TRYPANOSOMATA AND TRYPANOSOMIASIS.*

(*A Summary.*)

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Definition. — Trypanosomata are flagellate Protozoa causing a specific disease, Trypanosomiasis, in man and many of the lower Vertebrata.

History. — Valentine (Berne) discovered in 1841 the first trypanosoma. He observed it in the blood of the trout (*Salmo fario*). In 1842 and 1843 Gluge (Brussels), Mayer (Bonn), Gruby (Paris), published three works on the trypanosomata of the frog. It is for this organism that Gruby created the name Trypanosoma (τρύπανον, auger; σῶμα, body), the Greek plural for which would be Trypanosomata. Wedl, 1850, Chaussat, 1850, Ray-Lancaster, 1871, Rattig, 1875, described the parasite in the blood of Batrachians. Remark, 1842, Gros, 1845, Berg, 1845, Chaussat, 1850, described the organism in fishes. Gros in 1845 found it in the blood of the field mouse and of the mole. In 1850 Chaussat saw it in the blood of a black rat, but it was not until the work of Lewis in 1878 on the parasites in the rats of India that attention was really drawn to the trypanosomata of mammals. In 1880 Evans discovered trypanosomata in the blood of horses suffering with the well-known surra of India. He proved the parasite to be the cause of the disease. Wittich, 1881, discovered a trypanosoma in the blood of hamsters. Marchiafava and Celli in 1885 found a trypanosoma in the blood of a patient ill with malarial fever. Rouget, 1896, described the organism in the blood of a horse ill with dourine, though Voges says Chauvrat discovered it in 1892. Bruce described the organism of Nagana in 1897. Elmassian in 1901 first differentiated the trypanosomata of mal-de-caderas in South America. Theiler, in

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an article published by Bruce, is credited with the discovery of a new trypanosoma of cattle in South Africa.

CLASSIFICATION.

Trypanosomata belong to kingdom Animalia, sub-kingdom Protozoa, class Mastigophora, sub-class Flagellata, order Monadida, family Trypanosomidæ, genus Trypanosoma.

Class; Mastigophora, Butschli. — Class diagnosis — Protozoa. Microscopic organisms, whose bodies usually have a definite form, but are not always provided with a cell membrane; cilia are absent, but the adult stage possesses one or several flagella; mouth present or absent; usually only one nucleus, but never a paranucleus; usually one or several pulsating vacuoles; reproduction by division, with or without conjugation and encystation.

The systemic position and limits of this class are in doubt. They may be placed under rhizopods or near the ciliates. Some of the forms which certain authors admit to the class as animals others call plants. Some described as flagellates may be only developmental stages in the life cycle of other animals, as flagellate stages occur in the life cycle of some of the Mycetozoa and Sporozoa. Some species are exceedingly simple in character, others are much more complicated and give rise to more or less complex colonies.

Sub-classes of the Mastigophora. — 1. *Flagellata.* There is always a flagellum at the anterior end and frequently two, and others may be present on other parts of the body.

2. *Choanoflagellata* (Gr. χοάνη, a funnel), readily distinguished by the presence of a protoplasmic collar or cup, which surrounds the base of the flagellum, and both the collar and flagellum are contractile.

3. *Dinoflagellata* (Gr. δίνος, a whirl) generally possess two flagella, one extending freely from the anterior end of the body, the other lying in a groove which passes around the body transversely.

4. *Cystoflagellata* are marine forms having a cyst-like structure.

FLAGELLATA.

These are small organisms, usually with sharply defined mononuclear body, with a definite anterior end bearing one or more flagella; actively motile during life, but possessing power of encystment; reproduction by longitudinal division in free or encysted stage.

Flagellata represent the only sub-class of Mastigophora which at present comes into consideration as parasites. Owing to the minuteness of the objects and the difficulty of recognizing characters for classification, there is a wide difference of opinion among authors relative to the sub-division of the sub-class into orders, families, genera, and species.

Order: Monadida. — They are independently living organisms, in some cases forming colonies; arrangement of the flagella varies; one flagellum, usually anterior, or two equal flagella, or two unequal flagella, or one large and two small flagella; undulating membrane may be present.

Family: Trypanosomidæ, Doflein, 1901. — They are parasitic forms with one chief flagellum, directed anteriorly; in some forms a secondary flagellum directed posteriorly; body usually with two angles, and wound more or less in the form of a spiral, one angle of body provided with an undulating membrane. One nucleus and one centrosome present.

The family at present contains two genera which are distinguished as follows:

1. *Trypanosoma*, one flagellum, extending from the centrosome along the undulating membrane and becoming free at the anterior extremity.
2. *Trypanoplasma*, two flagella, one extending anteriorly, the other posteriorly.

GENUS TRYPANOSOMA, GRUBY, 1843.

The body is fusiform, presenting a lateral longitudinal undulating membrane, the thickened border of which terminates posteriorly in the posterior half of the body, in a centrosome, and is prolonged anteriorly in a free major

flagellum; nucleus generally anterior; there is a tendency to agglomeration by the posterior extremity, division longitudinal and unequal. It is parasitic in the blood of animals. Type species Trypanosoma rotatorium.

General characters. — The organisms all show very active eel-like movements and some motility. In some species the latter is slight, the parasites undulating with extreme rapidity, but covering so short a distance as to be easily followed under the microscope; while in others, especially Tr. Lewisi, the movements are so rapid that it is impossible to keep the parasite in the field. The flagellum at the anterior end varies greatly in length. It is always actively motile, pointed, and continuous with the thickened margin of the undulating membrane, ending at or near the centrosome. It may be entirely homogeneous, or it may contain from one to several distinct granules, extending well out from the body of the parasite. The undulating extends along one border of the organism from the centrosome in the posterior end to the anterior end, whence it continues as the free flagellum. Its folds and breadth vary with different species and with the age of the organism. The centrosome is at the posterior end and appears to have intimate associations with the flagellum and undulating membrane. Laveran and Mesnil say it is the center for external movements. Its varying distance from the posterior end has been used as a diagnostic point in determining species; but not much importance can be attached to this, for it has been shown that the posterior end of the organism is contractile. The protoplasm is homogeneous or granular depending on age, environment, and species. The granules may vary in number and size from a few small ones situated in the anterior portion to numerous large ones scattered through the protoplasm. The nucleus is usually in the anterior half of the parasite, and is generally round or oval. Trypanosomata are nourished through osmosis. Neither digestive vacuoles nor solid inclusions of any sort are found in them. They displace by their movements the red corpuscles. But they have never been seen to

attack them, either to surround them or take them within. McNeal and Novy have shown that hemoglobin is necessary for their growth.

