CHAPTER VI

ANIMAL PARASITES

Animal parasites are common in all countries, but are especially abundant in the tropics, where, in some places, almost every native is host for one or more species. Because of our growing intercourse with these regions the subject is assuming increasing importance in this country. Many parasites, hitherto comparatively unknown here, will probably become more common.

Some parasites produce no symptoms, even when present in large numbers. Others cause very serious symptoms. It is, however, impossible to make a sharp distinction between pathogenic and non-pathogenic varieties. Parasites which cause no apparent ill effects in one individual may, under certain conditions, produce marked disturbances in another. The disturbances are so varied, and frequently so indefinite, that diagnosis can rarely be made from the clinical symptoms. It must rest upon detection, by the naked eye or the microscope, of (*a*) the parasites themselves, (*b*) their ova or young progeny, or (*c*) some of their products.

Unlike bacteria, the great majority of animal parasites multiply by means of alternating and differently formed generations, which require widely different conditions for their development. The few exceptions are chiefly among the protozoa. Multiplication of parasites within the same host is thus prevented. In the case of the hook-worm, for example, there is no increase in the number of worms in the host's intestine, except through reinfection from the outside. The young are carried out of the intestine and must pass a certain period of development in warm, moist earth before they can again enter the human body and grow to maturity. In general, this alternation of periods of development takes place in one of three ways:

(1) The young remain within the original host, but travel to other organs, where they do not reach maturity, but lie quiescent until taken in by a new host. A good example is *Trichinella spiralis*.

(2) The young or the ova which subsequently hatch pass out of the host, and either (*a*) go through a simple process of growth and development before entering another host, as is the case with the hook-worm, or (*b*) pass through one or more free-living generations, the progeny of which infect new hosts, as is the case with *Strongyloides intestinalis*.

(3) The young or ova or certain specialized forms either directly (*e. g.*, malarial parasites) or indirectly (*e. g.*, tapeworms) reach a second host of different species, where a widely different process of development occurs. The host in which the adult or sexual existence is passed is called the *definitive* or final host; that in which the intermediate or larval stage occurs, the *intermediate* host. Man, for example, is the definitive host for *Tænia saginata*, and the intermediate host for the malarial parasites and *Tænia echinococcus*.

A few words concerning the classification and nomenclature of living organisms in general will be helpful at this place. Individuals which are alike *in all essential respects* are classed together as a *species*. Closely related species are grouped together to form a *genus;* genera which have certain characteristics in common make up a *family;* families are grouped into *orders;* orders into *classes;* and classes, finally, into the *branches* or *phyla*, which make up the *kingdom*. In some cases these groups are subdivided into intermediate groups—subphyla, subfamilies, etc., and occasionally, slight differences warrant subdivision of the species into *varieties*. The animal kingdom comprises nine branches: Protozoa, Porifera, Cœlenterata, Echinodermata, Vermidea, Arthropoda, Mollusca, Prochordata, and Chordata.

The scientific name of an animal or plant consists of two parts, both Latin or Latinized words, and is printed in italics. The first part is the name of the genus and begins with a capital letter; the second is the name of the species and begins with a lower case letter, even when it was originally a proper name. When there are varieties of a species, a third part, the designation of the variety, is appended. The author of the name is sometimes indicated in Roman type immediately after the name of the species. Examples: *Spirochæta vincenti*, often abbreviated to *Sp. vincenti* when the genus name has been used just previously; *Staphylococcus pyogenes albus; Necator americanus*, Stiles.

At the present time there is great confusion in the naming and classification of parasites. Some have been given a very large number of names by different observers, and in many cases different parasites have been described under the same name. The alternation of generations and the marked differences in some cases between male and female have contributed to the confusion, different forms of the same parasite being described as totally unrelated species.

The number of parasites which have been described as occurring in man and the animals is extremely large. Only those which are of medical interest are mentioned here. They belong to three phyla—Protozoa, Vermidea, and Arthropoda.

PHYLUM PROTOZOA

These are unicellular organisms, the simplest types of animal life. There is very little differentiation of structure. Each contains at least one, and some several nuclei. Some contain contractile vacuoles; some have cilia or flagella as special organs of locomotion. They reproduce by division, by budding, or by sporulation. Sometimes there is an alternation of generations, in one of which sexual processes appear, as is the case with the malarial parasites. The protozoa are very numerous, the subphylum Sarcodina alone including no less than 5000 species. Most of the protozoa are microscopic in size; a few are barely visible to the naked eye. One can gain a general idea of their appearance by examining water (together with a little of the sediment) from the bottom of any pond. Such water usually contains amebæ and a considerable variety of ciliated and flagellated forms.

The following is an outline of those protozoa which are of medical interest, together with the subphyla and classes to which they belong.

PHYLUM PROTOZOA

SUBPHYLUM I. **SARCODINA.**—Locomotion by means of pseudopodia.

CLASS **Rhizopoda.**—Pseudopodia form lobose or reticulose processes.

Genus.	*Species.*
Entamœba.	E. histolytica.
	E. tetragena.
	E. coli.
	E. buccalis.

SUBPHYLUM II. **MASTIGOPHORA (FLAGELLATA).**—Locomotion by means of flagella.

CLASS **Zoömastigophora.**—Forms in which animal characteristics predominate.

Genus.	*Species.*
Spirochæta.	Sp. obermeieri.
	Sp. vincenti.
	Sp. buccalis.
	Sp. dentium.
	Sp. refringens.
Treponema.	T. pallidum.
	T. pertenue.
Trypanosoma.	T. gambiense.
	T. cruzi.
	T. lewisi.
	T. evansi.
	T. brucei.
	T. equiperdum.
Leishmania.	L. donovani.
	L. tropica.
	L. infantum.
Cercomonas.	C. hominis.
Bodo.	B. urinarius.
Trichomonas.	T. vaginalis.
	T. intestinalis.
	T. pulmonalis.
Lamblia.	L. intestinalis.

SUBPHYLUM III. **SPOROZOA.**—All members parasitic. Propagation by means of spores. No special organs of locomotion.

CLASS **Telosporidia.**—Sporulation ends the life of the individual.

Genus.	*Species.*
Coccidium.	C. cuniculi.
Plasmodium.	P. vivax.
	P. malariæ.
	P. falciparum.
Babesia.	B. bigeminum.

SUBPHYLUM IV. **INFUSORIA.**—Locomotion by means of cilia.

CLASS **Ciliata.**—Cilia present throughout life.

Genus.	*Species.*
Balantidium.	B. coli.

SUBPHYLUM SARCODINA

Class Rhizopoda

These are protozoa the body substance of which forms changeable protoplasmic processes, or pseudopodia, for the taking in of food and for locomotion. They possess one or several nuclei.

1. Genus Entamœba.—(1) **Entamœba Histolytica.**—This organism is found, often in large numbers, in the stools of tropical dysentery and in the pus and walls of hepatic abscesses associated with dysentery, and is generally regarded as the cause of the disease. It is a colorless, granular cell, 20 to 40 μ in diameter (Fig. 110). It contains one or more distinct vacuoles, a round nucleus, which ordinarily is obscured by the granules, and frequently red blood-corpuscles and bacteria. When at rest its shape is spheric, but upon a warm slide it exhibits the characteristic ameboid motion, constantly changing its shape or moving slowly about. This motion is its most distinctive feature. If neutral red in 0.5 per cent. solution be run under the cover-glass, it will be taken up by the amebæ and other protozoa and render them conspicuous without killing them ("vital staining").

When the presence of amebæ is suspected, the stool should be passed into a warm vessel and kept warm until and during the examination. A warm stage can be improvised from a plate of copper with a hole cut in the center. This is placed upon the stage of the microscope, and one of the projecting ends is heated with

Fig. 110.—Amœba coli in intestinal mucus, with blood-corpuscles and bacteria (Lösch).

a small flame. Amebæ are most likely to be found in grayish or blood-streaked particles of mucus. Favorable material for examination can be obtained at one's convenience by inserting into the rectum a large catheter with roughly cut lateral openings. A sufficient amount of mucus or fecal matter will usually be brought away by it.

(2) **Other Entamebæ.**—*Entamœba coli*, a similar but somewhat smaller organism (10 to 20 μ), with less dis-

tinct pseudopodia and more distinct nucleus, has frequently been found in the stools of healthy persons. *E. tetragena* has recently been described. It apparently produces a chronic diarrhea and is not confined to the tropics. Another, *E. buccalis*, has been found in decaying teeth A number of similar organisms have been described as occurring in pus and in ascitic and other body fluids, but it is probable that in many cases, at least, the structures seen were ameboid body cells.

SUBPHYLUM MASTIGOPHORA (FLAGELLATA)

Class Zoömastigophora

The protozoa of this subphylum are provided with one or several whip-like appendages with lashing motion, termed flagella, which serve for locomotion and, in some cases, for feeding. They generally arise from the anterior part of the organism. Some members of the group also possess an undulating membrane—a delicate membranous fold which extends the length of the body, and somewhat suggests a fin. When in active motion this gives the impression of a row of cilia. The flagellata do not exhibit ameboid motion, and, in general, maintain an unchanging oval or spindle shape, and contain a single nucleus. The cytoplasm contains numerous granules and usually several vacuoles, one or more of which may be contractile. Encystment as a means of resisting unfavorable conditions is common.

1. Genus Spirochæta.—The spirochætæ appear to occupy a position midway between the bacteria and protozoa, but are more frequently described with the latter.

(1) **Spirochæta Recurrentis.**—This spirochæte was

described by Obermeier as the cause of relapsing fever. It appears in the circulating blood during the febrile attack, and, unlike the malarial parasite, lives in the plasma without attacking the red corpuscles. The organism is an actively motile spiral, 16 to 40 μ long, with three to twelve wide, fairly regular turns. It can be seen in fresh unstained blood with a high dry lens, being located by the commotion which it creates among

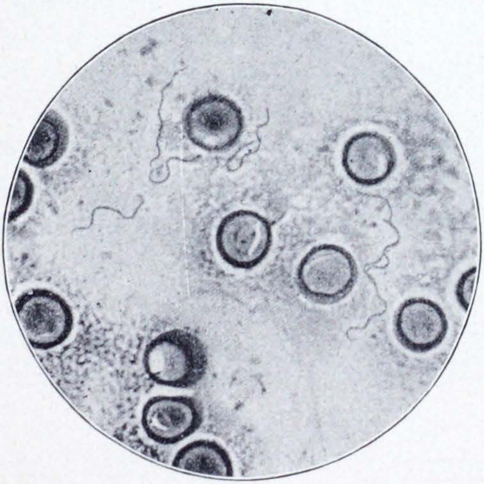


Fig. 111.—Spirochæte of relapsing fever ($\times$ 1000) (Karg and Schmorl).

the red cells. For diagnosis, thin films, stained with Wright's or some similar blood-stain, are used (Fig. 111).

Besides *Spirochæta recurrentis*, a number of distinct strains have been described in connection with different types of relapsing fever: *Sp. novyi* (Plate VII), *Sp. kochi*, *Sp. duttoni*, and *Sp. carteri*.

(2) **Spirochæta Vincenti.**—In stained smears from the ulcers of Vincent's angina (p. 380) are found what appear to be two organisms. One, the "fusiform bacil-

lus," is a slender rod, 6 to 12 μ long, pointed at both ends and sometimes curved. The other is a slender spiral organism, 30 to 40 μ long, with three to eleven comparatively shallow turns (Fig. 153). These were formerly thought to be bacteria, a spirillum and bacillus living in symbiosis. The present tendency is to regard them as stages or forms of the same organism, and to class them among the spirochætæ. The same organisms are quite constantly present in large numbers in ulcerative stomatitis and in noma. They are not infrequently found in small numbers in normal mouths.

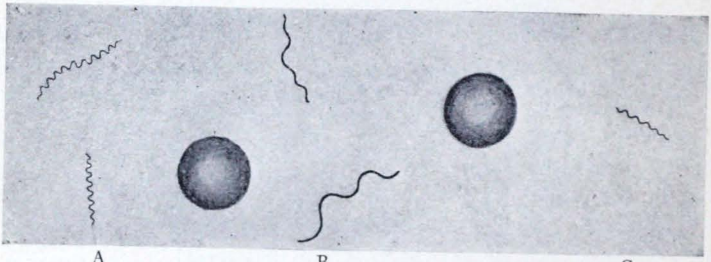


Fig. 112.—Spiral organisms: A, Treponema pallidum; B, Spirochæta refringens; C, Spirochæta dentium. Two red corpuscles are also shown (× 1200).

(3) **Other Spirochætæ.**—A number of harmless forms are of interest because of the possibility of confusing them with the more important pathogenic varieties. Of these, *Sp. buccalis* and *Sp. dentium* are inhabitants of the normal mouth. The former is similar in morphology to *Sp. vincenti*. *Sp. dentium* (Fig. 112) is smaller, more delicate, has deep curves, and may be easily mistaken for *Treponema pallidum*. It, also, stains reddish with Giemsa's stain. In suspected syphilitic sores of the mouth it is, therefore, important to make smears

from the tissue juices rather than from the surface (see p. 389). *Sp. refringens* is frequently present upon the surface of ulcers, especially about the genitals, and has doubtless many times been mistaken for *Treponema pallidum*. It can be avoided by properly securing the material for examination; but its morphology should be sufficient to prevent confusion. It is thicker than the organism of syphilis, stains more deeply, and has fewer and shallower curves (Fig. 112). Giemsa's stain gives it a bluish color.

