Diagnostic Medical Parasitology

MARKELL
VOGE

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By

EDWARD K. MARKELL, Ph.D., M.D. *Assistant Professor of Infectious Diseases, Division of Parasitology and Tropic Diseases, Department of Infectious Diseases, School of Medicine, University of California, Los Angeles*

And

MARIETTA VOGE, M.A., Ph.D. *Assistant Professor of Infectious Diseases, Division of Parasitology and Tropic Diseases, Department of Infectious Diseases, School of Medicine, University of California, Los Angeles*

WITH 115 FIGURES, INCLUDING 5 IN COLOR

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CHAPTER 1

Introduction

The Second World War, in which millions of servicemen were exposed to the infectious diseases of tropical and subtropical regions around the globe, resulted in a large increase in the time devoted to a study of those diseases in the medical curriculum. Chiefly, this was reflected in additional emphasis on the so-called parasitic diseases, as it is this large and diverse group of infections which more than any others characterize the warm areas, and which at that time were of outstanding military importance. With the end of the war, many men and women trained during their military service in the laboratory diagnosis of parasitic diseases were employed in civilian hospitals. The result was not only an increased awareness on the part of the physician, but also more efficient laboratory diagnosis, of the parasitic infections.

With the passage of time and ever-increasing pressure of a crowded medical curriculum, the time allocated to a study of the protozoan, helminth and arthropod parasites has been severely curtailed in many institutions. Correspondingly, time has depleted the ranks of laboratory technologists having good training in the field of parasitology. It is thus necessary for the medical student to attempt to acquire, in a brief space of time, both a clinical knowledge of the parasitic diseases, and some proficiency in their laboratory diagnosis.

The primary purpose of this book is to serve as a guide both to laboratory and to clinical diagnosis of the protozoan and helminthic diseases of medical importance in this country, and to a lesser extent to the arthropods in relation to disease. We cannot limit ourselves to those organisms indigenous to this country, or seen here with some frequency. Increasing world travel and speed of communica-

tion are introducing ever greater numbers of exotic diseases into our midst, and the physician cannot afford to confine his interest to those which are seen frequently, any more than he can ignore the less common types of malignant or metabolic diseases. While malaria, acquired in this country, is now practically non-existent, people returning from malarious areas may bring the infection with them, and even suffer their first clinical attacks in this country. Indeed, as was seen recently in California, such introduced malaria may be the source of local epidemics.

While intended primarily for the medical student, it is hoped that this book will prove equally useful to the medical technologist and all others concerned with the laboratory identification of the animal parasites of man. The success of the cooperative diagnostic efforts of the physician and laboratory technologist depends upon a mutual appreciation of their several problems. In the chapters dealing with technical methods, the problems of the technologist are discussed; the physician will be able better to utilize his laboratory service if he understands them. The manner in which parasitic organisms are acquired, and how they produce disease in man, is perhaps of no direct importance to the technologist. Yet, a basic understanding of these matters should not only make the technologist's work more interesting, but also enable him to do it better and more efficiently.

A word of explanation should be given concerning the illustrations. They are largely original, and have been planned to emphasize points of diagnostic importance. The drawings which accompany the chapter on intestinal protozoa are all made at the same magnification, to facilitate a comparison of size ranges between different organisms, and within a single species. Structures not of importance from the standpoint of identification have been omitted from the majority of drawings, with the purpose of emphasizing those features to which especial attention should be paid. Nuclear structure is of great importance in the identification of many species of intestinal protozoa, but the variation which may be encountered is often a source of confusion. Drawings of nuclei alone, illustrative of the range of nuclear variation in the different species, have been included. These are not drawn to scale, but are all shown at the same size. All drawings of the protozoa have been made from iron hematoxylin–stained preparations.

Any discussion of treatment has been expressly omitted.

Therapy of the various protozoan and helminthic diseases is best presented to the student as a part of his work in medicine, or more formally in a course in therapeutics, rather than at the time when he is first introduced to these agents of disease. Treatment of certain parasitic diseases is changing almost as rapidly as that of the bacterial infections, and it is essential for the physician to keep abreast of the advances in this field. Review articles on this subject are seen occasionally in the medical journals, in such publications as the *Annual Review of Medicine,* and in the *Tropical Diseases Bulletin.*

A listing of some of the more important texts and monographs, written in English, is given at the end of this chapter. Many of these are much more extensive treatments of the subject than is attempted here. Foremost among those published in this country are Belding's *Textbook of Clinical Parasitology,* and *Clinical Parasitology* by Faust and Russell. Textbooks of tropical medicine, while covering a wider range of subjects, usually devote a large proportion of space to a consideration of protozoan and helminthic diseases. One of the most up-to-date of these is the *Manual of Tropical Medicine* by Mackie, Hunter and Worth, while from the standpoint of clinical treatment Adams and Maegraith's *Clinical Tropical Diseases* leaves little to be desired.