Reproduction in flagellates generally takes place by simple division, either during the active condition of the individual, or after it has secreted a cyst about itself; in the latter case a division may result in two, but often in several new individuals. In the colonial Flagellata each individual in some genera divides a number of times and forms a colony. In some Flagellata there is a more complex method of reproduction which closely resembles the sexual. Some cells enlarge and become macrospores, others divide a large number of times and become microspores; these two kinds of spores fuse with one another, and then division results in a new colony.

Rabinovitsch and Kempner describe three modes of multiplication in Trypanosoma lewisi: longitudinal division, transverse division, and segmentation. Wasielewski and Senn by study of living preparations, and by employing an improved staining method, were able to show that the differences in arrangement of nuclei of the dividing cells, which was for the former workers a characteristic distinction between longitudinal and transverse division, were due to manipulation. They further showed that the characteristic feature of segmentation, namely, that division was initiated only after loss of the undulating membrane and flagellum, and rounding up of the parasite did not exist in reality. They thus established unequal longitudinal division as the only method of multiplication. This is confirmed by McNeal.

Prowazek announces in a few lines the observation of conjugation in Tr. Brucei without giving any description of the process. McNeal has found couples in such intimate contact that a magnification of x 3,000 fails to find any line of separation. Exchange of material from one cell to another has not been demonstrated, however, and the question is not yet settled.

Trypanosomata are found in all the body fluids, and are not present at the same time in enormous numbers in one part with but few in another. Animals having many parasites in the blood when killed show them also in the organs. The blood of animals suffering from the disease is always infectious by animal inoculation, although there are periods during its course when the parasites cannot be found for days by microscopic examination. McNeal and Novy have found that cultural methods will disclose the organism when microscopic examination has failed. Plimmer and Bradford and others state that the lymphatics near the point of inoculation first show the parasite, and that animal's blood may be infected for two days before the parasites are found in it. Martini regards the spleen, lymphatics, bone marrow, and to a less extent, the liver and kidneys, as the places for the destruction of the organism. Experiments to determine the place of multiplication tend to show the whole cycle takes place in the blood. Parasites live only a short time in the body after death. Within two hours signs of degeneration begin; the parasites shrink, assume irregular shapes and then disappear. Motile parasites are not usually found two hours post-mortem. In exceptional cases living parasites have been found as late as sixteen hours after death. After twenty-four hours — at longest forty-eight hours — the blood is not infectious. Trypanosomata are usually more numerous in the spleen, liver, and lymphatic glands than in the bone marrow, and are seldom present in the medullary canal. They are found in the serous fluids and exudates of the joints, but rarely in the urine.

Trypanosomata may be examined in the fresh state by taking a drop of blood from the infected animal; in mammals, the ear; in rats, the tail; in birds, the great vein on the inside of the wing; in reptiles, from the tail or toe; in batrachians, from the fore foot; and in fishes, from the tail fin. It is often necessary to examine many preparations and sometimes for several successive days. Kanthack, Durham, and Blandford have advised centrifugalizing the defibrinated

blood. The trypanosomata are found in the middle layer with leucocytes. In dourine the examination can be made from the bloody edematous fluid, and in sleeping sickness the blood and the cerebro-spinal fluid from a lumbar puncture should be examined. As trypanosomata are rare in this fluid, it should be centrifugalized before examination. In prolonged observations a hanging drop may be used, sealed with paraffin or vaseline. This is useful for studying the phenomenon of agglutination. The blood is mixed with physiological salt solution or physiological citrated solution to prevent coagulation, or with the serum of another animal. Francis advises, for Trypanosoma lewisi, letting the blood coagulate, the trypanosomata pass into the serum and can be studied free from blood corpuscles. After an hour the motility of the organism quiets down. To check the active movements, Plimmer and Bradford advise a drop of a 1–100 solution of gelatine to the drop of blood.

The organisms are stained with eosin and methylene blue by various methods. The most common and best is the Romanowsky or some of its modifications. Wright's modification of the Romanowsky's stain is used, but it does not bring out the eosin staining parts well. Wright's stain made with commercial wood alcohol as used by Wolbach is the best stain. It brings out the structure of the undulating membrane as no other stain does.

Laveran and Mesnil found that Tr. Lewisi died out in defibrinated blood in four or five days, while if placed in an ice-box at 5° C. to 7° C. they remain alive for fifty-two days. At —17° C. they die out in two hours, and the blood fails to infect animals. Hanging drops and capillary tubes kept at 5° C. to 10° C. for thirty-two to fifty-three days infected rats after a period of seven days. Tr. Brucei may be kept alive in the blood for two to three days, whereas in the dead body they die out in twenty-four hours. Laveran and Mesnil say blood mixed with physiological salt solution can be kept in a virulent state for three days at room

temperature. In undiluted blood they become inert in forty-eight hours, whereas if diluted with horse serum the parasites could be kept alive for three days. When kept in an ice-box at 5° C. and 7° C. they die in three days. When the blood is subjected to —50° for one-half to two hours and then melted, the organisms were still alive. Rouget says the trypanosoma of dourine in fresh blood preparations was motile for eighteen hours, but never after twenty-four hours. McNeal and Novy found that trypanosomata could be kept alive for several months when the infected blood was added to rat, rabbit, or guinea-pig blood. Bacteria soon destroy the organism, probably by altering the medium and more especially the hemoglobin. The fact that the trypanosomata lived in the rat blood when diluted with other blood for a considerable period, even at ordinary room temperature, whereas in undiluted blood they die out quite early, indicated that possibly multiplication took place.

Danilewsky is usually credited with having cultivated trypanosomata in capillary tubes, but this is not substantiated by any statement of his own. According to Rouget, Chalachkow is credited with growing the parasite on dog serum. Six days after inoculation he observed, besides the ordinary forms, new ones showing different degrees of development. McNeal and Novy were the first to use cultural methods successfully. They added sterile defibrinated blood to ordinary nutrient agar. For this purpose the agar was melted and then cooled to 50° C., when it was mixed with sterile defibrinated blood, usually two parts to one, after which the tube was slanted and allowed to solidify. The medium thus prepared was bright and contains unaltered hemoglobin. The latter appears to be essential, for when the blood agar was kept in the incubator the hemoglobin underwent a change, and it was no longer a useful medium.