2. Genus Treponema.—(1) **Treponema Pallidum.**—This is the organism of syphilis. Its description and methods of diagnosis will be found on p. 388.

(2) **Treponema pertenue,** morphologically very similar to *Treponema pallidum*, was found by Castellani in yaws, a skin disease of the tropics.

3. Genus Trypanosoma.—Trypanosomes have been found in the blood-plasma of a great variety of vertebrates. Many of them appear to produce no symptoms, but a few are of great pathologic importance. As seen in the blood, they are elongated, spindle-shaped bodies, the average length of different species varying from 10 to 70 μ. Along one side there runs a delicate undulating membrane, the free edge of which appears to be somewhat longer than the attached edge, thus throwing it into folds. Somewhere in the body, usually near the middle, is a comparatively pale-staining nucleus; and near the posterior end is a smaller, more deeply staining chromatin mass, the micronucleus or blepharoplast. A number of coarse, deeply staining granules, chromatophores, may be scattered through the cytoplasm. A flagellum arises in the blepharoplast, passes along the free edge of the undulating membrane, and is continued anteriorly as a free flagellum. These details of structure are well shown in Plate VII.

The life history of the trypanosomes is not well known. In most cases there is an alternation of hosts, various insects playing the part of definitive host.

Trypanosomes have been much studied of late, and many species have been described. Of these, only a few

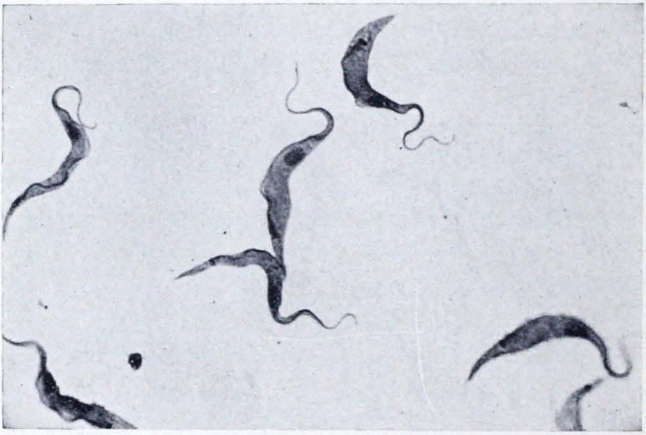


Fig. 113.—Trypanosoma lewisi in blood of rat. The red corpuscles were decolorized with acetic acid (× 1000) (photograph by the author from a slide presented by Prof. Novy).

have medical interest. At least two have been found in man.

Trypanosoma gambiense is the parasite of African "sleeping sickness." Its detection in the blood is described on p. 247.

Trypanosoma cruzi is a small form which has been found in the blood of man in Brazil.

Trypanosoma lewisi, a very common and apparently harmless parasite of gray rats, especially sewer rats, is interesting because it closely resembles the pathogenic forms, and is easily obtained for study. Its posterior end is more pointed than that of *T. gambiense.*

Trypanosoma evansi, T. brucei, and *T. equiperdum* produce respectively surra, nagana, and dourine, which are common and important diseases of horses, mules, and cattle in the Philippines, East India, and Africa.

4. Genus Leishmania.—The several species which compose this genus are apparently closely related to the trypanosomes, but their exact classification is undetermined. They have been grown outside the body and their transformation into flagellated trypanosome-like structures has been demonstrated. Calkins places them in the genus *Herpetomonas.*

(1) **Leishmania donovani** is the cause of kala-azar, an important and common disease of India. The "Leishman-Donovan bodies" are round or oval structures, 2 to 3 μ in diameter, with two distinct chromatin masses, one large and pale, the other small and deeply staining. The parasites are especially abundant in the spleen, splenic puncture being resorted to for diagnosis. They are readily found in smears stained by any of the Romanowsky methods. They lie chiefly within endothelial cells and leukocytes They are also present within leukocytes in the peripheral blood, but are difficult to find in blood-smears.

(2) **Leishmania tropica** resembles the preceding. It is found, lying intracellularly, in the granulation tissue of Delhi boil or Oriental sore.

(3) **Leishmania infantum** has been found in an obscure form of infantile splenomegaly in Algiers.

5. Genus Cercomonas.—(1) **Cercomonas hominis** has been found in the feces in a variety of diarrheal conditions, and in from 10 to 25 per cent. of healthy persons in tropical regions. It is probably harmless. The body is 10 to 12 μ long, is pointed posteriorly, and has a flagellum at the anterior end (Fig. 114). The nucleus is difficult to make out.

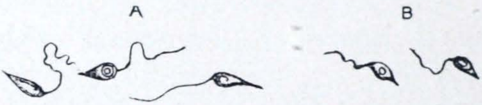


Fig. 114.—Cercomonas hominis (about × 500): A, Larger variety; B, smaller variety (Davaine).

6. Genus Bodo.—(1) **Bodo urinarius** is sometimes seen in the urine, darting about in various directions. It is probably an accidental contamination, or at most a harmless invader. It has a lancet-shaped body, about 10 μ long, and is somewhat twisted upon itself, with two flagella at the end.

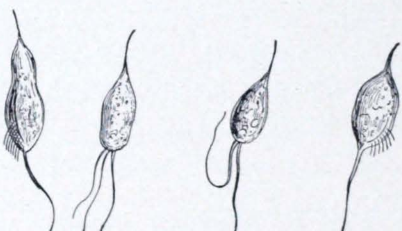


Fig. 115.—Trichomonas vaginalis (about × 1000) (after Kölliker and Scanzoni).

7. Genus Trichomonas.—(1) **Trichomonas Vaginalis.**—The acid discharge of catarrhal vaginitis sometimes contains this parasite in abundance. It is oval or pear-shaped, one to three times the diameter of a red blood-corpuscle in length, and has a cluster of flagella at one end (Fig. 115). As seen in fresh material it is not unlike a pus-corpuscle in size and general appearance, but is actively motile. When in motion the flagella are not easily seen. No pathogenic significance is ascribed to it in the vagina, but a few cases have been reported in which it was apparently the cause of a urethritis in the male. This and similar organisms, such as cercomonas

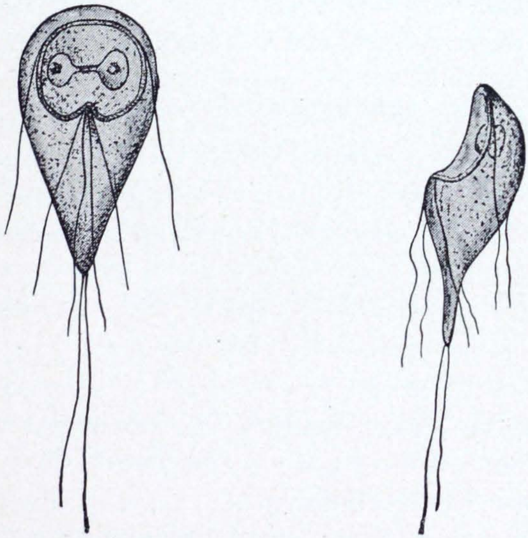


Fig. 116.—Lamblia intestinalis from the intestines of a mouse (about × 2000) (Grassi and Schweiakoff).

and bodo, might be mistaken for spermatozoa by the totally inexperienced worker.

(2) **Other Trichomonads.**—Various forms have been described, regarded by some as identical with *T. vaginalis*, by others as distinct species. Among these are *T. intestinalis*, sometimes found in the feces in diarrheal conditions, and *T. pulmonalis*, which has been encountered in the sputum of persons suffering from pulmonary gangrene and putrid bronchitis.

8. Genus Lamblia.—(1) **Lamblia intestinalis** is a very common parasite in the tropics, but is generally considered of little pathogenic importance. It is pear shaped, measures about 10 to 15 μ, and has a depression on one side of the blunt end, by which it attaches itself to the tops of the epithelial cells of the intestinal wall. Three pairs of flagella are arranged about the depression and one pair at the pointed end (Fig. 116).

SUBPHYLUM SPOROZOA

Class Telosporidia

All the members of this class are parasitic, but only a few have been observed in man, and only one genus, *Plasmodium*, is of much importance in human pathology. Propagation is by means of spores, and sporulation ends the life of the individual. In some species there is an alternation of generations, in one of which sexual processes appear. In such cases the male individual may be provided with flagella. Otherwise, there are no special organs of locomotion.

1. Genus Coccidium.—(1) **Coccidium cuniculi.**—This is a very common parasite of the rabbit and has been much studied; but extremely few authentic cases of infection in man have been reported. The parasite, which when fully developed is ovoid in shape and measures about 30 to 50 μ in length and has a shell-like integument, develops within the epithelial cells of the bile-passages. Upon reaching adult size it divides into a number of spores or merozoites which enter other epithelial cells and repeat the cycle. A sexual cycle outside the body, which suggests that of the malarial parasite, but does not require an insect host, also occurs.

Infection takes place from ingestion of the resulting sporozoites.

2. Genus Plasmodium.—This genus includes the malarial parasites which have already been described (p. 248).

3. Genus Babesia.—The proper position of this genus is uncertain. It is placed among the flagellates by some. The chief member is *Babesia bigeminum*, the cause of Texas fever in cattle. It is a minute, pear-shaped organism, lying in pairs within the red blood-corpuscles. An organism, *B.* (or *Piroplasma*) *hominis*, described as occurring in the red cells in "tick-fever" of Montana, is also placed in this genus, but its pathogenicity and even its existence are questionable.

SUBPHYLUM INFUSORIA

Class Ciliata

The conspicuous feature of this class is the presence of cilia. These are hair-like appendages which have a regular to-and-fro motion, instead of the irregular lashing motion of flagella. They are also shorter and more numerous than flagella. Most infusoria are of fixed shape and contain two nuclei. Contractile and food-vacuoles are also present. Encystment is common. Only one species is of medical interest. Certain ciliated structures, which have been described as infusoria, notably in sputum and nasal mucus, were probably ciliated body cells.

1. Genus Balantidium.—(1) **Balantidium Coli.**—This parasite, formerly called *Paramœcium coli*, is an occasional inhabitant of the colon of man, and sometimes produces diarrhea. It is an oval organism, about 0.1 mm. long, is covered with cilia, and contains a bean-shaped macronucleus, a globular micronucleus, two contractile vacuoles, and variously sized granules (Fig. 117).

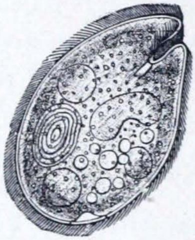


Fig. 117.—Balantidium coli (about × 300) (after Eichhorst).

Its ordinary habitat is the rectum of the domestic pig, where it apparently causes no disturbance. It probably reaches man in the encysted condition.

PHYLUM VERMIDEA

Of the worms, many species are parasitic in man and the higher animals. In some cases man is the regular host; in others, the usual habitat is some one of the animals, and the occurrence of the worm in man is more or less accidental. Such are called *incidental parasites*. Only those worms that are found in man with sufficient frequency to be of medical interest are mentioned here.

PHYLUM VERMIDEA

SUBPHYLUM I. **PLATYHELMINTHES.**—Flat-worms.

CLASS **Trematoda.**—Flukes. Unsegmented, leaf shaped.

Genus.	*Species.*
Fasciola.	F. hepatica.
Dicrocœlium.	D. lanceatum.
Opisthorchis.	Op. felineus.
	Op. sinensis.
Paragonimus.	P. westermani.
Schistosomum.	S. hæmatobium.
	S. japonicum.

CLASS **Cestoda.**—Tapeworms. Segmented, ribbon shaped.

Genus.	*Species.*
Tænia.	T. saginata.
	T. solium.
	T. echinococcus.
Hymenolepis.	H. nana.
Dipylidium.	D. caninum.
Dibothriocephalus.	D. latus.

SUBPHYLUM II. **NEMATHELMINTHES.**—Round-worms.

CLASS **Nematoda.**—Unsegmented, cylindric or fusiform.

Genus.	*Species.*
Anguillula.	A. aceti.
Ascaris.	A. lumbricoides.
Oxyuris.	O. vermicularis.
Filaria.	F. bancrofti.
	F. philippinensis.
	F. perstans.
	F. diurna.
	F. medinensis.
Uncinaria.	U. duodenalis.
Necator.	N. americanus.
Strongyloides.	S. intestinalis.
Trichinella.	T. spiralis.
Trichocephalus.	T. trichiuris.

SUBPHYLUM PLATYHELMINTHES

Class Trematoda

The trematode worms, commonly known as "flukes," are flat, unsegmented, generally tongue- or leaf-shaped worms. They are comparatively small, most species averaging between 5 and 15 mm. in length. They possess an incomplete digestive tract, without anus, and are provided with one or more sucking disks by means of which they can attach themselves to the host. Some are also provided with hooklets. Nearly all species are hermaphroditic, and the eggs of nearly all are operculated (provided with a lid), the only important exception being *Schistosomum hematobium*, the egg of which has a characteristic spine. Development takes place by alternation of generations, the intermediate generation occurring in some water animal: mollusks, amphibians, fishes, etc.

