ON THE NATURAL OCCURRENCE OF HERPETOMONADS (LEPTOMONADS) IN THE BLOOD OF A FISH, *DENTEX ARGYROZONA,* AND ITS SIGNIFICANCE

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During the years 1908-1916, we were conducting researches, individually and in collaboration, on the life-cycles of Trypanosomes, Crithidia and Herpetomonas and their significance in the evolution of disease. After working on the life-histories of the Protozoa used by us, we were able to show, by direct experiment, that Herpetomonads and Crithidia could be inoculated into or fed to vertebrates and produce therein pathogenic effects resembling those of leishmaniasis. We also found natural occurrences of herpetomonads in mice, and in 1916 summarized our experimental conclusions to date, when war-work on the diagnosis of protozoal diseases completely stopped our further progress. Recently (Jan.-Feb., 1919), while working at the St. James Marine Aquarium, near Cape Town, we were agreeably surprised to find a Herpetomonas in the heart blood of a freshly killed silver-fish, *Dentex argyrozona.* Subsequently, the same organism was found in small numbers in the blood and organs of three more Dentex, a total of 4 out of 41 examined containing the Herpetomonas, but in each case the infection was scanty.

As no mention of such a parasite in the blood of fish can be found in the literature available, we propose to describe the Herpetomonas, and to name it *Herpetomonas denticis.* It is true, that, morphologically, it is somewhat difficult to separate this species from others, but the occurrence of physiological species or races is known, and such may be the case here. New and unexpected methods of research in future may shed further light on physiological species; at present we think that we are justified in giving a separate name to the Herpetomonas of *Dentex argyrozona* for purposes of reference. We regret that, as a result of our comparative studies, we are unable to accept a biflagellate character as diagnostic of the genus Herpetomonas, and it is also unfortunate that *Leptomonas gracilis,* the type species of Leptomonas, has not been studied by modern methods. We therefore accept Butschli's (1884) definition of the genus Herpetomonas.

As indicated in the introduction, the herpetomonads were found in the blood and organs of freshly killed silver-fish, *Dentex argyrozona*. Fresh preparations of the blood taken direct from the heart were examined both directly and by the aid of the paraboloid condenser, and stained preparations of both heart blood and internal organs of the fish were examined. Fixation by exposure to osmic acid vapor and formalin vapor followed by absolute alcohol, and direct wet fixation with Schaudinn, Carnoy or Bouin-Duboscq fluids were tried, while Giemsa, Delafield's hematoxylin and iron-hematoxylin stains were used. The most useful preparation was one fixed with osmic vapor and absolute alcohol and stained with Giemsa's solution.

DISTRIBUTION OF THE PARASITE IN THE HOST

At no time was *Herpetomonas denticis* abundant in any fish that we examined. It was seen very rarely and with difficulty in life. The parasites were most often found in stained smears made from the heart blood of the host. A few were found in the spleen and liver. Other organs were rarely infected, though a few parasites have been observed in a preparation from the kidney of one host. Intracellular forms have not been observed.

The herpetomonads were present both in the flagellate and the non-flagellate phases. As a general statement, flagellate forms were more common in the heart blood and non-flagellate forms in preparations of organs such as the spleen.

No herpetomonads were seen in the gut of any of the Dentex, though careful search was made. No marked pathologic effects on the hosts were observed.

MORPHOLOGY

The flagellate form of *Herpetomonas denticis* is small, the body measuring from 5 to 24μ long and 1.5 to 2.5μ broad (see photomicrographs). The variation in length is rather great, the short-bodied forms being apparently younger. The flagellum is often longer than the body, especially in young flagellate forms, as, for example, in a parasite whose body length was 7.5μ and length of free flagellum was 16μ. The posterior (non-flagellate) end of the body was sometimes pointed, at other times rounded.

The general cytoplasm was almost homogeneous, though in some specimens a finely alveolar structure was seen. Chromated granules may be seen in some specimens.

The nucleus was karyosomatic in some cases, and finely granular in others, the structure varying as we have pointed out previously, with the degree of activity of the cell-life. Prior to periods of great

multiplicative activity, the nucleus usually becomes finely granular in a flagellate, and such changes can be observed in the living organism under favorable conditions.

