eyepiece. This ocular micrometer consists of a disc of glass with a scale engraved on it. This disc is placed in an eyepiece, resting on the internal stop or diaphragm and positioned so that the numbers on the disc can be read; if necessary, the stop is then moved up or down a little until the scale is in focus. Special micrometer eyepieces are procurable, fitted with a scale and an arrangement by which the scale can be focused accurately. When the disc is in proper position and focus, the divisions and numbers on it can be easily read on looking down the tube of the microscope. So that the ocular micrometer can be used to measure objects, it is calibrated by comparing it with a stage micrometer, which is a microscope slide that has divisions of a known value in microns engraved on it. The scale on the stage micrometer consists usually of 1 mm divided into a hundred parts, so that each division is equal to a hundredth part of a millimetre, *i.e.* 0·01 mm = 10μm (Fig. 2).

Calibration of the ocular micrometer with the aid of the stage micrometer is carried out as follows: the ocular micrometer having been placed in the selected eyepiece, the stage micrometer slide is put on the microscope stage and the scale on it is focused. There are now two scales in focus, that of the ocular and that of the stage micrometer (Fig. 2). These are superimposed one on the other, so that one of the lines of the ocular micrometer scale coincides exactly with a line on the stage micrometer scale; a second point is then selected, as far away from the first point as possible, where there is another pair of lines which exactly coincide. The numbers