1. Genus Fasciola.—(1) **Fasciola Hepatica.**—The "liver fluke" inhabits the bile-ducts of numerous herbivorous animals, especially sheep, where it is an important cause of disease. It brings about obstruction of the bile-passages, with enlargement and degeneration of the liver

Fig. 118.—Fasciola hepatica, about two-thirds natural size (Mosler and Peiper).

—"liver rot." A species of snail serves as intermediate host. The worm is leaf shaped, the average size being about 2.8 by 1.2 cm. The anterior end projects like a beak (head-cone 3 to 4 mm. long) (Fig. 118). Ova appear in the feces. They are yellowish brown, oval, operculated, and measure about 0.13 by 0.07 mm.

2. Genus Dicrocœlium.—(1) **Dicrocœlium lanceatum** is often associated with the liver fluke in the bile-passages of animals, but is neither so common nor so widely distributed geographically. It has rarely been observed in man. It is smaller (length about 1 cm.) and more elongated. The long diameter of the eggs is about 0.04 mm.

3. Genus Opisthorchis.—(1) **Opisthorchis felineus** inhabits the gall-bladder and bile-ducts of the domestic cat and a few other animals. Infection in man has been repeatedly observed in Europe, and especially in Siberia. The body is flat, yellowish-red in color, and almost transparent. It measures 8 to 11 mm. by 1.5 to 2 mm. The eggs are oval, with a well-defined operculum at the narrower end, and contain a ciliated embryo when deposited. They measure about 30 by 11 μ.

(2) **Opisthorchis sinensis,** like the preceding fluke, inhabits the gall-bladder and bile-ducts of domestic cats and dogs. It is, however, much more frequent in man, being a common and important parasite in certain parts of Japan and China. The number present may be very great; over 4000 were counted in one case. The parasite resembles *Op. felineus* in shape and color. It is 10 to 14 mm. long and 2.5 to 4 mm. broad. The eggs have a sharply defined lid and measure 27 to 30 by 15 to 17 μ. When they appear in the feces they contain a ciliated embryo. The intermediate host is unknown.

4. Genus Paragonimus.—(1) **Paragonimus westermani,** called the "lung fluke," is also a common parasite of man in Japan, China, and Korea. It is likewise found in dogs, cats, and pigs in these countries, and, according to Ward and Stiles, in North America also. It inhabits the lung, causing the formation of small cavities. Moderate hemoptysis is the principal symptom. Ova are readily found in the sputum; the worms themselves are seldom seen, except *postmortem*. The worms are faint reddish-brown in color, egg shaped with the ventral surface flattened, and measure 8 to 10 mm. by 4 to 6 mm. The ova, which are found in the sputum, are thin shelled, brownish yellow, and average about 0.093 by 0.057 mm. Little is known of the development outside the body.

5. Genus Schistosomum.—(1) **Schistosomum Hæmatobium.**—This trematode, frequently called *Bilharzia hæmatobia*, is an extremely common cause of disease (bilharziasis or Egyptian hematuria) in northern Africa, particularly in Egypt.

Unlike the other flukes, the sexes are separate. The male is 12 to 14 mm. long and 1 mm. broad. The body is flattened and the lateral edges curl ventrally, forming a longitudinal groove, in which the female lies (Fig. 119).

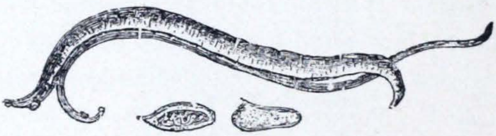


Fig. 119.—Schistosomum hæmatobium, male and female (about × 4) with eggs (about × 70) (von Jaksch).

The latter is cylindric in shape, about 20 mm. long and 0.25 mm. in diameter. The eggs are an elongated oval, about 0.15 mm. long, yellowish in color, and slightly transparent. They possess no lid, such as characterize the eggs of most of the trematodes, but are provided with a thorn-like spine which is placed at one end or laterally near the end.

In man the worm lives in the veins, particularly the portal vein and the veins of the bladder and rectum, leading to obstruction and inflammation. The eggs penetrate into the tissues and are present in abundance in the mucosa of the bladder and rectum. They also appear in the urine and feces. The mode of infection is unknown.

(2) **Schistosomum japonicum** resembles the preceding morphologically, but both the male and female are smaller. The ova present no spines and somewhat resemble those of *Uncinaria duodenalis.* It was discovered in Japan in 1904 and is apparently common in that country. It probably inhabits the arteries.

Class Cestoda

The cestodes, or tapeworms, are very common parasites of both man and the animals. In the adult stage they consist of a linear series of flat, rectangular segments (proglottides), at one end of which is a smaller segment, the scolex or head, especially adapted by means of sucking discs and hooklets for attachment to the host. The series represents a colony, of which the scolex is ancestor. The proglottides are sexually complete individuals (in most cases hermaphroditic), which are derived from the scolex by budding. With the exception of the immature segments near the scolex, each contains a uterus filled with ova.

The large tapeworms, *Tænia saginata*, *T. solium*, and *Dibothriocephalus latus*, are distinguished from one another mainly by the structure of the scolex and the uterus. The scolex should be studied with a low-power objective or a hand lens. The uterus is best seen by pressing the segment out between two plates of glass.

All the tapeworms pass a larval stage in the tissues of an intermediate host, which is rarely of the same species as that which harbors the adult worm. From the ova which have developed in the proglottides of the adult worm, and which pass out with the feces of the host, there develop embryos, or *oncospheres*, each provided with three pairs of horny hooklets. When the oncosphere is taken into the intestines of a suitable animal, it penetrates to the muscles or viscera and there forms a cyst in which develop usually one, but sometimes many, scolices, which are identical with the head of the adult worm. When the flesh containing this cystic stage is eaten without sufficient cooking to destroy the scolices, the latter attach themselves to the intestinal wall and produce adult tapeworms by budding.

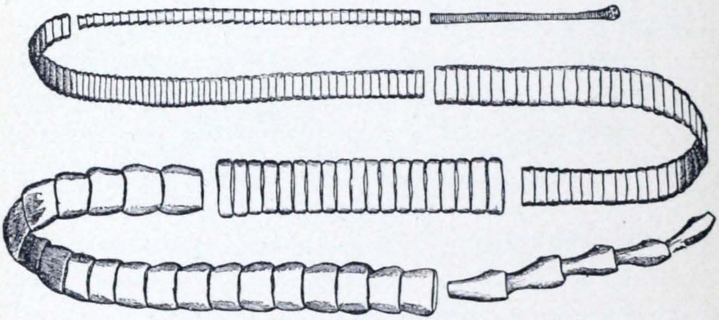


Fig. 120.—Tænia saginata (Eichhorst).

Ordinarily, only the adult stage occurs in man. In the case of *Tænia echinococcus* only the larval stage is found. *T. saginata* and *T. solium* may infect man in either stage, although the cystic stage is very rare.

Since the head, or scolex, is the ancestor from which the worm is formed in the intestine, it is important, after giving a vermifuge, to make certain that the head has been passed with the worm. Should it remain, a new worm will develop.

The principal tapeworms found in man belong to the genera Tænia, Hymenolepis, and Dibothriocephalus.

1. Genus Tænia.—(1) **Tænia Saginata or T. Mediocanellata** (Fig. 120).—This, the beef tapeworm, is the common tapeworm of the United States. Its length sometimes exceeds twenty-five feet. The middle segments measure about 6 by 15 mm. The scolex is about the size of a pin-head, and is surrounded by four sucking discs, but has no hooklets (Fig. 122). The uterus extends along the midline of the segment and gives off about twenty branches upon each side (Fig. 129).

Fig. 121.—Eggs of Tænia saginata, magnifications 100, 250, and 500 diameters (photographs by the author).

The larval stage is passed in the muscles of various animals, especially cattle.

The scolex is ingested with the meat, its capsule is dissolved by the digestive juices, and it attaches itself to the intestinal wall by means of its suckers. It then develops into the mature worm.

The ova are present in the stools of infected persons, often in great numbers They are spheric or ovoid, yellow in color, and have a thick, radially striated shell (Fig. 121). Their greatest diameter is 30 to 40 μ (about four or five times the diameter of a red blood-corpuscle).

Vegetable cells, which are generally present in the feces, are often mistaken for them.

(2) **Tænia solium,** the pork tapeworm, is very rare in this country. It is usually much shorter than *Tænia saginata.* The scolex is surrounded by four sucking discs, and has a projection, or rostellum, with a double row of horny hooklets (Fig. 123). The uterus has only seven to ten branches (Fig. 129).

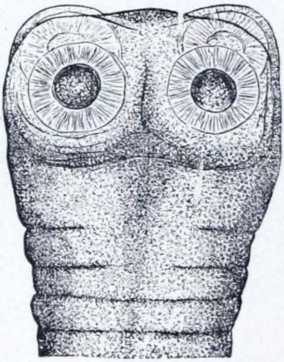


Fig. 122.—Head of Tænia saginata (Mosler and Peiper).

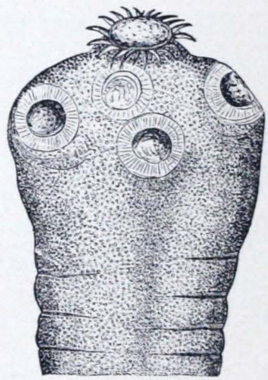


Fig. 123.—Head of Tænia solium (Mosler and Peiper).

The cysticercus stage occurs ordinarily in the muscles of the pig, but is occasionally seen in man, most frequently affecting the brain and eye (*Cysticercus cellulosæ*).

The ova closely resemble those of *Tænia saginata*, but are a little smaller (Fig. 130).

(3) **Tænia Echinococcus.**—The mature form of this tapeworm inhabits the intestines of the dog and wolf. The larvæ develop in cattle and sheep ordinarily, but are sometimes found in man, where they give rise to echinococcus or "hydatid" disease. The condition is unusual

in America, but is not infrequent in Central Europe and is common in Iceland and Australia.

The adult parasite is 2.5 to 5 mm. long and consists of only four segments (Fig. 124). It contains many ova. When the ova reach the digestive tract of man the embryos are set free and find their way to the liver, lung, or other organ, where they develop into cysts, thus losing their identity. The cysts may attain the size of a child's head. Other cysts, called "daughter-cysts," are formed within these. The cyst-wall is made up of two layers, from the inner of which develop larvæ which are identical with the head, or scolex, of the mature parasite. These are ovoid structures 0.2 to 0.3 mm. long. Each has four lateral suckers and a rostellum surmounted by a double circular row of horny hooklets. The rostellum with its hooklets is frequently invaginated into the body.

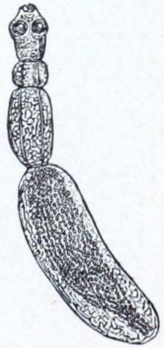


Fig. 124.—Tænia echinococcus; enlarged (Mosler and Peiper).

Diagnosis of echinococcus disease depends upon detection of scolices, free hooklets which have fallen off from degenerated scolices, or particles of cyst-wall, which is characteristically laminated and usually has curled edges. The lamination is best seen at the torn edge of the membrane. These can be found in fluid withdrawn from the cysts or, less frequently, in the sputum or the urine, when the disease involves the lung or kidney (Figs. 59 and 125). The cysts are sometimes "barren," growing to a considerable size without producing scolices.

The cyst fluid is clear, between 1.009 and 1.015 in specific gravity, and contains a notable amount of sodium chlorid, but no albumin.

2. Genus Hymenolepis.—(1) **Hymenolepis nana,** the dwarf tapeworm (Fig. 126), is 1 to 1.5 cm. in length and 0.5 to 0.7 mm. in breadth at the widest part. The head is globular and has a rostellum with a crown of 24

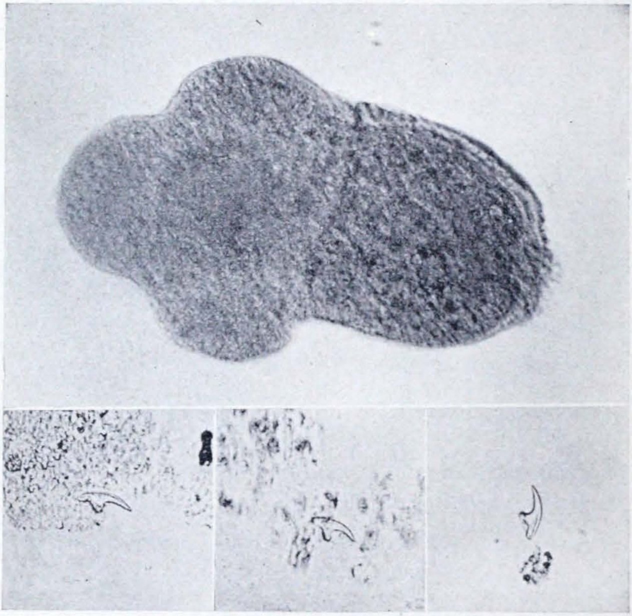


Fig. 125.—Scolex and hooklets of Tænia echinococcus in fluid from hepatic cyst (× 300) (photographs by the author).

to 30 hooklets. There are about 150 segments The eggs are round or oval, 30 to 40 μ in diameter, and resemble those of *Tænia saginata*. The worm is common in Europe and America. It is most frequent in children and is generally present in large numbers, producing considerable digestive and nervous disturbances. The mode of infection is unknown.