The blepharoplast, or kinetic nucleus of some authors, is distinct and often bar-like, but sometimes is slightly curved or rounded, the latter appearance probably being due to an end-on view. The organella may be surrounded by a less deeply staining area of cytoplasm.

The flagellum arises in the neighborhood of the blepharoplast but not from it. The root of the single flagellum is usually well marked.

The non-flagellate stages are small, oval or somewhat pyriform bodies, possessing a nucleus and distinct blepharoplast. The small oval or leishmaniform parasites measure 2.5 to 4.5μ by 1.5 to 2.5μ. Larger forms, elongating into flagellates though still lacking a flagellum, may be somewhat longer and broader.

Multiplication by fission occurs among both flagellate and non-flagellate forms. Division begins in the blepharoplast and is followed by division of the nucleus. Longitudinal fission was seen in flagellate parasites and in several intermediate elongating forms.

The occurrence of division shows that the herpetomonad could increase in numbers in the Dentex, and so was more than a mere conservation of the organism.

SIGNIFICANCE OF NATURAL HERPETOMONADS IN VERTEBRATES

The significance of the findings of herpetomonads in the blood of representatives of most classes of vertebrates is most important. The published results of our personal work on the life-histories of Herpetomonads and Crithidia have been strongly indicative that leishmaniases—such as kala-azar, dermo-mucosal and dermal leishmaniases—were really due to herpetomonads being able to live in the blood of vertebrates. Further, we have conducted experiments on the inoculation and the feeding of herpetomonads and a few crithidia to all classes of vertebrates with positive results. Laveran and Franchini have performed similar experiments and Laveran has shown that Leishmania can be inoculated into cold-blooded vertebrates. These various experiments have been carefully discussed recently by Laveran in his monograph on "Leishmanioses." We have had the good fortune to find herpetomonads occurring naturally in the blood of mice and of Dentex.

At present, herpetomonads have been found occurring naturally in the blood of the following vertebrates.

(1) Man. In 1913 a herpetomonad was described by Franchini from the blood and internal organs of man. Unfortunately, the name *Haemocystozoon brasiliense* was given to the organism. An allied parasite was recently found by M. Léger in French Guiana, herpeto-

monad and trypanosome forms being seen. It should also be mentioned here that herpetomonad flagellate forms of Leishmania have been seen in man.

(2) Mice. Herpetomonads were seen in the blood of Gambian mice by Dutton and Todd in 1903, while Fantham and Porter published similar observations on the natural occurrence of herpetomonads in the blood of English mice in 1915, these having been seen from time to time during the previous six years.

(3) Pigeons. Natural infections of these birds by a herpetomonad was found by Edmond and Etienne Sergent in 1907 in Algeria.

(4) Reptiles. Natural infection of geckos in Algeria with herpetomonads in the blood was found in 1914 by Sergent, Lemaire and Senevet. The Herpetomonads of geckos was also found by Chatton and Blanc in Tunis in 1918. Marcel Léger in 1918 found herpetomonads in the blood of small lizards belonging to the genus Anolis in Martinique.

(5) Fishes. A herpetomonas occurring in the blood and internal organs of *Dentex argyrozona* is now recorded by us.

As before mentioned, we were able to produce herpetomoniasis experimentally in all groups of vertebrates from Pisces to Mammalia. From the foregoing list, it will be seen that herpetomonads in nature have a similarly wide distribution in vertebrates, having been found in the blood of representatives of all the great groups of vertebrates except Amphibia, in which they will doubtless sooner or later be detected. However, it should again be pointed out that in no case in vertebrates is the flagellate form numerous, the leishmaniform phase being the one most seen, and relatively few vertebrates seem to be infected. In some cases it may be necessary to culture the blood in order to detect the parasite. Artifically induced herpetomoniasis resembles visceral leishmaniasis in its insidious onset and pathogenic effects such as feverish attacks, splenic enlargement often accompanied by hepatic enlargement, emaciation, progressive anemia and leucopenia.