Some of the English language journals devoted to parasitology and tropical medicine are also listed. The *Tropical Diseases Bulletin* has already been mentioned. This monthly abstracting journal, published in England, is invaluable. It lists, under headings of the various etiological agents, the world-wide literature of tropical medicine, and publishes periodic summaries of work in certain fields as well as occasional comprehensive clinical reviews.

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Tropical Diseases Bulletin

CHAPTER 2

Parasites, Parasitism and Host Relations

If we except certain of the simplest plants, there are no living organisms which do not depend in one way or another upon other kinds of life. Most plants are able to build up their own food from simple inorganic constituents by means of photosynthesis. Nevertheless, even those plants which possess chlorophyll are affected by many other organisms, and may under normal conditions depend for their continued existence on certain of these. Thus, terrestrial plants in nature utilize inorganic compounds in the soil which are made available to them through the activities of bacteria. Animals, not possessed of chlorophyll, must depend ultimately upon the photosynthetic activities of plants. Some nourish themselves, entirely or in part, directly upon plant materials; in other instances there is a long chain of animal-eat-animal, but with a first link in the chain dependent upon plant materials.

In view of the tremendous numbers and diversity of living things, and the varied circumstances of their existence, it is not surprising that they should obtain their nourishment in many different ways. It is also entirely logical that these various methods should have basic similarities, so that frequently it is difficult to draw a firm line between one method of nutrition and another. Many terms have been devised to describe the relationships which exist between different kinds of plants and animals, at the fundamental food-seeking or food-supplying level. These terms are not always used by everyone to denote the same thing, with the result that they may lead to confusion rather than clarity. We need not

concern ourselves here with many terms which have been created to designate slight differences in relationship, and shall adopt somewhat rigid definitions of those which we do consider. However, it must be emphasized that any one organism may at different times exhibit different nutritional habits, or at the same time obtain its nutriment in more than one way. If a definition is helpful in the understanding of a biological process, it is worth while, but it should never be allowed to channel or limit one's ideas.

In a consideration of the major nutritional relationships between different species, we will limit ourselves to those involving different kinds of animals, with the understanding that much, but not all, of what is said may be extended to cover animal-plant interrelationships as well. Fundamentally, there are two different ways in which an animal may obtain food at the expense of other animals. It may attack another living animal, consuming part or all of its body for nourishment, and in the process frequently but not necessarily killing it. This process is known as predation; the attacker is the predator and the victim the prey. Or an animal may derive its nutrition from already-dead animals, either devouring those dead of natural causes or taking the leavings of a predator. Animals which subsist in this manner are known as scavengers. Some animals are pure predators, others pure scavengers, but many predators are not averse to an occasional bit of scavenging. Some animals always seek their food by their own efforts, or in association with others of their own species. This is the most conspicuous and perhaps the most common way in which animals go about obtaining food; it is this large group to which we commonly refer when we speak of scavengers and predators. However, other animals, still in essence predators or scavengers, have become so modified that they are unable to obtain food except in close association, either continuous or at intervals, with members of another species. This association of two species, perhaps primarily for food-getting on the part of one or both members of the group, is known as symbiosis.* Symbiosis means literally "living together" and may also involve protection or other advantages to one or both partners. Different forms of symbiosis may be distinguished, on the basis of whether or not the

* The definitions given here for symbiosis, commensalism and mutualism differ from those used by many authors. However, they conform to the recommendations of the Committee on Terminology of the American Society of Parasitologists (1937). It is to be hoped that these recommendations will be generally adopted.

association is detrimental to one of the two partners. Commensalism, from the Latin for "eating at the same table," denotes an association which is beneficial to one partner and at least not disadvantageous to the other. A specialized type of commensalism, known as mutualism, is seen where such associations are beneficial to both organisms. Parasitism, on the contrary, is a symbiotic relationship in which one animal, the host, is to some degree injured through the activities of the other animal, the parasite. Parasitism, like other forms of symbiosis, necessarily involves an intimate relationship between the two species, and it is this close and prolonged contact which differentiates parasitism from the predatory activities of many non-parasites.