3. Genus Dipylidium.—(1) **Dipylidium caninum,** sometimes called *Tænia elliptica*, is a very common tapeworm of dogs and cats. It is about 20 cm. long and 2 to 3 mm. broad. The intermediate host is the flea or louse. Infection of human beings is not common, and is mostly confined to children, who are probably infected from the dog licking their mouths or from getting lice or fleas into their mouths.

Fig. 126.—Hymenolepis nana, about natural size (Mosler and Peiper).

4. Genus Dibothriocephalus.—(1) **Dibothriocephalus latus,** the fish tapeworm, sometimes reaches fifty feet in length, although it is generally not more than half so long. When several worms are present, they are much smaller. It is common in some countries of Europe, especially Ireland, and in Japan, but is very rare in this country.

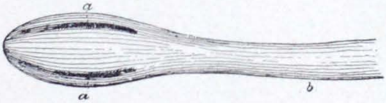


Fig. 127.—Head of Dibothriocephalus latus (about $\times 9$): *a*, *a*, Head grooves; *b*, neck (Blanchard).

The head is about 1 mm. broad and is not unlike the bowl of a spoon in shape. It is unprovided with either suckers or hooklets, but has two longitudinal grooves which serve the same purpose (Fig. 127). The length of the segments is generally less than their breadth, mature segments measuring about 3 by 10 or 12 mm. The uterus, which is situated in the center of the segment, is roset shaped (Fig. 129) and brown or black in color.

The larval stage is found in fish, especially the pike.

Fig. 128.—Ova of Dibothriocephalus latus (× 250 and 500). The lids were forced open by pressure upon the cover-glass (photographs by the author).

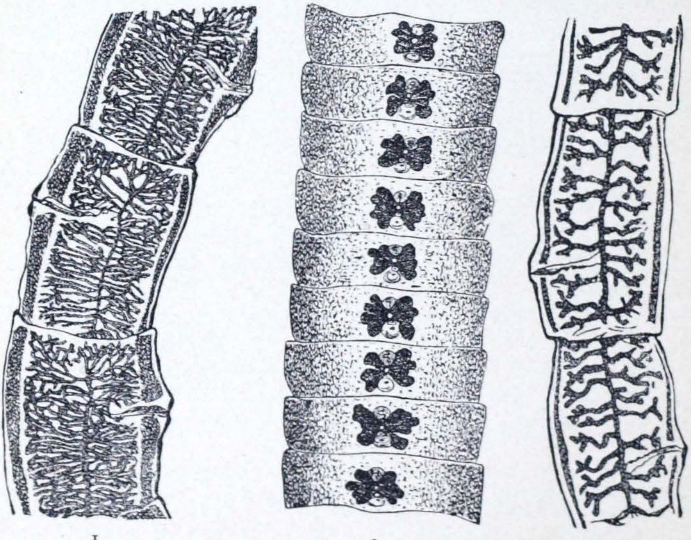


Fig. 129.—Segments of—(1) Tænia saginata; (2) Dibothriocephalus latus; (3) Tænia solium, showing arrangement of uterus.

The ova are characteristic. They measure about 45 by 70 μ, are brown in color, and are filled with small spherules. The shell is thin and has a small hinged lid at one end. As the eggs appear in the feces the lid is not easily seen, but it may be demonstrated by sufficient pressure upon the cover-glass to force it open (Fig. 128). The only other operculated eggs met with in man are those of the fluke-worms.

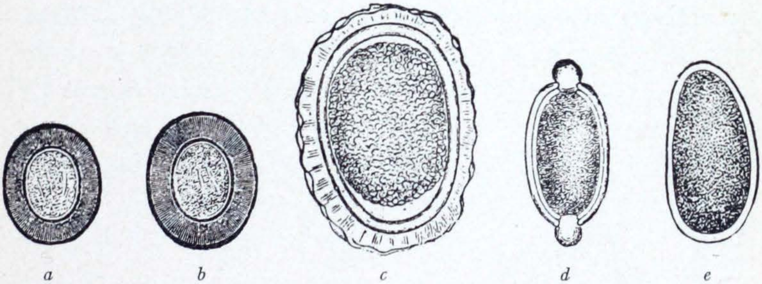


Fig. 130.—Comparative size of eggs of intestinal parasites (about ×400): *a*, Tænia solium; *b*, Tænia saginata; *c*, Ascaris lumbricoides; *d*, Trichocephalus trichiurus; *e*, Oxyuris vermicularis (after Strümpell).

Dibothriocephalus latus is interesting clinically because it often causes a very severe grade of anemia, which may be indistinguishable from pernicious anemia.

SUBPHYLUM NEMATHELMINTHES

Class Nematoda

The nematodes, or round-worms, are cylindric or fusiform worms, varying in length, according to species, from 1 mm. to 40 or 80 cm. As a rule, the sexes are separate. The male is smaller and more slender than the female. In a few cases the female is viviparous; in most cases she deposits ova which are characteristic, so that the finding of a single egg may establish the diagnosis. Except in a few instances the young are different from the adult, and must pass a certain larval stage of development before again reaching a host.

An intermediate host is, however, necessary with only a few species.

1. Genus Anguillula.—(1) **Anguillula Aceti.**—This worm, commonly called the "vinegar eel," is usually present in vinegar. A drop of the vinegar, particularly of the sediment, will frequently show great numbers, all in active motion: males, about 1 or 1.5 mm. long; females, somewhat larger and frequently containing several coiled embryos; and young, of all sizes up to the adult (Fig. 60).

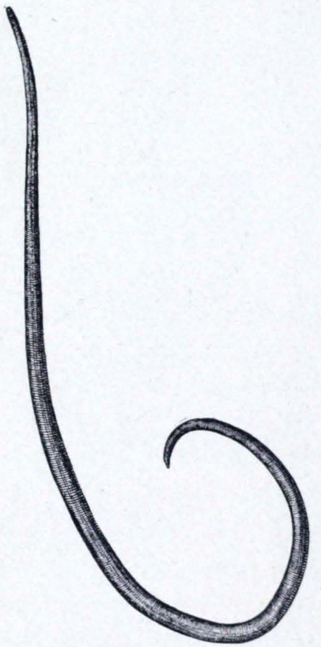


Fig. 131.—Ascaris lumbricoides (female) (Mosler and Peiper).

The vinegar eel is never parasitic, but is occasionally met with as a contamination in the urine (see p. 171), and has there been mistaken for the larva of filaria or strongyloides.

2. Genus Ascaris.—(1) **Ascaris Lumbricoides.**—The female is 20 to 40 cm. long and about 6 mm. thick (Fig. 131); the male, a little more than half as large. Their color is reddish or brown. They are the common "round-worms" so frequently found in children. Their habitat is the small intestine. Large numbers are sometimes present.

The diagnosis is made by detection of the worms or ova in the feces. The latter are generally numerous. They are elliptic, measuring about 50 by 70 μ, and have an unsegmented protoplasm (Fig. 132). The shell is thick and is surrounded by an uneven gelatinous envelop which is often stained with bile.

The eggs do not hatch in the intestine of the original host. They pass out in the feces and, after a variable period, usually about five weeks, come to contain an embryo which remains within the shell until ingested by a new host. The embryo is very resistant and may

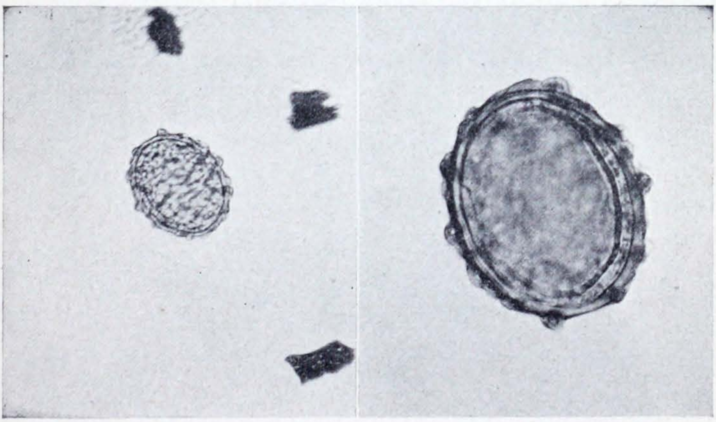


Fig. 132.—Ova of Ascaris lumbricoides (×250 and 500) (photographs by the author).

remain alive within the shell for years. Upon reaching the intestine of the new host it hatches out and develops into the adult worm.

3. Genus Oxyuris.—(1) **Oxyuris Vermicularis.**—This is the "thread-worm" or "pin-worm" which inhabits the colon and rectum, especially of young children. Its presence should be suspected in all unexplained cases of pruritus ani. The female is about 1 cm. long; the male, about 0.6 cm. (Fig. 133).

The worms are not infrequently found in the feces; the ova, rarely. The latter are best found by scraping the skin at the margin of the anus, where they are deposited by the female, who wanders out from the rectum for this purpose, this producing the troublesome itching. They are asymmetrically oval with one flattened side, are about 50 μ in length, and often contain a partially developed embryo. The diagnosis is best made by giving a purgative and searching the stool for the adult worms.

Infection takes place through swallowing the ova. Auto-infection is likely to occur in children; the ova cling to the fingers after scratching and are thus carried to the mouth.

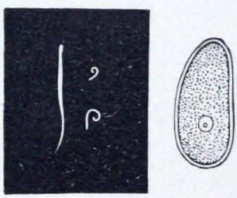
a  b

Fig. 133.—Oxyuris vermicularis and egg: *a*, Male and female, natural size; *b*, egg (about × 250) (after Heller).

4. **Genus Filaria.**—(1) **Filaria Bancrofti.**—The adults are thread-like worms, the male about 4 cm., the female about 8 cm., long. They live in pairs in the lymphatic channels and glands, especially those of the pelvis and groin, and often occur in such numbers as to obstruct the flow of lymph. This is the most common cause of elephantiasis. Infection is very common in tropical countries, especially in Samoa, the West Indies, Central America, and the Isthmus of Panama. It is said that in Samoa 50 per cent. of the natives are infected.

The female is viviparous, and produces vast numbers of embryos, which appear in the circulating blood. The name *Filaria sanguinis hominis*, which is commonly applied to them, is incorrect, since they do not constitute a species. These embryos are about as wide as a red corpuscle and 0.2 to 0.4 mm. long (Fig. 99), and are very actively motile. They are found in the peripheral blood only at night, appearing about 8 P. M., and reaching their maximum number—which is

Fig. 134.—Embryo of Filaria bancrofti in chylous hydrocele fluid; length, 300 μ; width, 8 μ. A number of red blood-corpuscles also appear (studied through courtesy of Dr. S. D. Van Meter).

sometimes enormous—about midnight. If the patient change his time of sleeping, they will appear during the day. Infection is carried by a variety of mosquito, which acts as intermediate host. Diagnosis rests upon detection of embryos in the blood, as described on p. 256.

The embryos are sometimes found in urine and chylous fluids from the serous cavities. Their motion is then usually less active than when in blood. That shown in Fig. 134 was alive sixty hours after removal of the fluid. Embryos were present in the blood of the same patient.

A number of other filariæ whose larvæ appear in the blood are known, some of them only in the larval stage. Among these are *Filaria philippinensis* and *F. perstans*, which exhibit no periodicity, and *F. diurna* and *F. loa*, whose embryos appear in the blood during the day. The adult of the last named is especially frequent in the orbit and beneath the conjunctiva.

(2) **Filaria medinensis,** the "guinea-worm," is a very interesting and important worm of Africa and southern Asia. It is thought to be the "fiery serpent" which molested the Children of Israel in the Wilderness.

The larva probably enters the body through the skin or gastro-intestinal tract. It wanders about in the subcutaneous tissues until maturity, producing slight, if any, symptoms. The male has only recently been discovered. It is only 4 cm. long. It dies soon after the female is impregnated. The adult female is a very slender, yellowish worm, about 50 to 80 cm. long, its appearance somewhat suggesting a catgut suture. When gestation is complete the greater part of the female's body consists of a uterus filled with embryos. The female then travels to the feet or ankles of the host and there produces a red nodule and, finally, an ulcer, from the center of which her head protrudes. Through this great numbers of embryos are discharged whenever it comes in contact with water. Little damage is done unless the worm is pulled out, when the embryos are set free in the tissues and cause serious disturbances.

When discharged the embryos seek out a small crustacean, cyclops, which serves as intermediate host.

5. Uncinaria Duodenalis and Necator Americanus.—These, the Old and the New World hook-worm respectively, are among the more harmful of the animal parasites. They inhabit the small intestine, often in great numbers, and commonly produce a severe and often fatal anemia. The presence of a few, however, may cause slight, if any, disturbance.