The blood was usually drawn from the carotids into sterile pipettes. A glass rod was passed through the cotton plug before sterilizing and by means of this the blood was readily defibrinated. The transfer of the defibrinated blood to the

melted agar was made with sterile Pasteur bulb pipettes. In order to secure from the rat the largest possible yield of trypanosomatous blood without bacterial contamination, the best procedure is to draw it directly from the heart into a sterile pipette.

With reasonable care blood agar can be thus prepared without the least danger of bacterial contamination. As soon as the blood agar cools and becomes solidified the tube is placed upright so as to allow as much water of condensation as possible to collect. This water is then inoculated with the otherwise sterile trypanosomic blood. When the inoculated tubes are to be kept at room temperature for weeks and months, it is necessary to prevent desiccation. This can be done best by sealing wax — rubber caps are not so useful. In culture experiments at incubator temperature, a different procedure was found necessary. The tightly rolled cotton plugs were first cut short, then charred in a flame, and pushed inside the tube. A number of these were then placed in a large desiccator, or preferably in a Novy anaërobic plate apparatus, on the bottom of which was some cotton well soaked in mercuric chloride. In this way an abundance of moisture and oxygen was provided for, and at the same time desiccation of the tubes prevented. By wiping down the outside of the tubes with mercury solution practically all danger of mould contamination is avoided. The growth at incubator temperature is more rapid, reaching a maximum in eight to twelve days, and death takes place in three weeks. Cultures at room temperature retain their vitality for weeks and months. Rat trypanosomata will grow on blood agar of 1 to 2 or 1 to 5 or even 1 to 10. The best conditions obtain with a 2 to 1 blood agar.

Culture of Tr. Brucei. — Tr. Brucei will only exceptionally grow on a medium which contains less than one-half its volume of blood. Sometimes only one or two tubes will develop out of a large number, although the medium is the same in all the tubes. For isolation from the living animals a nutrient agar of the following composition is prepared:

Extractives of 125 grams chopped beef in distilled water .	1,000 cc.
Agar	20 g.

Peptone	20 g.
Common salt	5 g.
Normal Na_2Co_3 solution	10 cc.

The agar thus prepared is distributed into tubes or small Erlenmeyer flasks and sterilized in the autoclave. To one volume of the sterile melted agar, cooled to about 60° C., two volumes of naturally sterile defibrinated rabbit's blood is added; thoroughly mixed and allowed to solidify. Tubes are allowed to solidify in the inclined position; flasks upright. Into the small amount of liquid which collects on the surface, pure Nagana blood is inoculated, and here development takes place.

Culture of Tr. Evansi. — The medium employed for the cultivation of Tr. Evansi was the same as that employed for the cultivation of Tr. Lewisi and Tr. brucei. Tubes inoculated January first in the Philippines were kept in cold storage three days and then shipped to Ann Arbor, where they arrived February eighth, or thirty-eight days later. There was a good growth, but all attempts at inoculating animals or obtaining sub-cultures failed. This is due to the fact that the organisms were thirty-eight days old when received.

Culture of Trypanosomata of birds. — The method employed was essentially the same as that used in Tr. Brucei and Tr. Evansi. In the work on Tr. Brucei, only eight per cent of infected animals gave cultures of this organism. This is due in large part to the concentration of meat extract used in the preparation of agar. An excess of meat extract inhibits, possibly by over stimulation, the development of the initial culture, whereas a smaller amount favors growth. After the growth is once obtained the media is the same as for Tr. Brucei.

Agar cooled to about 50° C., with two volumes of defibrinated rabbits added, is used. The water of condensation is inoculated with a drop of blood taken from the heart of the bird by means of a drawn-out tube pipette. In some instances, as when large birds are used, the blood may be drawn from the median vein by means of a sterile syringe. The cotton plug of the tube is cut short, moistened with mercuric chloride, and the tube covered with a rubber cap, after which it is placed at 25° C. for about a week. Growing trypanosomata may be recognized on the third day. On the sixth to seventh day, they are usually extremely abundant and very motile. Trypanosomata of birds differ in culture markedly from each of the three other organisms as well as among themselves. When cultivation is attempted, it is always advisable to inoculate three or four tubes of the medium for the reason that it may happen that but one out of a set of such tubes may develop.

Culture of Tr. rotatorium. — The blood of frogs and toads was taken to make blood agar. The blood was first examined to see that it was free

from parasites. The animal was then etherized and placed in $HgCl_2$ solution (1–1,000) for fifteen minutes, rinsed in distilled water, opened with all precautions, the blood from the heart taken with a sterile pipette, and mixed rapidly with water of condensation on slanted agar tubes (made with meat extract, peptone, and slightly alkaline to litmus). Two or three drops of blood were used for each tube. The tubes were sealed with rubber stoppers and allowed to stand five to ten days, so that contamination with bacteria might be detected.

The blood of a frog infected with Trypanosoma rotatorium collected in the same manner was mixed with that in the blood agar tubes prepared and tested as just described. The tubes were kept at room temperature. Growth took place after two weeks, and was never luxuriant. Arrangement in rosettes was never seen. One generation only of subcultures grew. All the cultures soon died. Inoculations failed.

Inoculations. — Inoculations of blood diluted with physiological salt solutions, defibrinated blood or pure blood are best made intraperitoneally. The animal must be susceptible, and the organisms must be in good condition and young. Old organisms will not infect. Young animals are more easily infected than old.

TRYPANOSOMIASIS.

Trypanosomiasis is such a widespread disease and affects so many animals that a great many names have been given it. Musgrave and Clegg give a list of eighty-two names used to designate the disease, but the list is not complete.

Symptoms. — Trypanosomiasis is usually characterized by a period of incubation, followed in most animals by a remittent, intermittent, or less frequently, relapsing fever; by the presence of trypanosomata in the circulating blood, which in some animals are in proportion to the temperature, by progressive anemia and emaciation, by catarrhal condition of the mucous membranes of the eyes and nose, by roughness of the hair, which in many instances falls out, by subcutaneous edema, more commonly of the posterior extremities, genitals, and belly. In the later stages, paresis of the posterior extremities is very common.

Pathology. — In addition to evidence of severe anemia, certain changes in the spleen are found, the most constant being enlargement and a peculiar mottling. There are also lymphatic hyperplasia, peculiar yellowish gelatinous subcutaneous and subserous infiltrations, an enlarged liver, and an accumulation of fluid in the serous cavities.