Parasitism as a way of life may be the only possibility for a given organism, or it may be but one alternative. An organism which cannot survive in any other manner is called an obligate parasite. A facultative parasite is an organism which may exist in a free-living state, or as a commensal, and if opportunity presents itself may become parasitic. It is implicit in this term that the organism does not of necessity have to be a parasite at any stage of its existence. Some animals are obligatory parasites at one or more stages of their life cycles, but free-living at others. The term "temporary parasite" is sometimes applied to such animals. Parasites living within the host may be distinguished as endoparasites, while those which are found upon the surface of the body are called ectoparasites.

Small organisms, such as mosquitoes, which must periodically seek out other and larger forms on which to nourish themselves, have occasionally been called "intermittent parasites." This unhappy use of the term "parasite" comes from the assumption that a predator must be larger and stronger than its prey, whereas a parasite is small and weak. This generalization is certainly true of most predators and parasites, or at least of the most obvious ones. However, the essence of the parasitic relationship, which separates it from predation, is the protracted and intimate association between parasite and host. The association between the mosquito and its victim is neither prolonged nor intimate. Cameron (1956) refers to those blood-sucking arthropods which lead an independent existence except for occasional nutritional forays as micro-predators.

Many organisms customarily considered to be parasites are actually commensals. *Entamoeba coli* lives within the lumen of the intestine, subsists there upon the bacterial flora of the gut, and does its host no appreciable harm. This is a symbiotic relationship

in which no advantage or disadvantage accrues to the host, whereas the ameba is supplied with food and protected from harm. Other cases are less definite. There is considerable controversy over the question of whether *Entamoeba histolytica* is at all times parasitic or if it can at times have a purely commensal relationship with its host.

Adaptations to Parasitism

The parasitic relationship probably evolved very early in the history of living organisms. We know little about how such relationships arose, but may hypothesize that we can see in the facultative parasite one possible initial step along the road to obligate parasitism. The possibility of the adaptation of a parasitic mode of existence may depend upon what is known as "pre-adaptation," or evolutionary changes which make possible existence in an environment otherwise unsuitable. Such pre-adaptive changes might be in the nature of increased resistance to the enzymatic activities of the host. Further physiological adaptations to parasitism might involve the loss of enzymes or enzyme systems which are then supplied by the host. Such losses may be expected to make a parasitic or at least symbiotic relationship obligatory.

Certain groups of parasites exhibit profound morphological adaptations to their way of life. As might be expected, these modifications are more striking in those groups which are wholly parasitic than in those containing both free-living and parasitic species. Organs not necessary to a parasitic existence are frequently lost. The only class of Protozoa which contains nothing but parasitic forms is the Sporozoa. Members of this class have no locomotor organelles, although these structures are present in one form or another in all other classes of Protozoa, even in their parasitic representatives. Most of the free-living turbellarian flatworms are provided with a ciliated epidermis in the adult stage. Cilia are not found on the parasitic members of this group, or on the related, but strictly parasitic, trematodes and cestodes. A digestive tract, of moderate complexity in the turbellarians, is generally reduced in the trematodes and is absent in the cestodes. The reproductive system is very highly developed in the two latter groups; this seems a reflection of the difficulties inherent in transfer of these organisms to new hosts. Specialized attachment organs in the form of suckers and hooks have been developed in the parasitic flatworms. Body

size may be greatly affected by the parasitic state. Although we think of parasites as small organisms, many of them are much larger than their free-living relatives. The majority of free-living turbellarians are under half a centimeter in length, and while some land planarians may reach a half meter, none approaches the length of 10 meters or more seen in some tapeworms. Most free-living nematodes barely attain naked-eye visibility as adults, but *Ascaris* can reach 35 cm. and *Dracunculus* as much as a meter in length.

Specialized mechanisms for effecting entrance into the body or tissues are seen in some parasites. *Entamoeba histolytica* elaborates a proteolytic enzyme which aids its penetration of the intestinal mucosa. No such enzyme has been found in the commensal *E. coli*. The cercarial stage in the life cycle of the blood fluke is able to penetrate through the skin of man to produce infection. This it does with the aid of penetration glands which produce an enzyme capable of digesting the skin. The embryo of *Hymenolepis nana*, before developing into a cysticercoid larva, penetrates an intestinal villus with the help of the six hooklets which it bears.

Increased reproductive capacity has been already mentioned as characterizing two parasitic groups in contrast to their free-living relatives. Most metazoan parasites exhibit such an increase, which in some cases involves larval stages as well as adults. The chances of a particular ovum successfully infecting a new host are usually very small, and if more than one host species is involved, the chance of successful completion of the cycle becomes still smaller. If a parasite is successful in infecting an intermediate host, it is obviously advantageous if the larval stage which develops there can multiply to produce many additional organisms, capable of infecting the definitive or a second intermediate host. Such a modification is seen in the trematodes and many of the cestodes, where a single ovum develops in the intermediate host into a larva which in turn produces many larvae of a more advanced kind.