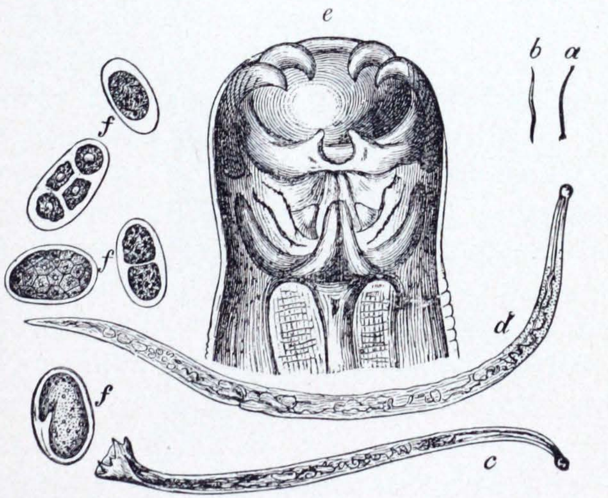


Fig. 135.—Uncinaria duodenalis: *a*, Male (natural size); *b*, female (natural size); *c*, male (enlarged); *d*, female (enlarged); *e*, head; *f*, *f*, *f*, eggs (after v. Jaksch).

Uncinaria duodenalis is common in southern Europe and in Egypt. The body is cylindric, reddish in color, and the head is bent sharply. The oral cavity has six hook-like teeth. The female is 12 to 18 mm. long and the tail is pointed. The male is 8 to 10 mm. long and the posterior end is expanded into an umbrella-like pouch, the caudal bursa. The eggs are oval and have a thin, smooth, transparent shell. As they appear in the feces the protoplasm is divided into 2, 4, 8, or more rounded segments (Fig. 135). They measure 32 to 40 μ by 55 to 65 μ.

Necator americanus is very common in subtropical America, including the southern part of the United States and the West Indies. In Porto Rico 90 per cent. of the rural population is infected. Isolated cases, probably imported, have been seen in most of the Northern

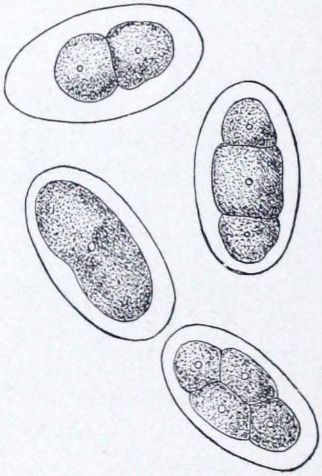


Fig. 136.—Four eggs of the New World hook-worm (Necator americanus), in the one-, two-, and four-cell stages. The egg showing three cells is a lateral view of a four-cell stage (about × 350) (after Stiles).

States. The American hook-worm is smaller than the Old World variety, the male being 7 to 9 mm. long, the female 9 to 11 mm. The four ventral hook-like teeth are replaced by chitinous plates. There are also differences in the caudal bursa of the male, and in the situation of the vulva in the female. The ova (Fig. 136) resemble those of *Uncinaria duodenalis*, but are larger, 36 to 40 μ by 67 to 75 μ.

The life-history of the two worms is probably the same.

The ova pass out with the feces, and, under favorable conditions of warmth and moisture, develop an embryo which hatches within a few days. The resulting larvæ pass through a stage of development in warm moist earth, growing to a length of 0.5 to 0.6 mm., and moulting twice. They are then ready to infect a new host. In some cases they probably reach the host's intestine by way of the mouth, with food or water; but the usual route is probably that established by Loos. When moist earth containing the larvæ comes in contact with the skin, they penetrate into the subcutaneous tissues. This is favored by retention of mud between the toes of those who go barefooted. When the larvæ are abundant a dermatitis is induced ("ground itch"). From the subcutaneous tissue they pass by way of lymph- and bloodstreams to the lungs. Here they make their way into the smaller bronchi, are carried by the bronchial mucus to the pharynx, and are swallowed. They thus ultimately reach the small intestine, where they develop into mature worms.

The diagnosis of hook-worm infection, which is assuming increasing importance in this country, must rest upon detection of ova in the feces. The worms themselves seldom appear except after thymol and a cathartic. A small portion of the feces, diluted with water if necessary, is placed upon a slide, covered, and searched with a 16 mm. objective. A higher power may rarely be necessary to positively identify an egg, but should not be used as a finder. The eggs are nearly always typic, showing a thin but very distinct shell, a clear zone, and a segmented protoplasm, and after having once been seen are not easily mistaken. In severe infections eggs may be found in every microscopic field; in most cases, even though comparatively mild, they can be found on the first slide examined. It is seldom necessary to search more than half a dozen slides. When they are scarce, some method of sedimenting the feces may be tried, but this is rarely necessary.

6. Genus Strongyloides.—(1) **Strongyloides Intestinalis.**—Infection with this worm is by no means so rare in this country as the few clinical reports would indicate. It is very common in subtropical countries, notably in Italy and in southern China. It seems probable that the parasite is the cause of "Cochin China diarrhea," although some authorities regard it as harmless.

The adult worm, which reproduces by parthenogenesis, is about 2 mm. long. It inhabits the upper portion of the small intestine, but neither it nor the ova appear in the stool unless an active diarrhea exists. Ordinarily the eggs hatch in the intestines, and when infection is severe embryos can be found in the feces in large numbers. These are the "rhabditiform embryos," which measure about 0.40 by 0.02 mm. They are actively motile, and are best found by making a small depression in the fecal mass, filling it with water, and keeping in a warm place (preferably an incubator) for twelve to twenty-four hours. The embryos will collect in the water, and can be easily found by transferring a drop to a slide and examining with a 16 mm. objective. The inexperienced worker should make sure that the worms *move*, or he may be misled by the vegetable spines which are generally present in the feces. Certain of these spines (notably those from the skin of a peach) closely resemble small worms.

Outside the body the rhabditiform embryos develop into a free-living, sexually differentiated generation. The young of this generation are the more slender "filariform embryos" (Fig. 137). Infection can occur either through these embryos of the free-living generation or by direct transformation of rhabditiform into filariform embryos, and these into the parthenogenic parasitic adult.

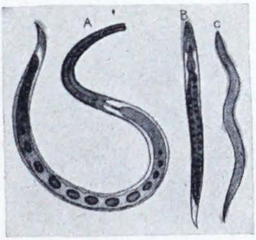


Fig. 137.—Strongyloides intestinalis: A, Mature female; B, rhabditiform larva; C, filariform larva (after Braun).

7. Genus Trichinella.—(1) **Trichinella Spiralis.**—This is a very small worm, not exceeding 3 mm. in length when fully developed. Infection in man occurs from ingestion of insufficiently cooked pork, which contains encysted embryos. Ordinary "curing" of pork does not kill them. These reach maturity in the small intestine. Soon after copulation the males die, and the females penetrate into the mucous membrane. They live in this situation about six weeks, giving birth to great numbers of young, averaging as high as 1500 from a single female. The larvæ migrate to the striated muscles, chiefly near the tendinous insertions, where they grow to a length of about 0.8 mm., and finally become encysted. In this condition they may remain alive and capable of developing for as long as twenty-five years.

Trichiniasis is generally accompanied by a marked eosinophilia. The diagnosis is made by teasing out upon a slide a bit of muscle, obtained preferably from the outer head of the gastrocnemius, the insertion of the deltoid,

Fig. 138.—Trichinella spiralis (larvæ) from head of right gastrocnemius muscle; seventh week of disease (two-thirds objective; eye-piece 4) (Boston).

or the lower portion of the biceps. The coiled embryos can easily be seen with a 16 mm. objective (Fig. 138). The embryos can be found in the blood (p. 257) before they have reached their final resting-place in the muscles.

Fig. 139.—Trichocephalus trichiurus: *a*, Female; *b*, male (natural size) (Heller).

8. Genus Trichocephalus.—(1) **Trichocephalus Trichiurus.**—This, the "whip-worm," is 4 or 5 cm. long. Its anterior portion is slender and thread-like, while the posterior portion is thicker (Fig. 139). It is widely distributed geographically, and is one of the most common of intestinal parasites in this country. It lives in the large intestine, especially the cecum, with its slender extremity embedded in the mucous membrane. Whip-worms do not, as a rule, produce any symptoms, although gastro-intestinal disturbances, nervous symptoms, and anemia have been ascribed to them. They, as well as many other intestinal parasites, are probably

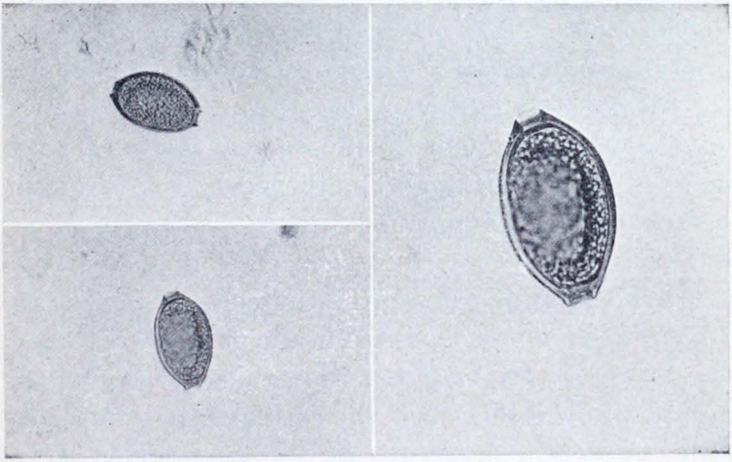


Fig. 140.—Ova of Trichocephalus trichiurus (× 250 and 500) (photographs by the author).

an important factor in the etiology of appendicitis, typhoid fever, and other intestinal infections. The damage which they do to the mucous membrane favors bacterial invasion.

The number present is usually small. The worms themselves are rarely found in the feces. The ova, which are not often abundant, are easily recognized. They are brown, ovoid in shape, about 50 μ long, and have a button-like projection at each end (Fig. 140).

PHYLUM ARTHROPODA

The arthropoda which are parasitic to man belong to the classes Arachnoidea and Insecta. They are nearly all external parasites, and the reader is referred to the standard works upon diseases of the skin for descriptions. The several species of the louse (*Pediculus capitis*, *P. vestimenti*, *P. pubis*), the itch mite (*Sarcoptes scabiei*), and the small organism (*Demodex folliculorum*) which lives in the sebaceous glands, especially about the face, are the most common members of this group.

A number of flies may deposit their ova in wounds or in such of the body cavities as they can reach, and the resulting maggots may cause intense irritation. Ova may be swallowed with the food and the maggots appear in the feces. Probably most important is the "screw worm," the larva of *Chrysomyia macellaria*, infection with which is not rare in some parts of the United States. The ova are most commonly deposited in the nasal passages, and the larvæ, which may be present in great numbers, burrow through the soft parts, cartilage, and even bone, always with serious and often with fatal results.

CHAPTER VII

MISCELLANEOUS EXAMINATIONS

PUS

PUS contains much granular débris and numerous more or less degenerated cells, the great majority being polymorphonuclear leukocytes—so-called "pus-corpuscles." Eosinophilic leukocytes are common in gonorrheal pus and in asthmatic sputum. Examination of pus is directed chiefly to detection of bacteria.

When very few bacteria are present, culture methods, which are outlined in Chapter VIII, must be resorted to. When considerable numbers are present, they can be detected and often identified in cover-glass smears. Several smears should be made, dried, and fixed, as described on p. 407.

One of these should be stained with a bacterial stain. Löffler's methylene-blue and Pappenheim's pyronin-methyl-green are especially satisfactory for this purpose. These stains are applied for one-half minute to two minutes or longer, without heating; the preparation is rinsed in water, dried, mounted, and examined with an oil-immersion lens. Another smear should be stained by Gram's method. These will give information concerning all bacteria which may be present, and frequently no other procedure will be necessary for their identification.

The most common pus-producing organisms are *staphylococci* and *streptococci*. They are both cocci, or spheres, their average diameter being about 1 μ. Staphylococci are commonly grouped in clusters, often compared to bunches of grapes (Fig. 141). There are several varieties which can be distinguished only in cultures. Streptococci are arranged side by side, forming chains of variable length (Fig. 142). Sometimes there are only three or four individuals in a chain; sometimes a chain

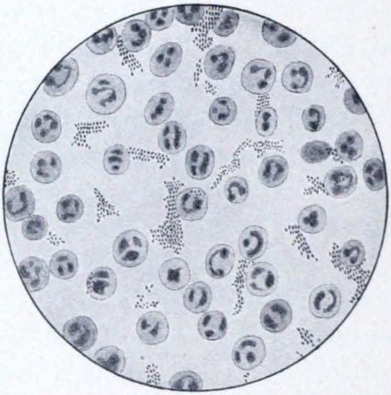


Fig. 141.—Staphylococcus pyogenes albus from an abscess of the parotid gland (Jakob).

is so long as to extend across several microscopic fields. Streptococci are more virulent than staphylococci, and are less commonly met. Both are Gram-positive. Their cultural characteristics are given on p. 415.