Trypanosomata Vermes. — Butschli found trypanosomata in the intestinal canal of a nematode (Tribolus gracilis). They were 33 μ in length and were sometimes observed in stellate colonies. They have also been reported in the intestinal canal of leeches (Hirudinea), having been sucked in from the blood of some higher animal.

Trypanosomata in Arthropoda. — Trypanosomata have been found in flies, gnats, lice, fleas and other Hexapods, these having obtained them from higher animals, and serving also to spread the disease.

Trypanosomiasis of Vertebrates.

Pisces history. — In 1841 Valentine discovered a trypanosoma in the blood of a trout (Salmio fario). Remark, 1842, saw the organism in a pike (esoxlucius) and in many fresh water fishes. Gros in 1845 saw the parasite in a number of fishes. The list of fishes, both fresh water and marine, in which trypanosomata have been found is a long one. The Trypanosomata are rare in cartilaginous sea fish and numerous in the bony sea fish. The parasites have been described by many observers, among whom are Berg, Wedl, Chaussat, Mitrophanov, Danilewsky, Chalachnikov, Kruse, Lingnard, Sabrazes, Laveran, Mesnil, Hofer, Leger, and Plehn.

Technic. — Blood is obtained by cutting two or three rays of the caudal fin. The organisms will live in a physiological salt solution for four or five days. For stained preparations it is necessary to open the fish while still alive, take a drop of blood from the heart, dry rapidly over an alcohol lamp and fix with alcohol or alcohol and ether. Air at the sea is so humid that if the blood is left to dry the parasites become deformed. In dead fish the blood and trypanosomata are quickly deformed.

Classification. Trypanosoma Remaki.

Trypanosoma Remaki has a large geographical distribution and is divided into two varieties named, according to size, *parva* and *magna*.

Tr. Remaki *parva* is 28 to 30 μ long, body 15 to 20 μ. It may reach a length of 42 μ.

Tr. Remaki *magna* is 45 to 57 μ long, body 26 to 28 μ. It colors a deeper blue than the *parva* and is thicker. The structure is otherwise

similar to the *parva*. These large forms are not division forms, for a single sign of division has never been seen; they may be older forms of the *parva*. When multiplication occurs in the parva, the parasite enlarges and division may begin at the nucleus, more often at the centrosome. The centrosome enlarges and divides into two, which are united by a bridge. At the same time the flagella divides at the base and then throughout its length.

Trypanosoma Danilewsky. — Danilewsky found this form in the carp (Cyprinus carpio). It is 35 to 45 μ in length, 3 μ thick. The undulating membrane is large with many folds. The protoplasm contains numerous chromatin granules of various sizes. Multiplication has never been seen.

Trypanosoma tincæ. — Doflein found it in the tench (Tinca tinca), which were sick and dying in great numbers. In fresh blood this trypanosoma is very motile and is almost always coiled up. It is about 35 μ in length. The undulating membrane is large and has many folds. Some forms show division of centrosome and commencing division of flagella.

Tr. Abramis. — Trypanosomata have been found in the bream (Abramis brama). Not much is known about this form.

Trypanosoma granulosum. — The first description of trypanosoma in the eel (Anguilla vulgaris) was that of Sabrazes and Muratet of Bordeaux. The largest forms reach from 44 to 80 μ in length. The centrosome is spherical and of good size. The undulating membrane is well expanded and bordered by a sharply defined flagella. The protoplasm is filled with granules of good size of deep violet blue. Granules are sometimes massed around the nucleus, which becomes hard to see. The nucleus is a violet red and sometimes occupies the breadth of the body, sometimes it is slender and is close to the concave side. Sabrazes kept these Trypanosoma a week at 10° C. to 19° C., in vitro and saw them multiply.

Trypanosoma soleæ. — The trypanosoma of soles (Solea vulgaris) is 40 μ long, of which the body is 32 μ and the flagella only 8 μ. The protoplasm contains some chromatic granules toward the posterior extremity, and shows some fine longitudinal striations.

Trypanosoma scylæ. — Laveran and Mesnil found a trypanosoma in the dogfish (Scylium canacula and Scylium stellare). It is from 70 to 75 μ long, the flagella 14 μ. The protoplasm is a strong blue, the undulating membrane a pale blue. The centrosome is smaller than that of Tr. Soleæ. It is very rare.

Tr. rajæ. — Trypanosomata, found by Laveran and Mesnil in Rajæ, are 75 to 80 μ long, flagella 20 μ long, about 6 μ in thickness. Multiplication forms have not been seen. It is similar to Trypanosoma Scylæ.

TRYPANOPLASMA. — Trypanoplasma are found in the Cyprinides (carp and minnows). There are two species.

Trypanoplasma Borelli. — The body of Trypanoplasma Borelli is flattened, often bent in a circle. The concave side is thick and takes a deeper color. The color of the posterior end is deep blue, while that of the anterior end is clear. It is 20 μ long and 3 μ thick. There are two masses of chromatic granules, one globular, the nucleus, on the convex side, and the other, the centrosome, on the concave side. The centrosome gives rise to two flagellæ which issue from the same pole; one runs posterior and one anterior. The trypanoplasma of the minnows is more pathogenic and has large grains of pigment, but is otherwise like Trypanoplasma Borelli.

Trypanoplasma Cyprini. — Mlle. M. Plehn found this form in two carps. This form caused an epidemic in Germany. The flagellæ are of unequal length; the anterior is more than one-half the length of the body, the posterior one-quarter the length of the body. Multiplication forms are rare.

Physiology of the Trypanoplasma. — Leger says the posterior flagellum is the helm, the anterior flagellum a feeler, and the undulating membrane the locomotive apparatus.

Methods of infection. — It is probable that trypanosomiasis of fish is spread by ectoparasites which fix themselves on the gills or on the surface of the body between the scales.

Symptoms. — Hofer describes the symptoms of the disease in the carp. The fish lie on the side for weeks, the head and tail drooping. If they are picked up, they swim around a while and then fall on the side, breathing slowly and feebly. With the weakness there is a loss of flesh.

Leger describes the symptoms in the minnow. The fish becomes pale, swells up, becomes motionless, takes no nourishment and dies. Similar symptoms have been observed by Plehn and Doflein.