Effects of Parasites upon the Host

A parasite, by definition, is an organism which to some degree injures its host. However, we have already found that many organisms which are loosely termed parasites are in reality commensals. Some may be at times truly parasitic and at other times commensal in their relationship to the host. In many instances it cannot be said with certainty whether or not an organism injures the host. Even if we can be fairly sure that some injury is produced, we may not be

able to detect it. Thus a distinction has grown up between hookworm disease and hookworm infection, on the basis of the presence or absence of clinical symptoms. Overt symptoms of infection with this parasite may depend upon the number of worms present, upon the nutritional status of the host or both.

Injury to the host may be brought about in a wide variety of ways. Some of these mechanisms are common to all parasites, even if this term is used in its broad sense to include bacteria, viruses and fungi. The most widespread type of injury is that brought about by interference with the vital processes of the host, through the action of secretions, excretions or other products of the parasite. Such interference is probably largely or exclusively on the level of the host enzyme systems. Parasites producing such effects may be in the tissues or organs of the host, in the blood stream, within the gastrointestinal tract, or may even be ectoparasitic. Invasion and destruction of host tissue may be distinguished from injury which does not involve gross physical damage, although both types of injury reflect biochemical changes brought about in the host tissue by the parasites. When the giant intestinal fluke, *Fasciolopsis buski,* is present in large numbers, absorption from the intestinal tract of its secretions and excretions may lead to severe disturbances in the host. *Entamoeba histolytica* erodes the intestinal wall, destroying the tissues locally by means of a proteolytic enzyme. Malarial parasites invade and multiply in red blood cells, which are destroyed in the process. The helminth parasites, by virtue of their size, may damage the host in other ways impossible for the smaller parasites. In addition to the widespread effects of absorption of its products, *Fasciolopsis buski* may produce severe local damage to the intestinal wall by means of its powerful suckers. *Ascaris* may perforate the bowel wall, cause intestinal obstruction if present in large numbers, and invade the appendix, bile duct or other organs. Some parasites exert their effects by depriving the host of essential substances. Thus hookworms suck blood, and by so doing may deprive the host of more iron than is replaced by his diet, and so bring about an anemia. The broad fish tapeworm, *Diphyllobothrium latum,* selectively removes vitamin B_{12} from the alimentary tract. The lack of sufficient quantities of this vitamin also produces anemia.

Effects of the Host on the Parasite

The effects of the parasite on the host are more obvious than those which operate in the reverse direction, but the latter are

nonetheless important. The genetic constitution of the host may profoundly influence the host-parasite relationship. It is now well known that there are racial variations in resistance to certain strains of *Plasmodium vivax*. There is also considerable evidence which suggests that possession of the sickle cell trait, an inherited characteristic, is also associated with increased resistance to infection with the malarial parasite, *P. falciparum*.

The diet or nutritional status of the host may be of major importance in determining the outcome of a parasitic infection (Frye, 1955). A high protein diet has been found to be unfavorable for the development of many intestinal protozoa, while a diet low in protein was shown by Elsdon-Dew (1953) to favor the appearance of symptoms of amebiasis and the complications of this disease. It has been shown that a carbohydrate-rich diet favors the development of certain tapeworms, and the presence of carbohydrate in the diet is known to be essential for some of these worms. The general nutritional status of the host may be of considerable importance both in determining whether or not a particular infection will be accompanied by symptoms and in influencing their severity if present. Major nutritional disturbances may influence resistance through their effects upon the immune mechanisms of the host.

While the fundamental immune processes are generally considered to be the same in infection with the animal parasites as in bacterial, viral and mycotic infections, the details are much better known for bacteria and viruses than for the larger forms. Every species of animal is naturally resistant to infection with many organisms which parasitize different species. As we have seen in the case of certain strains of malaria, resistance may also be a racial phenomenon. In some cases it has been possible to adapt parasites to hosts which they normally infect poorly, or not at all. This does not necessarily involve changes in the host's natural resistance, but rather changes in the parasite. Acquired immunity can be demonstrated in many parasitic diseases. This is generally found to be at a lower level than that produced by bacteria and viruses. Absolute immunity to reinfection, such as is generally seen following infection with smallpox, measles, whooping cough and a number of other viral and bacterial diseases, occurs but rarely following protozoal infections and probably never with helminth infections of man. Primary infection with *Leishmania* seems to confer a degree of immunity to reinfection. While many protozoal and helminthic infections confer no long-lasting immunity to reinfection, they do