Should bacteria resembling *pneumococci* be found, Buerger's or Smith's method for capsules (p. 55) should be tried. When these are not available, capsules can usually be shown by the method of Hiss. The dried and fixed smear is covered with a stain composed of 5 c.c. saturated alcoholic solution gentian-violet and 95 c.c.

distilled water, and heated until steam rises. The preparation is then washed with 20 per cent. solution of copper sulphate, dried, and mounted in Canada balsam.

Pneumococci may give rise to inflammation in many locations (see p. 54). When they form short chains, demonstration of the capsule is necessary to distinguish them from streptococci.

If tuberculosis be suspected, the smears should be stained by one of the methods for the *tubercle bacillus*

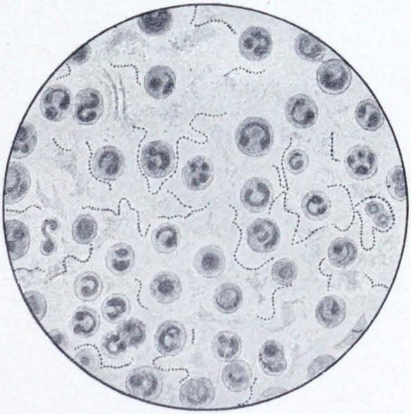


Fig. 142.—Streptococcus pyogenes from a case of empyema (Jakob).

(pp. 49 and 51), or guinea-pigs may be inoculated. The bacilli are generally difficult to find in pus, and bacteria-free pus would suggest tuberculosis.

Gonococci, when typic, can usually be identified with sufficient certainty for clinical purposes in the smear stained with Löffler's methylene-blue or, much better, Pappenheim's pyronin-methyl-green. They are coffee-bean-shaped cocci which lie in pairs with their flat surfaces together (Fig. 144). They lie for the most part within pus-cells, an occasional cell being filled with them, while the surrounding cells contain few or none. A few are found outside of the cells. It is not usual to find gonococci when many other bacteria are present, even

Fig. 143.—Diplococcus pneumoniæ from ulcer of cornea (obj. one-twelfth oil immersion) (study through courtesy of Dr. C. A. Oliver) (Boston).

though the pus is primarily of gonorrheal origin. Whenever the identity of the organism is at all questionable, Gram's method should be tried. In rare instances it

Fig. 144.—Gonococci in urethral pus (McFarland).

may be necessary to resort to cultures. The gonococcus is distinguished by its failure to grow upon ordinary media (see p. 416).

Gonococci are generally easily found in pus from untreated acute and subacute gonorrheal inflammations—conjunctivitis, urethritis, etc.—but are found with difficulty in pus from chronic inflammations and abscesses, and in urinary sediments.

PERITONEAL, PLEURAL, AND PERICARDIAL FLUIDS

The serous cavities contain very little fluid normally, but considerable quantities are frequently present as a result of pathologic conditions. The pathologic fluids are classed as transudates and exudates.

Transudates are non-inflammatory in origin. They contain only a few cells, and less than 2.5 per cent. of albumin, and do not coagulate spontaneously. The specific gravity is below 1.018. Micro-organisms are seldom present.

Exudates are of inflammatory origin. They are richer in cells and albumin, and tend to coagulate upon standing. The specific gravity is above 1.018. Bacteria are generally present, and often numerous. The amount of albumin is estimated by Esbach's method, after diluting the fluid. Bacteria are recognized by cultures, animal inoculation, or stained smears.

Exudates are usually classed as serous, serofibrinous, seropurulent, purulent, putrid, and hemorrhagic, which terms require no explanation. In addition, chylous and chyloid exudates are occasionally met, particularly in the peritoneal cavity. In the chylous form the milkiness is due mainly to the presence of minute fat-droplets, and is the result of rupture of a lymph-vessel usually from obstruction of the thoracic duct. Chyloid exudates are milky chiefly from proteins in suspension, or fine débris from broken-down cells. These exudates are most frequently seen in carcinoma and tuberculosis of the peritoneum.

Cytodiagnosis.—This is diagnosis from a differential count of the cells in a transudate or exudate, particularly one of pleural or peritoneal origin.

The fresh fluid, obtained by aspiration, is centrifugalized for at least five minutes; the supernatant liquid is

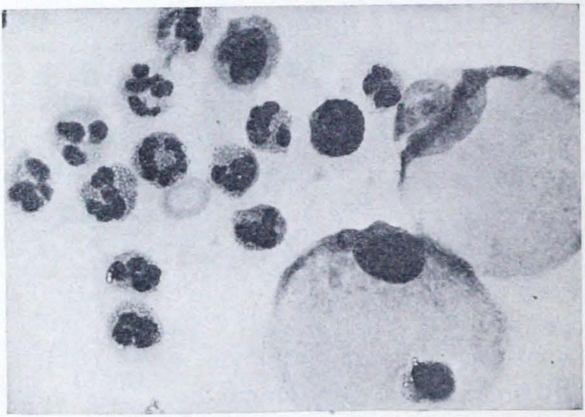


Fig. 145.—Cytodiagnosis. Polymorphonuclear leukocytes and swollen endothelial cells from acute infectious non-tuberculous pleuritis (Percy Musgrave; photo by L. S. Brown).

poured off; and cover-glass smears are made and dried in the air. The smears are then stained with Wright's blood-stain, to which one-third its volume of pure methyl-alcohol has been added. Cover the smear with this fluid for one-half minute, then dilute with 8 or 10 drops of water, and let stand about two minutes. Wash gently in water, and dry by holding the cover-glass between the fingers over a flame. Mount in balsam and examine with an oil-immersion objective.

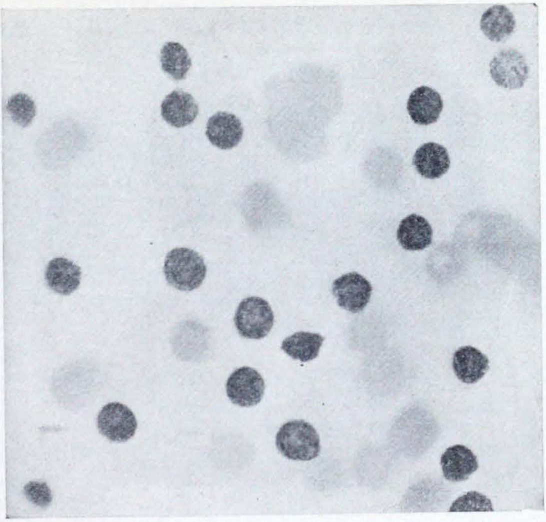


Fig. 146.—Cytodiagnosis. Lymphoid cells from pleural fluid; case of tuberculous pleuritis (Percy Musgrave; photo by L. S. Brown).

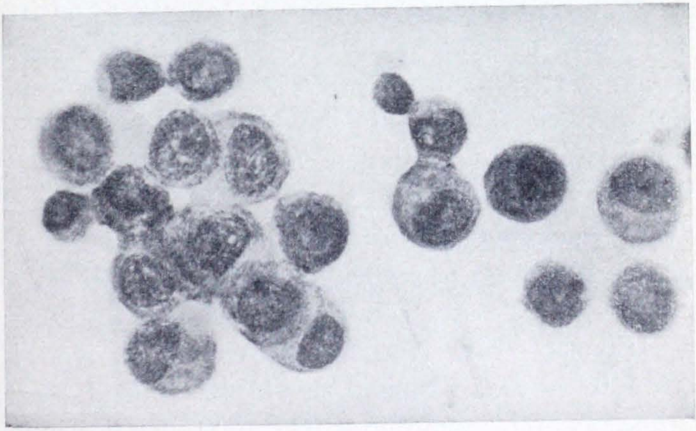


Fig. 147.—Cytodiagnosis. Endothelial cells from transudate or mechanical effusion (Percy Musgrave; photo by L. S. Brown).

Predominance of polymorphonuclear leukocytes (pus-corpuscles) points to an acute infectious process (Fig. 145).

Predominance of lymphocytes (Fig. 146) generally signifies tuberculosis. Tuberculous pleurisy due to direct extension from the lung may give excess of polymorphonuclears owing to mixed infection.

Predominance of endothelial cells, few cells of any kind being present, indicates a transudate (Fig. 147). Endothelial cells generally predominate in carcinoma, but are accompanied by considerable numbers of lymphocytes and red blood-corpuscles.

CEREBROSPINAL FLUID

Examination of the fluid obtained by lumbar puncture is of value in diagnosis of certain forms of meningitis.

Tubercle bacilli can be found in the majority of cases of tuberculous meningitis. The sediment, obtained by thorough centrifugalization or by coagulation and treatment with antiformin, is spread upon slides and stained by one of the methods already given. A considerable number of smears should be examined. In doubtful cases inoculation of guinea-pigs must be resorted to.

The *Diplococcus intracellularis meningitidis* is recognized as the cause of epidemic cerebrospinal fever, and can be detected in the cerebrospinal fluid of most cases, especially those which run an acute course. Cover-glass smears from the sediment should be stained by the method for the gonococcus (p. 369). The meningococcus is an intracellular diplococcus which often cannot be distinguished from the gonococcus in stained smears (Fig. 148). It, also, decolorizes by Gram's method. The presence of such a diplococcus in meningeal exudates is, however, sufficient for its identification.

Various organisms have been found in other forms of meningitis—the pneumococcus most frequently. In

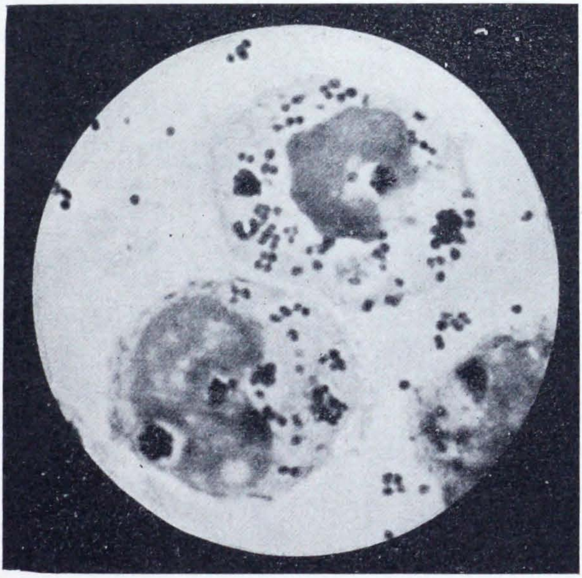


Fig. 148.—Diplococcus intracellularis meningitidis in leukocytes (× 2000) (Wright and Brown).

some cases no micro-organisms can be detected even by culture methods.

ANIMAL INOCULATION

Inoculation of animals is one of the most reliable means of verifying the presence of certain micro-organisms in fluids and other pathologic material, and is helpful in determining the species of bacteria which have been isolated in pure culture.

Clinically, it is applied almost exclusively to demonstration of the tubercle bacillus when other means have failed or are uncertain. The guinea-pig is the most suitable animal for this purpose. When the suspected material is fluid and contains pus, it should be well centrifugalized, and one or two cubic centimeters of the sediment injected by means of a large hypodermic needle into the peritoneal cavity or underneath the loose skin of the groin. Fluids from which no sediment can be obtained must be injected directly into the peritoneal cavity, since at least 10 c.c. are required, which is too great an

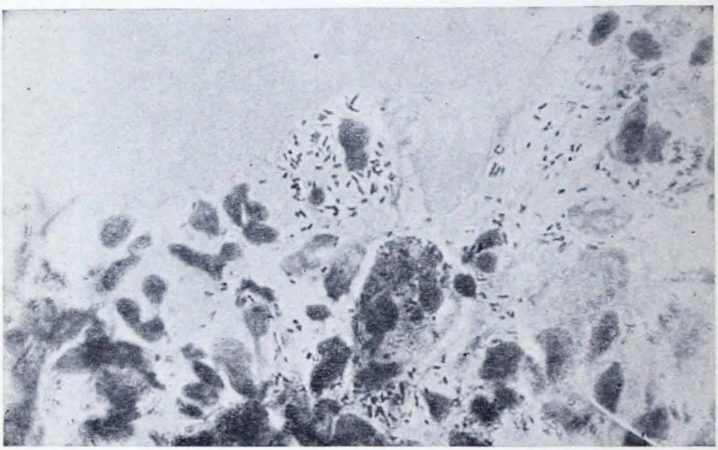


Fig. 149.—Influenza bacilli in spinal fluid. Case of meningitis (× 1000) (photograph by the author).

amount to inject hypodermically. Solid material should be placed in a pocket made by snipping the skin of the groin with scissors, and freeing it from the underlying tissues for a short distance around the opening. When the intraperitoneal method is selected, several animals must be inoculated, since some are likely to die from peritonitis caused by other organisms before the tubercle bacillus has had time to produce its characteristic lesions.

The animals should be killed at the end of six or eight weeks, if they do not die before that time, and a careful postmortem examination should be made for the characteristic pearl-gray or yellow tubercles scattered over the peritoneum and through the abdominal organs, particularly the spleen, and for caseous inguinal and retroperitoneal lymph-glands. The tubercles and portions of the caseous glands should be crushed between two slides, dried, and stained for tubercle bacilli. The bacilli are difficult to find in the caseous material.