AMPHIBIA. History. — Gluge in 1842 discovered the presence of trypanosomata in frogs. In 1843 Mayer and Gruby described the parasite and Gruby gave the name it

now holds. Other observers are Chaussat, Wedl, Lieberkuhn, Ray, Lancaster, Rattig, Gaule, Grassi, Danilewsky, Chalachnikov, Ziemann, Laveran and Mesnil, Dutton and Todd, Ed. and Et. Sergent. Ziemann was the first to use the Romanowsky method of staining. This method gives a blue stain to protoplasm of animal organisms, and a red to the nucleus. He succeeded in staining the nucleus and centrosome, but did not stain the undulating membrane nor the flagellum.

Trypanosomata are more often found in frogs in summer, especially in August in this part of the world. The males of *Rana esculenta* have twice as many parasites as the females. Gruby says that the proportion of males infected is smaller than that of females.

Trypanosomata rotatorium. — The pleomorphism of this organism has drawn the attention of many observers. Danilewsky distinguished at least four varieties. Chalachnikov distinguished two groups. The thickened edge of the undulating membrane is in relation with a clear space, in the center of which is the nucleus. There are two varieties of Tr. rotatorium, according to Laveran and Mesnil, one with the surface covered with numerous divisions, the other flat with smooth surface. The flagellum is short in comparison with the body. The posterior end is round and blunt at times, or may be short or very long. Trypanosoma of frogs present an extreme variety of forms. It is 40 to 60 μ long and 5 to 40 μ wide. Some have an oval form, round at both ends with a short free flagellum, an undulating membrane which extends one-half the length of the body, and is 50 to 60 μ long and 30 to 40 μ wide. Multiplication forms were not seen.

Lewis and Williams, who grew cultures of trypanosomata of frogs, give the following description: Cultural forms of Tr. rotatorium show organisms of a very long oval form, the bodies of largest being 2 x 18 μ, the flagellum nearly as long as the body. Only the largest forms showed a trace of undulating membrane. Motility was not very pronounced. Numerous small forms were seen. The centrosome was at the anterior end, the nucleus at the posterior end.

Another form was 3 μ x 16 μ, flagellum hardly half as long as the body, undulating, not distinct on small forms. Large forms, similar to Tr. rotatorium, except that the flagellum was lacking, occurred, but were rare.

Trypanosomata inopinatum. — This variety measures 25 to 30 μ and 3 μ wide. It resembles very much Tr. Lewisi. It is stockier, less tapering. Its nucleus is in the middle of the body, while in Tr. Lewisi it is in the

anterior half. The undulating membrane does not have folds in it, but appears more rigid than that of Tr. Lewisi.

Mode of infection. — The mode of infection is not known, but may be by some ectoparasite. In experimental work it is almost impossible to infect frogs. Laveran and Mesnil succeeded once out of seven or eight times, while up to that time it was thought impossible.

Trypanosomata Diemyctyli. — This parasite will be described in a special paper (see this number of the Journal).

REPTILIA. History. — From the existence of trypanosomata in the digestive tract of Ixodes testudinis, an ectoparasite of turtles, Leydig, 1857, concluded that they existed in the blood of turtles. Kunstler, in 1883, discovered a Trypanosoma in the blood of a mud turtle. In 1902 Laveran and Mesnil found in an Asiatic turtle a trypanosoma to which they gave the name of Tr. damoinæ.

Trypanosoma Damoinæ. —This organism is 32 μ long including flagellum, and about 4 wide. The protoplasm is finely granular with some larger chromatin granules toward the posterior end. Multiplication forms have not been seen.

Trypanosoma Lacertiliæ. — Gehrke has seen a trypanosoma in a lizard.

Trypanosoma Ophidiæ. — Dutton and Todd have seen trypanosomata in snakes and turtles in Gambia, Africa. They mention two varieties, one long, the other short.

Modes of infection. — The trypanosomata are spread by ectoparasites like Ixodes.

AVES. History. — Danilewsky published his article on Trypanosomata of birds in 1888. Laveran, in 1903, published his observations on Trypanosomata of the common brown owl (Syrnium ahico). Dutton and Todd described trypanosomata of Crithagra and Estrelda of Gambia, and Hanna wrote of the trypanosomata of the pigeon and crow of India. The trypanosomata Eberthi that Kent found in the digestive tube of the hen is a Trichomonas. Ed. and Et. Sergent, 1904, studied the blood of a large number of Algerian birds, and found them in only three species. Schaudinn, 1904, published his studies on alternation of

generations and change of host of trypanosomata and Spirochætæ. In March, 1905, McNeal and Novy published the best work on trypanosomata of birds, and by means of pure cultures were able to prove that Schaudinn had "mixed cultures" when he evolved his ideas in the paper above mentioned.

Technic. — Blood for examination is obtained from the marginal vein on the inner side of the wing. The feathers are removed, the skin washed with a little water, and the small vein cut with a pair of sharp pointed scissors. The injury is so slight that the smallest bird can be examined every few days for some time. The detection of trypanosomata in fresh blood is difficult, as they are scarce, yet this method is, as a rule, more delicate than the examination of stained specimens because the motility of the organism is likely to draw attention to it, while in stained specimens the organism may be concealed by a mass of cells.

The bird trypanosomata do not stain readily. The nucleus may be colorless or nearly so. The flagella are usually very indistinct.

The injection of the trypanosoma cultures can be made subcutaneously or into the breast muscle. The intraperitoneal method is dangerous and was abandoned for intrapleural injections. For this purpose the needle of the syringe is inserted obliquely through the percular angle into the right pleural cavity. Relatively large doses, even one-half cubic centimeter, can be thus introduced into a small bird, such as a sparrow. The feathers over the wish-bone should be removed, and the skin washed previous to making injections.

Trypanosoma avium. — This form is found in blackbirds, bluebirds, blue-jays, orioles, robin, English sparrow, and song sparrow. There are two strikingly different forms, the multiplication, rosettes, and the free-swimming, darting, thread-like spirochætes. The spindle-shaped cells of the rosettes were 10 μ long and about 3 μ wide. The spirochæte form is 30 to 60 μ long and 5 to 1 μ wide.

Trypanosomata Mesnili. — The native trypanosoma is characterized by its large size and great bulk, and by a wide rounded posterior extremity. The length of the body is 50 μ, width, 8 μ.

Trypanosomata Laverani. — It is a wide spindle which measures 20 μ in length and 6 μ wide.

Tr. Johnstoni. — Dutton and Todd found this form in the estrelda estrelda. Its length is 36 to 38 μ, and 1 μ wide.

Tr. Paddæ. — In 1905 Dr. Levaditi found in the blood of a Padda aryzivora a new form. It is 30 μ long and 5 μ wide. The flagellum is very short.

Mode of infection. — This infection is spread by the *Culex pipiens* and may be fatal as seen in paddæ.

MAMMALIA. Trypanosomata Lewisi. — Mention is made as early as 1845 of trypanosomata in rats and hamsters. In 1850 Chaussat saw the organism in *Mus rattus*. The parasites were rare in young rats, but were nearly always present in the adults. In 1877 Lewis found the organism in the brown rats of Calcutta. The first good description of Tr. Lewisi is that of Crookshank in 1886. Then followed the work of Rabinowitsch and Kempner in 1899, Wasielewski and Senn, 1900, Laveral and Mesnil, 1900–1901, Jürgens, 1902, Francis, 1903, Martini, 1903, McNeal and Novy, 1903, McNeal, 1904, and Smedley, 1905.

Geographical distribution. — Tr. Lewisi has been found in every part of the world. Crookshank found twenty-five per cent of London rats infected. Rabinowitsch and Kempner found forty-one per cent infected in Berlin. Laveran and Mesnil found four per cent infected in Paris. At Krommenie, Holland, ninety per cent were infected. At Bordeaux, one hundred per cent; in India, twenty-nine per cent; in Manila, twenty-five to sixty-five per cent were infected. In Japan, South America, and Madagascar the infection is present. In this country, Ann Arbor, Philadelphia, Detroit, Lincoln, Nebraska, San Francisco, have infected rats, while none have been found in Washington and Boston.

Trypanosoma Lewisi. — The adult Tr. Lewisi is 27–28 μ long, flagellum included, and 1.5 to 2 μ wide. Unlike the dividing forms, the whole body takes part in the undulating movement. The posterior tip is often feebly stained. The centrosome is situated about 3 or 4 μ from the posterior

tip. It measures 1 μ in length by 2/3 μ in width, and is placed transversely, extending across the entire width of the cell at this point. The flagellum at times appears to originate in this body, but the study of more favorable specimens clearly shows a chromatic space between them. There is an intimate connection, however, for these structures remain attached when all the rest of the cell has disintegrated. The flagellum extends in a smooth curve along the convex border of the parasite, supporting the undulating membrane, and is prolonged about 7 μ beyond the anterior tip as a free whip. The oval nucleus presents a chromatic network in a lighter nucleoplasm. At times a grouping of the chromatic into twelve chromasomes is apparent.

Smedley gives the following differences between the cultural and parasitic forms:

Cultural forms.	*Parasitic forms.*
Exceedingly active.	Very active.
Very variable in size and shape, generally spindle or pear-shaped.	Size only varies within narrow limits. Body is slightly fusiform, and has sharp pointed extremities.
Nucleus variable in position.	Nucleus is invariably situated at the middle of the anterior half of the body.
Centrosome is found close to the nucleus, or at a variable distance anterior to it. It is usually elongated.	Centrosome is found at a short distance from the posterior extremity, and is usually round.
Undulating membrane is not developed.	Undulating membrane is well developed, and is usually thrown into one or two folds.
Flagellum is frequently very long. Its basal portion is very short, owing to the position of the centrosome.	Flagellum much shorter, relatively to the length of the body of the parasite.

Susceptibility of other animals to Tr. Lewisi. — Tr. Lewisi, when injected into guinea-pigs, increases slightly, and then gradually disappears. The organism will live in no other animal.

Active immunity. — A single infection with Tr. Lewisi renders the rats free from parasites thereafter. Francis notes

an exception, but the second infection lasted only three days.

Passive immunity.— It has been found that the serum of rats which have been immunized by one or more inoculations of trypanosome blood does give protection to other rats within certain limits. If one cubic centimeter of immune serum is added in vitro to one cubic centimeter of trypanosome blood, and the mixture injected into a fresh rat, no infection will follow.

Mode of infection. — The infection is spread by fleas and probably in the case of wild rats by the eating of infected rats, as rats are prone to eat each other.

Trypanosomiasis of smaller mammals. — Trypanosomata have been found in the mouse, the rabbit, the guinea-pig, the hamster, the mole, and other small mammals.

TRYPANOSOMIASIS IN THE HORSE. — There are five well-known forms of Trypanosomiasis in horses: Nagana, Surra, Mal-de-Caderas, Dourine, and Trypanosomiasis of horses in Gambia.

Nagana. — The duration of the disease produced by Tr. Brucei varies with the species of animal. From this point of view one can divide mammals into three groups. 1. Animals in which it produces an acute disease: mouse, rat, vole, marmotte, hedgehog, dog, monkey. 2. Animals in which it produces a subacute disease: rabbit, guinea-pig, wood-mouse, dormouse, equides, pig. 3. Animals in which it produces a chronic disease: cattle, goats, and sheep.

Geographical distribution. — Nagana is confined to Africa and the Island of Mauritius. In Africa it is found in German and English East Africa, Congo, Nubia, Somaliland, Soudan, Zambesi, and Zululand.

TRYPANOSOMA BRUCEI. — The differential characteristics of this organism are summarized by Smedley as follows:

CULTURAL FORMS.

Tr. Lewisi.	*Tr. Brucei.*
1. Spindle-shaped or pear-shaped. Very variable in size, usually 3 to 5 or 14 to 16 μ excluding the flagellum. Smaller and larger forms frequently seen.	1. Resemble forms found in blood of infected animals, but shorter and more pointed. More constant in shape and size than Tr. Lewisi, measure 15 to 20 μ excluding the flagellum.
2. Move with greater rapidity, generally in straight lines. The body is not curved or bent.	2. Movements much shorter and are generally of a wiggling character.
3. Protoplasm clear and homogeneous, rarely it contains a large single vacuole.	3. Protoplasm soon becomes slightly granular, invariably contains two or three large vacuoles.
4. Flagellum very long and active, often quite rapid except where it issues from the body.	4. Comparatively short but very active.
5. Undulating membrane absent, unless it is developed in a minute form at base of flagellum.	5. Well developed; its contractions passing around the cell in a spiral direction.
6. Colonies form large masses of cells which are symmetrically arranged with their anterior extremities directed centrally. Huge colonies visible macroscopically as small whitish granules are formed by the coalescence of several colonies.	6. Colonies of small size and much less numerous. The younger colonies may present a symmetrical rosette-like appearance, the flagella being directed outward. In older colonies the Tr. are closely packed together but somewhat irregularly arranged. Secondary massing together of the colonies does not occur to any extent.
7. Tr. multiply rapidly. Cultures swarm with colonies and free forms and remain alive for	7. Tr. are never so numerous and degenerate rapidly, the culture being generally dead at

three months or longer. Cultures retain their virulence for some time.	the end of two months. Cultures rapidly lose their virulence, the time taken to do so depending on the temperature of incubation.