THE MOUTH

Micro-organisms are always present in large numbers. Among these is *Leptothrix buccalis* (Fig. 150), which is

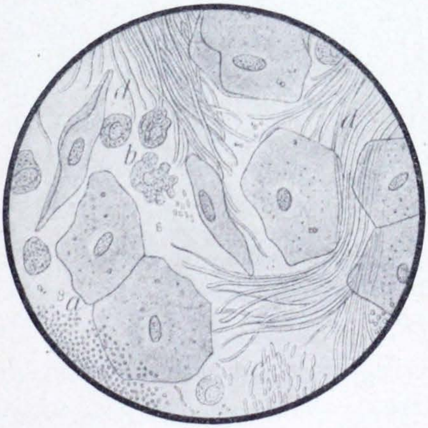


Fig. 150.—Gingival deposit (unstained): *a*, Squamous epithelial cells; *b*, leukocytes; *c*, bacteria; *d*, Leptothrix buccalis (Jakob).

especially abundant in the crypts of the tonsils and the tartar of the teeth. The whitish patches of *Pharyngomycosis leptothrica* are largely composed of these fungi. They are slender, segmented threads, which generally, but not always, stain violet with Lugol's solution, and are readily seen with a 4 mm. objective. At times they are observed in the sputum and stomach fluid. In the former they might be mistaken for elastic fibers; in the latter, for Boas-Oppler bacilli. In either case, the reaction with iodin will distinguish them.

Thrush is a disease of the mouth seen most often in children, and characterized by the presence of white patches upon the mucous membrane. It is caused by the thrush fungus, *Oïdium albicans*. When a bit from one

Fig. 151.—Thrush fungus (Oïdium albicans) (Jakob).

of the patches is pressed out between a slide and cover and examined with a 4 mm. objective, the fungus is seen to consist of a network of branching segmented hyphæ with numerous spores, both within the hyphæ and in the meshes between them (Fig. 151). The meshes also contain leukocytes, epithelial cells, and granular débris.

Acute pseudomembranous inflammations, which occur chiefly upon the tonsils and nasopharynx, are generally caused by the diphtheria bacillus, but may result from streptococcic infection. In many cases diphtheria bacilli can be demonstrated in smears made from the membrane and stained with Löffler's methylene-blue or 2 per cent. aqueous solution of methyl-green. They are straight or curved rods, which vary markedly in size and outline, and stain very irregularly. A characteristic form is a palely tinted rod with several deeply stained granules (metachromatic bodies), or with one such

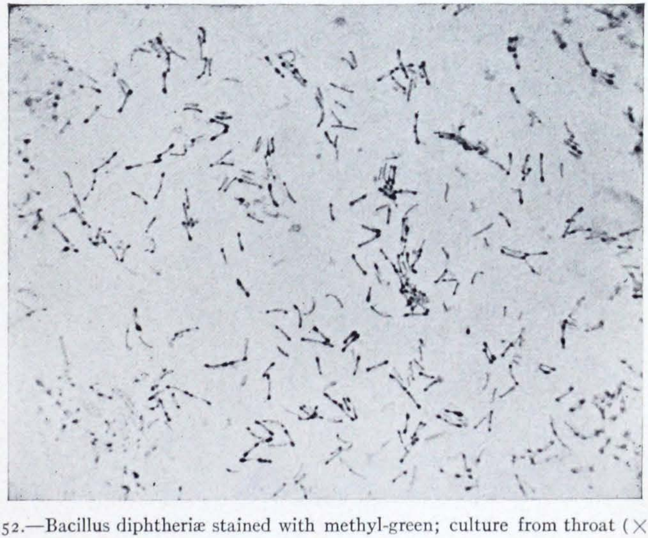


Fig. 152.—Bacillus diphtheriæ stained with methyl-green; culture from throat (× 1000) (photograph by the author).

granule at each end (Fig. 152). They stain by Gram's method. It is generally necessary, and always safer, to make a culture upon blood-serum, incubate for twelve hours, and examine smears from the growth.

Vincent's angina is a pseudomembranous and ulcerative inflammation of mouth and pharynx, which when acute may be mistaken for diphtheria, and when chronic is very apt to be mistaken for syphilis. Stained smears from the ulcers or membrane show large numbers of spirochætæ and "fusiform bacilli," giving a striking and characteristic picture (Fig. 153). The "bacillus" is spindle shaped, more or less pointed at the ends, and about 6 to 12 μ long. The spirillum is a very slender, wavy thread, about 30 to 40 μ long. Diluted analin-gentian-violet makes a satisfactory stain. Further description is given on p. 331.

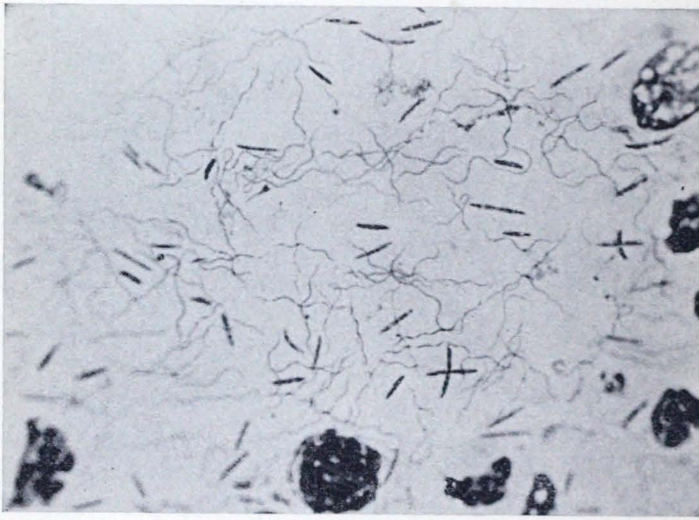


Fig. 153.—Spirochæta vincenti from case of ulcerative stomatitis (× 1200).

Tuberculous ulcerations of mouth and pharynx can generally be diagnosed from curetings made after careful cleansing of the surface. The curetings are well rubbed between slide and cover, and the smears thus made are dried, fixed, and stained for tubercle bacilli. Since there is much danger of contamination from tuberculous sputum, the presence of tubercle bacilli is significant only in proportion to the thoroughness with which the ulcer was cleansed. The diagnosis is certain when the bacilli are found within groups of cells which have not been dis-associated in making the smears.

THE EYE

Staphylococci, *pneumococci*, and *streptococci* are probably the most common of the bacteria to be found in non-specific conjunctivitis and keratitis. Serpiginous ulcer of the cornea is generally associated with the pneumococcus (Fig. 143).

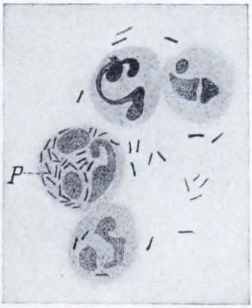


Fig. 154.—Conjunctival secretion from acute contagious conjunctivitis; polynuclear leukocytes with the bacillus of Weeks; P, phagocyte containing bacillus of Weeks (one-twelfth oil-immersion, ocular iii) (Morax).

The usual cause of acute infectious conjunctivitis ("pink-eye"), especially in cities, seems to be the *Koch-Weeks bacillus*. This is a minute, slender rod, which lies within and between the pus-corpuscles (Fig. 154), and is negative to Gram's stain. In smears it cannot be distinguished from the influenza bacillus, although its length is somewhat greater.

The *diplobacillus of Morax and Axenfeld* gives rise to an acute or chronic blepharoconjunctivitis without follicles or membrane, for which zinc sulphate seems to be a specific. It is widely distributed geographically and is common in many regions. The organism is a short, thick diplobacillus, is frequently intracellular, and is Gram-negative (Fig. 155). A delicate capsule can sometimes be made out.

Early diagnosis of gonorrheal ophthalmia is extremely important, and can be made with certainty only by detection of *gonococci* in the discharge. They are easily found in smears from untreated cases. After treatment is

Fig. 155.—The diplobacillus of Morax and Axenfeld (from a preparation by Dr. Harold Gifford).

begun they soon disappear, even though the discharge continues.

Pseudomembranous conjunctivitis generally shows either *streptococci* or *diphtheria bacilli*. In diagnosing diphtheritic conjunctivitis, one must be on his guard against the *Bacillus xerosis*, which is a frequent inhabitant of the conjunctival sac in healthy persons, and which is identical morphologically with the diphtheria bacillus.

The clinical picture is hence more significant than the microscopic findings.

Various micro-organisms—bacteria, molds, protozoa—have been described in connection with trachoma, but the specific organism of the disease is not definitely known.

Herbert has called attention to the abundance of eosinophilic leukocytes in the discharge of *vernal catarrh.* He regards their presence in considerable numbers as very helpful in the diagnosis of this disease.

THE EAR

By far the most frequent exciting causes of acute otitis media are the pneumococcus and the streptococcus. The finding of other bacteria in the discharge generally indicates a secondary infection, except in cases complicating infectious diseases, such as typhoid fever, diphtheria, and influenza. Discharges which have continued for some time are practically always contaminated with the staphylococcus. The presence of the streptococcus should be a cause of uneasiness, since it much more frequently leads to mastoid disease and meningitis than does the pneumococcus. The staphylococcus, bacillus of Friedländer, colon bacillus, and Bacillus pyocyaneus may be met in chronic middle-ear disease.

In tuberculous disease the tubercle bacillus is present in the discharge, but its detection offers some difficulties. It is rarely easy to find, and precautions must always be taken to exclude the smegma and other acid-fast bacilli (p. 53), which are especially liable to be present in the ear. Rather striking is the tendency of old squamous cells to retain the red stain, and fragments of such cells may mislead the unwary.

PARASITIC DISEASES OF THE SKIN

Favus, tinea versicolor, and the various forms of ringworm are caused by members of the fungus group. To demonstrate them, a crust or a hair from the affected area is softened with a few drops of 20 per cent. caustic soda solution, pressed out between a slide and cover, and examined with a one-sixth objective. They consist of a more or less dense network of hyphæ and numerous round or oval refractive spores. The cuts in standard works upon diseases of the skin will aid in differentiating the members of the group.

MILK

A large number of analyses of human and cows' milk are averaged by Holt as follows, Jersey milk being excluded because of its excessive fat:

	Human Milk. Normal variations, per cent.		Human Milk. Average, per cent.	Cows' Milk. Average, per cent.
Fat	3.00 to	5.00	4.00	3.50
Sugar	6.00 to	7.00	7.00	4.30
Proteins	1.00 to	2.25	1.50	4.00
Salts	0.18 to	0.25	0.20	0.70
Water	89.82 to	85.50	87.30	87.50
	100.00	100.00	100.00	100.00

The reaction of human milk is slightly alkaline; of cows', neutral or slightly acid. The specific gravity of each is about 1.028 to 1.032. Human milk is sterile when secreted, but derives a few bacteria from the lacteal ducts. Cows' milk, as usually sold, contains large numbers of bacteria, the best milk rarely containing fewer than 10,000 per cubic centimeter. Microscopically, human milk is a fairly homogeneous emulsion of fat, and is practically destitute of cellular elements.

Chemic examination of milk is of great value in solving the problems of infant feeding. The sample examined

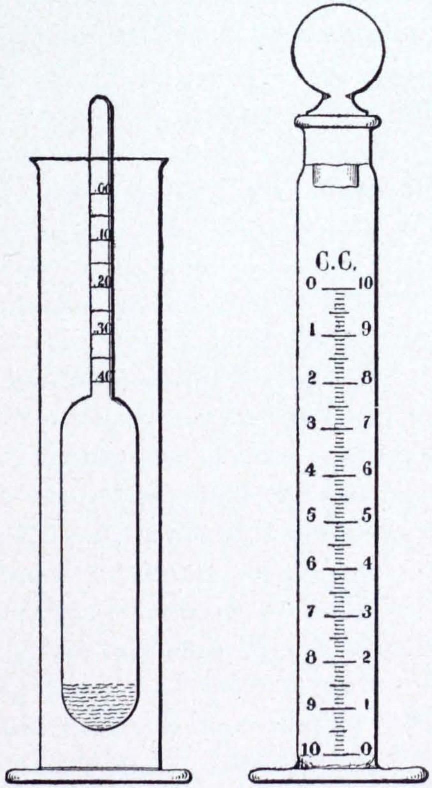


Fig. 156.—Holt's milk-testing apparatus.

should be the middle milk, or the entire quantity from one breast. The fat and protein can be estimated roughly, but accurately enough for many clinical purposes by means of Holt's apparatus, which consists of a 10 c.c. cream gage and a small hydrometer (Fig. 156). The cream gage is filled to the o mark with milk, allowed to stand for twenty-four hours at room temperature, and the percentage of cream then read off. The percentage of fat is three-fifths that of the cream. The protein is then approximated from a consideration of the specific gravity and the percentage of fat. The salts and sugar very seldom vary sufficiently to affect the specific gravity, hence a high specific gravity must be due to either an increase of protein or decrease of fat, or both, and vice versâ. With normal specific gravity the protein is high when the fat is high, and vice versâ. The method is not accurate with cows' milk.

Fig. 157.—Tube for milk analysis.

For more accurate work the following methods, applicable to either human or cows' milk, are simple and satisfactory.