STAINED PREPARATIONS.

8. Protoplasm stains pale blue.	8. Protoplasm stains a deeper blue and frequently contains deeply stained granules.
9. Centrosome usually rod-shaped and situated either at margin of nucleus or just anterior to it.	9. Centrosome much smaller, round or elongated, and often difficult to distinguish from the other granules. Generally situated at the posterior end.
10. Flagellum long and thick and projects for almost its entire length from the cell. It measures two to four times the length of the body.	10. Flagellum is short and fine and can be traced backward along the free border of undulating membrane to end in centrosome. It measures three to five microns.

Symptoms. — Bruce describes the symptoms of Nagana as follows: Fever of a remittent or intermittent type; catarrhal secretions from the nose and eyes; staring of the coat and edema of the abdominal region, the prepuce and the posterior extremities. The animal becomes markedly emaciated and has a dejected appearance, the head hangs, the hair becomes very rough, and in places falls out, the mucous membranes of the eyes and genitals become very pale, and there is generally a slight opacity of the cornea. Just before death the animal falls to the ground and dies apparently without suffering.

Pathology. — Bruce mentions the anatomic lesions in Nagana as a gelatinous atrophy of the subcutaneous tissues, subserous and subcutaneous ecchymosis and an enlargement of the spleen.

Mode of infection. — The disease is spread by the bite of the tsetse fly (Glossina morsitans).

Treatment. — Arsenic is used and also trypanred. Human serum, serum from animal origin, and serum from animals immune to Trypanosomiasis.

SURRA. — This form of Trypanosomiasis is scattered over the greater part of the world. It occurs in India, Annam, Korea, Persia, Java, Philippines, Abyssinia, Egypt, Madagascar, Togo, Chili, Marajo Island, Paraguay, Uruguay, in North America and Australia. It is caused by Tr. Evansi.

Tr. Evansi. — The surra organisms differ from the Nagana in the following respects: 1. They are larger, their average length is 25 to 35 μ, the flagellum is very long. 2. They are very actively motile and moved either backward or forward. 3. The protoplasm of the anterior portion of the cell contained a large number of small granules or globules about .3 to .5 in diameter, of a yellowish or greenish tint; in forms dividing longitudinally, these globules are arranged in parallel lines, one row in each half of the dividing parasites. 4. There was an entire absence of rosettes and colonies.

Symptoms. — The period of incubation is apparently six to eight days. The coat is staring, the submaxillary glands may swell, emaciation is constant, lacrymation may be profuse toward the end of the disease, petechiæ on the eyelids, hemorrhages into the anterior chamber, and opacity of the cornea are present, there are cartarrhal discharges from the nose, petechiæ, and injection of the nasal septum, ulcerations inside the nose, thickening of schneiderian membrane, stringhalt, muscular tremors and crossing of the legs are due to some effusion on the spinal cord or on particular nerves; there is often a voracious appetite and great thirst. Gurgling and tympanitis are present. The temperature is remittent, intermittent, and sometimes a relapsing type.

Pathology. — There are marked anemia and leucocytosis, serous effusions into the peritoneal cavity, petechiæ, jaundice, fatty changes in the muscles, liver, spleen, kidneys, and other organs, with a large deposit of fat under the skin.

Complications. — The complications are pneumonia, nephritis, pleurisy, sudden collapse, palpitation, pericarditis,

hemorrhages, miscarriage, edema of the extremities and under surface of the abdomen, and effusion in the joints.

Mode of infection. — The disease is spread by flies — Tabanus tropicus and Stomoxys calcitrans.

DOURINE. — This disease occurs in Syria, Algeria, Morocco, Tripoli, Bohemia, France, Germany, Hungary, Spain, Turkey, and the United States. It is caused by Tr. equiperdum.

Tr. Equiperdum. — It is 25 to 28 μ long, being shorter and smaller than Tr. Brucei. The protoplasm is less intensely colored than the other pathogenic trypanosomata. The protoplasmic granulations are never observed as in the case with Tr. Brucei.

Symptoms. — This disease has a chronic and an acute form. The chronic form has three stages: 1. The stage of edema in the genital and abdominal regions. 2. The period of plaques, places that look as if a piece of metal had been slipped under the skin, as large at times as the palm of the hand. 3. The period of profound anemia and paraplegia.

Mode of infection. — It is usually spread by coition, rarely by biting flies.

Mal-de-Caderas. — This disease is limited to South America. The trypanosoma that causes it was discovered by Dr. Elmassian in May, 1901, Dr. Voges, Oct. 3, 1901, confirmed. It is caused by the Trypanosoma equinum.

Tr. Equinum. — In the fresh state Tr. equinum is very much like Tr. Evansi or Tr. Brucei. It is 22 to 24 μ long and 1.5 μ wide. Multiplication forms become 28 to 30 μ long and 5 μ wide. The characteristic feature is the centrosome, which is so small that its presence has been denied.

Symptoms. — Horses are never known to recover from this disease. It is a chronic disease lasting two to five

months in the horse, and six to twelve months in asses and mules. There is an intermittent fever, rapid progressive emaciation, albumen and blood in the urine, the red blood corpuscles diminish and the microscopic changes of pernicious anemia appear; the lymphocytes and especially the eosinophiles increase in number; the most marked symptom is a symmetrical or asymmetrical paresis of the hind legs; defæcation and urination difficult; dyspnea; appetite and extreme thirst to the last; gradual extension of paralysis to other parts of the body and frequent edema.

Pathology. — Enlargement of spleen and lymphatic glands; serofibrinous exudations in body cavities; liver enlarged and engorged with blood; heart flabby; lungs often with hemorrhagichoids and subpleural emphysematous patches; catarrhal condition of the respiratory passages and pathologic changes in the spinal cord.

Treatment. — Treatment without success. Quinine, methylene blue, arsenic, enterol, potassic permanganate and other drugs have been used.

Mode of infection. — By biting insects in the rainy season when flies are most abundant. These varieties are Tabanus and Stomoxys calcitrans.

Trypanosomiasis of Horses of Gambia. — This disease was discovered by Dutton and Todd in 1902. There were few horses affected, and they came from Senegal. It is caused by Tr. dimorphon.

Symptoms. — The symptoms differ in that there is no edema, and no staring of the coat. The parasites are very few in the blood and may be absent for long periods of time. At the end of the disease, the animal is extremely feeble, respiration very painful, sweating continually, with light convulsions just before death.

Tr. dimorphon. — There are three forms, a tadpole form, a long form, and a stumpy form. The tadpole form is 1 to 3 μ long, the long form 26 to 30 μ and the stumpy form 16 μ and 3 μ wide.

Mode of infection. — It is spread by the Glossina palpalis perhaps, but it is not known.

GALZEIKTE. — This disease occurs in cattle and is epidemic in South Africa, in Transvaal. It is produced by the Trypanosoma theileri, which is a distinct organism producing a distinct form of trypanosomiasis. All attempts to infect other animals have failed.

Tr. Theileri. — The large forms measure 60 to 70 μ long and 4 to 5 μ wide; the small forms are 25 to 30 μ long and 2 to 3 μ wide.

Symptoms. — There is an acute form of this disease and a subacute form. In from three to five days after inoculation there is a fever which lasts a number of days and then returns to normal. The symptoms are those of a severe anemia and the mortality is about twelve per cent.

Pathology. — The anemia and the enlargement of the spleen and lymphatic glands are the striking features of this disease. The heart is flabby with subserous petechiæ. The lungs present a hypostatic edema. The spleen is enlarged and soft.

Mode of infection. — The disease is spread by Hippobosca rufipes, which is very common in South Africa.

SLEEPING SICKNESS. — Sleeping sickness is a disease caused by Trypanosoma Gambiense. It occurs in Africa only. The first account of the disease was given by Winterbottom in 1803, who observed it on the west coast of Africa. From that time to the present it has been described by many observers. In 1902 Dutton and Todd published their work

in Senegambia, which gives the first modern account of the organism and the disease.

Tr. Gambiense. — The organism is 17 to 28 μ long and 1 to 2 μ wide. The protoplasm often contains chromatic granules which are distinguished by their size and number. They are often present in pairs.

In every case of sleeping sickness the organisms are found in the blood or cerebro-spinal fluid or both. There is a very evident connection between cases with slight symptoms and those with symptoms of an advanced stage. The latent period may be as long as two to five years or longer. The change from the latent stage to the advanced condition is very gradual. The duration of the disease after its recognition by friends is from two to four months. No native who has shown definite and constant signs of ill-health has recovered.

Dutton and Todd say that they have only observed eight fatal cases of Congo sickness in which necropsies showed no obvious secondary infection. They do not think they have sufficient evidence to state that death is produced by trypanosomata alone. Secondary bacterial infections seem to determine the fatal issue of many cases.

Complications. — The complications are: Purulent meningitis, pleurisy and pneumonia, pneumonia and localized tubercle of lungs, localized gangrene of lung, enlarged caseating and breaking down glands in abdomen, dysenteric ulceration of bowel, universally adherent pericardium, and infiltration of pus in femoral, inguinal, and internal glands (gonorrheal).

Mode of infection. — It is spread by Glossina palpalis.

Koch divides trypanosomata into two groups: one composed of Tr. Lewisi and Tr. Theileri, the other of the remaining trypanosomata. These groups are distinguished by the fact that the organisms of the first are constant in their morphological characteristics, their virulence and their relation

to domestic animals. Rat trypanosomata can be passed on exclusively to rats, and Theiler's organism to no other species of animal but cattle. Hence he draws the conclusion that they have been exclusively restricted to their own particular host, and consequently are most particularly suited to their host, thereby acquiring special properties and becoming a distinct species.

Quite different are the relations of the trypanosomata of the second group, to which Tr. Brucei, Tr. Evansi, Tr. equinum, and Trypanosomata of man belong; they are not sharply separated morphologically from each other, their virulence fluctuates over a wide range, and they are not exclusively found in one host. From these facts he draws the conclusion that the parasites of the second group have lived but a short time in their host, that they are not completely adapted to it, and have not yet developed into a distinct species.

BIBLIOGRAPHY.

The best work and only book is Laveran and Mesnil, Trypanosomes et Trypanosomiasis, 1904. The United States Department reports by Musgrave and Clegg, Salmon and Stiles, and Francis give good illustrations, good classifications, and good accounts of the disease. The Thompson-Yates Laboratory reports give the best accounts of human trypanosomiasis. McNeal and Novy, Smedley and Williams give the results of their work on cultures of Tr. Lewisi, Tr. Brucei, Tr. Evansi, of birds and of frogs. Doflein's book on Parasitic Protozoa gives a good classification, and Koch's address gives a good account of the infection in general.

The classification used in this compilation is that of Weysse.

Francis. Hyg. Lab. Bulletin No. 11, 1903.

Musgrave and Clegg. Dept. of Interior, No. 5, 1903.

Salmon and Stiles. Bureau of Animal Industry Bulletin No. 42, 1902.

Report of Supt. of Gov. Lab. in Philippines, 1903.

Lewis and Williams. Am. Med., ix, No. 12, 491, 1905.

Novy and McNeal. Victor Vaughan Book, 1903, 549.

Smedley. Jour. of Hyg., v, No. 1, 1905.

Koch. Deutsch Med. Wochenschrift, 1904, Nov. 17.

Jakinoff. Centralblatt f. Bakt., Orig., xxxvii, 1904.

Greig and Gray. British Med. Journal.

Kilborne. Jour. Ass. Military Surgeons, xiv, No. 4, 1904.

Head. Jour. of Comp. Path. and Therap., September, 1904.

Holmes. Jour. of Comp. Path. and Therap., December, 1904.

Petrie. Centralblatt für Bakt., xxxv, No. iv.

McNeal and Novy. Jour. Inf. Dis., March 1, 1905, ii, No. 2.
Dutton, Todd and Christy. Thompson-Yates & Johnston Lab., 1905.
Sergent. Annales de l'Institut Pasteur, January, 1905.
Voges. Zeitschrift f. Hyg., 1902, xxxix, 357.
McNeal. Jour. Inf. Dis., 1904, i, No. 4.
Hofer. Handbuch der Fischkrankheiten, 1904.
Leger. Bull. Inst. Pasteur, 1904, 1905.

The Bulletin de l'Inst. Pasteur has had reviews of all the recent work on Trypanosomes.