Fat.—*Leffmann-Beam Method.*—This is essentially the widely used Babcock method, modified for the small quantities of milk obtainable from the human mammary gland. The apparatus consists of a special tube which fits the aluminum shield of the medical centrifuge (Fig. 157) and a 5 c.c. pipet. Owing to its narrow stem, the tube is difficult to fill and to clean. Exactly 5 c.c. of the milk are introduced into the tube by means of the pipet, and 1 c.c. of a mixture of equal parts of concentrated hydrochloric acid and amyl-alcohol is added and well mixed. The tube is filled to the o mark with concentrated sulphuric acid, adding a few drops at a time and agitating constantly. This is revolved in the centrifuge at 1000 revolutions a minute for three minutes, or until the fat has separated. The percentage is then read off upon the stem, each small division representing 0.2 per cent. of fat.

Proteins.—*T. R. Boggs' Modification of the Esbach Method.*—This is applied as for urinary albumin (p. 105), substituting Boggs' reagent for Esbach's. The reagent is prepared as follows:

(1)	Phosphotungstic acid	25 gm.
	Distilled water	125 c.c.
(2)	Concentrated hydrochloric acid	25 c.c.
	Distilled water	100 c.c.

When the phosphotungstic acid is completely dissolved, mix the two solutions. This reagent is quite stable if kept in a dark glass bottle.

Before examination, the milk should be diluted according to the probable amount of protein, and allowance made in the subsequent reading. For human milk the optimum dilution is 1 : 10; for cows' milk, 1 : 20. Dilution must be accurate.

Lactose.—The protein should first be removed by acidifying with acetic acid, boiling, and filtering. Purdy's method may then be used as for glucose in the urine (p. 112); but it must be borne in mind that lactose reduces copper more slowly than glucose, and longer heating is, therefore, required; and that 35 c.c. of Purdy's solution is equivalent to 0.0268 gm. lactose (as compared with 0.02 gm. glucose).

It is frequently desirable to detect formalin, which is the most common preservative added to cows' milk. Add a few drops of dilute ferric chlorid solution to a few cubic centimeters of the milk, and run the mixture gently upon the surface of some strong sulphuric acid in a test-tube. If formaldehyd be present, a bright red ring will appear at the line of contact of the fluids. This is not a specific test for formaldehyd, but nothing else likely to be added to the milk will give it.

SYPHILITIC MATERIAL

In 1905 Schaudinn and Hoffmann described the occurrence of a very slender, spiral micro-organism in the lesions of syphilis. This they named *Spirochæta*

Fig. 158.—Treponema pallidum (× 1000) (Leitz $\frac{1}{12}$ oil-immersion objective and Leitz dark-ground condenser).

pallida, because of its low refractive power and the difficulty with which it takes up staining reagents. The name was later changed to *Treponema pallidum*. Its etiologic relation to syphilis is now universally admitted. It is found in primary, secondary, and tertiary lesions, but is not present in the latter in sufficient numbers to be of value in diagnosis.

Treponema pallidum is an extremely slender, spiral, motile thread, with pointed ends. There is a flagellum at each end, but it is not seen in ordinary preparations. The organism varies considerably in length, the average being about 7 μ, or the diameter of a red blood-corpuscle; and it exhibits three to twelve, sometimes more, spiral curves, which are sharp and regular and resemble the curves of a corkscrew (Figs. 112, 158, 159). It is so delicate that it is difficult to see even in well-stained

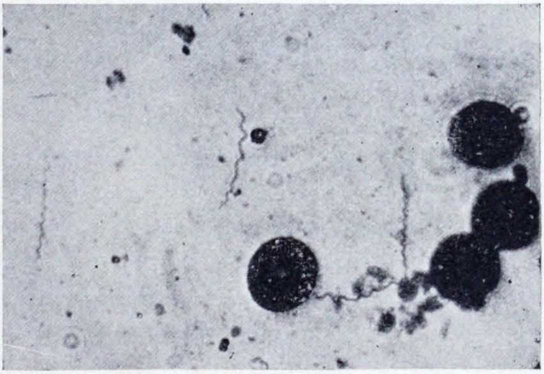


Fig. 159.—Treponema pallidum and Spirochæta refringens ($\times$ 1200) (Leitz oil-immersion objective).

preparations; a high magnification and careful focusing are, therefore, required. Upon ulcerated surfaces it is often mingled with other spiral micro-organisms, which adds to the difficulty of its detection. The most notable of these is *Spirochæta refringens*, described on p. 333.

Treponema pallidum is most easily demonstrated in chancres and mucous patches, although the skin lesions —papules, pustules, roseolous areas—often contain large numbers. Tissue-juice from the deeper portions of the lesions is the most favorable material for examination, because the organisms are commonly more abundant than upon ulcerated surfaces and are rarely accompanied by other micro-organisms. After cleansing, the surface is gently scraped with a curet or rubbed briskly with a swab of cotton or gauze. In a few moments serum will exude. The rubbing should not be so vigorous as to bring the blood, because the corpuscles may hide the treponema. Very thin cover-glass smears are then made from the serum.

Staining Methods.—**Giemsa's stain** is probably the most widely used. It is best purchased ready prepared. Smears are fixed in absolute alcohol for fifteen minutes. Ten drops of the stain are added to 10 c.c. of faintly alkaline distilled water (1 drop of a 1 per cent. solution of potassium carbonate to 10 c.c. of the water), and the fixed smear is immersed in this diluted stain for one to three hours or longer. It is then rinsed in distilled water, dried, and mounted. In well-stained specimens *Treponema pallidum* is reddish, most other micro-organisms, bluish. More intense staining may be obtained by gently warming the stain.

Wright's blood-stain, used in the manner already described (p. 222) except that the diluted stain is allowed to act upon the film for fifteen minutes, gives good results.

Silver Method.—The silver impregnation method has long been used for tissues. Stein has applied it to smears as follows:

1. Dry the films in the incubator at 37° C. for three or four hours.

2. Immerse in 10 per cent. silver nitrate solution, in diffuse daylight for some hours, until the preparation takes on a metallic luster.

3. Wash in water, dry, and mount.

The organisms are black against a brownish background.

India-ink Method.—A drop of India-ink of good grade (Günther and Wagner's recommended) is diluted with 1 to 5 drops of water. A loopful of this is mixed on a slide with a similar quantity of serum from the suspected lesion. The mixture is then spread over the slide and allowed to dry. After drying, it is examined with an oil-immersion lens. Micro-organisms, including *Treponema pallidum*, appear clear white on a brown or black background, much as they do with the dark ground condenser (Fig. 158). Because of its extreme simplicity this method has been favorably received. It cannot, however, be absolutely relied upon, since, as has been pointed out, many India-inks contain wavy vegetable fibrils which might easily mislead a beginner, and sometimes, indeed, even an experienced worker.

Dark ground illumination (see p. 21) may be used to study the living organisms in fresh tissue juices. This offers a satisfactory means of diagnosis, but since the instrument is expensive the practitioner will rely upon one or more of the staining methods just enumerated.

SEMEN

Absence of spermatozoa is a more common cause of sterility than is generally recognized. In some cases they are present, but lose their motility immediately after ejaculation.

Semen must be kept warm until examined. When it must be transported any considerable distance, the method suggested by Boston is convenient. The fresh semen is placed in a small bottle, to the neck of which a string is attached. This is then suspended from a button on the trousers, so that the bottle rests against the skin of the inguinal region. It may be carried in this way for hours. When ready to examine, place a small quantity upon a warmed slide and apply a cover. The spermatozoa are readily seen with a 4 mm. objective (Fig. 57). Normally, they are abundant and in active motion.

Detection of semen in stains upon clothing, etc., is often important. The finding of spermatozoa, after soaking the stain for an hour in normal salt solution or

Fig. 160.—Seminal crystals (medium size) (×750) from a stain on clothing. A single thread ⅛ inch long was used in the test, the stain being three years and four months old (Peterson and Haines).

dilute alcohol, and teasing in the same fluid, is absolute proof that the stain in question is semen, although it is not possible to distinguish human semen from that of the lower animals in this way. A little eosin added to the fluid will bring the spermatozoa out more clearly.

Florence's Reaction.—The suspected material is softened with water, placed upon a slide with a few drops of the reagent, and examined at once with a medium power of the microscope. If the material be semen, there will be found dark-brown crystals (Fig. 160) in the form of rhombic platelets resembling hemin crystals, or of needles, often grouped in clusters. These crystals can also be obtained from crushed insects, watery extracts of various internal organs, and certain other substances, so that they are not absolute proof of the presence of semen. Negative results, upon the other hand, are conclusive, even when the semen is many years old.

The reagent consists of iodin, 2.54 gm.; potassium iodid, 1.65 gm.; and distilled water, 30 c.c.

DIAGNOSIS OF RABIES

In view of the brilliant results attending prophylactic treatment by the Pasteur method, early diagnosis of rabies (hydrophobia) in animals which have bitten persons is extremely important.

The most reliable means of diagnosis is the production of the disease in a rabbit by subdural or intracerebral injection of a little of the filtrate from an emulsion of the brain and medulla of the suspected animal. The diagnosis can, however, usually be quickly and easily made by microscopic demonstration of Negri bodies. Whether these bodies be protozoan in nature and the cause of the disease, as is held by many, or whether they be products of the disease, it is certain that their presence is pathognomonic.

Negri bodies are sharply outlined, round, oval, or somewhat irregular structures which vary in size, the extremes being 0.5 and 18 μ. They consist of a hyalin-like cytoplasm, in which when properly stained one or more chromatin bodies can usually be seen. They are situated chiefly within the cytoplasm of the large cells of the central nervous system, the favorite locations being the multipolar cells of the hippocampus major (Ammon's horn). In many cases they suggest red blood-corpuscles lying within nerve-cells.

Probably the best method of demonstrating Negri bodies is the impression method of Langdon Frothingham, which is carried out as follows:

(1) Place the dog's brain[1] upon a board about 10 inches square, and divide into two halves by cutting along the median line with scissors.

(2) From one of the halves cut away the cerebellum and open the lateral ventricle, exposing the Ammon's horn.

(3) Dissect out the Ammon's horn as cleanly as possible.

(4) Cut out a small disc at right angles to the long axis of the Ammon's horn, so that it represents a cross-section of the organ.

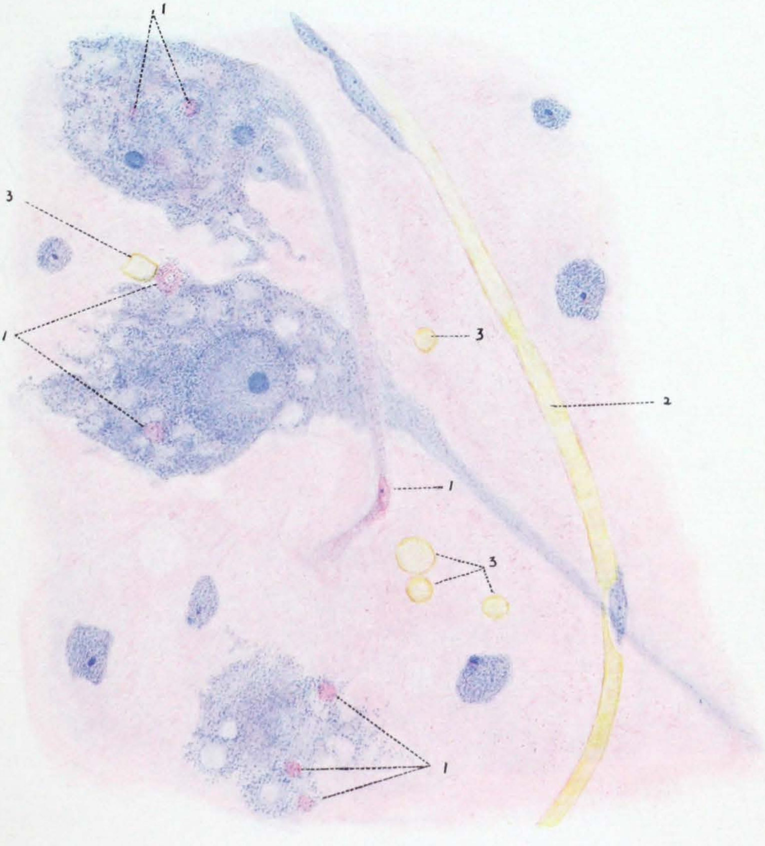
(5) Place this disc upon the board near the edge, with one of the cut surfaces upward.

(6) Press the surface of a thoroughly clean slide upon the disc and lift it suddenly. The disc (if its exposed surface has not been allowed to become too dry) will cling to the board, leaving only an impression upon the slide. Make several similar impressions upon different portions of the slide, using somewhat greater pressure each time. Impressions are also to be made from the cut surface of the cerebellum, since Negri bodies are sometimes present in the Purkinje cells when not found in the Ammon's horn.

(7) Before the impressions dry, immerse in methyl-alcohol for one-half to two minutes.

[1] For Dr. Frothingham's method of removing a dog's brain see American Journal of Public Hygiene for February, 1908.

PLATE XIII



Nerve-cells containing Negri bodies.

Hippocampus impression preparation, dog. Van Gieson stain; × 1000. 1, Negri bodies; 2, capillary; 3, free red blood-corpuscles (courtesy of Langdon Frothingham).