The mode of entry of the herpetomonads into the blood of the Dentex examined by us is, unfortunately, not certain. It may be due to the inoculative action of an ectoparasite such as a leech. In the case of fresh-water fishes, herpetomonads might be introduced into their blood by aquatic biting Hemiptera such as Nepa. The entry of a natural intestinal parasite into the blood from the gut seems to be excluded, as, in every case, we carefully examined the gut contents of the fishes dissected by us, but found no indication of the presence of a Herpetomonas as a natural parasite in the guts. We may remark here that we always made a practice of examining before use the

dejecta and blood of the vertebrate animals subsequently used by us in our previous experiments on induced herpetomoniasis, and on no occasion did we find a Herpetomonas occurring naturally in the gut of the vertebrate. On one occasion, in the cloaca of two specimens of *Lacerata vivipara,* we found a uniflagellate monad, but it lacked a blepharoplast, and hence was not a Herpetomonas. Bayon (1915) states that he found a Herpetomonad in the cloaca of a *Chameleon pumilus* on Robben Island, while M. Léger (1918) states that he found a herpetomonad in the rectum of a lizard, *Anolis* sp. in Martinique. It is possible that these herpetomonads in the hind gut of lizards may have been acquired from ingested infected flies, and have passed thence into the blood of the lizards. It is also possible that in the catching of the fly on the tongue of the lizard, herpetomonads may have been liberated from the insect and have passed through the mucous membrane of the tongue of the vertebrate. On the other hand, they may have been inoculated into the blood of the lizard directly by the action of a bloodsucking fly or other Arthropod. We do not consider, on the evidence available, that herpetomonads are natural parasites of the gut of vertebrates, though they may be acquired from invertebrates by way of the gut and pass therefrom into other organs.

In our experience, when a herpetomonad is introduced into a vertebrate host, it may be able to exist either as a somewhat heavy infection that tends to die out and hence is transitory in the vertebrate, or it may become established in so attenuated a form that the pathogenic effects of its presence are not detected unless the resistance of the host is suddenly diminished. Again, owing to periodicity of multiplicative periods of the parasites, they may only be capable of detection in the blood of the host at certain seasons. The accidental, successful introduction of herpetomonad parasites by the agency of certain insects may thus afford an explanation of sporadic outbreaks of such diseases as kala-azar or other form of leishmaniasis.

When a herpetomonad gains access to and proves capable of multiplying in a vertebrate, though the infection may prove to be sparse, as in the case of *H. denticis,* it indicates that while there is still difficulty for the herpetomonad to live in the blood of the vertebrate, yet an attempt is being made that may become more successful—and perhaps more obvious—in the future. In other words, the presence of a natural herpetomonad in the blood and organs of Dentex indicates a further example of the habituation of a flagellate of invertebrates to life in a vertebrate host.

The herpetomonads, indeed, show wonderful powers of adaptation, one of the most plastic being *H. davidi,* natural to the gut of certain plant-frequenting insects, which is able to live in the latex of certain Euphorbiaceous plants.

Such diseases as leishmaniases in vertebrates need not be regarded as necessarily being conveyed by any one specific insect carrier of herpetomonads, but as being transmitted more or less accidentally into a susceptible subject by the agency of any insect capable of being heavily parasited with herpetomonads, and of passing these flagellates into vertebrates.

The leishmaniases are herpetomoniases in which the dominant stage of the causal agent in the vertebrate is the rounded, resting, non-flagellate leishmaniform stage. The leishmaniases are allied in causal agency, pathology and treatment (by tartar emetic) with the trypanosomiases, wherein the dominant stage of the causal trypanosome in the vertebrate is the flagellate stage, but resting, non-flagellate, leishmaniform parasites also occur in the internal organs of the vertebrate.

The occurrence of natural herpetomonads in vertebrates, and the ability to infect vertebrates experimentally with herpetomonads—entailing pathogenic results resembling leishmaniasis in the case of warm-blooded hosts—present an interesting chapter in the evolution of disease.

SUMMARY

A new flagellate, *Herpetomonas denticis,* n. sp., occur naturally in the blood of fish, *Dentex argyrozona,* from St. James, near Cape Town. The parasite was also seen in spleen, liver and kidneys of the fish. Flagellate forms, 5 to 24μ long, and 1.5 to 2.5μ broad, occurred in the heart blood, and rounded, non-flagellate, leishmania-like forms were seen in the internal organs. Multiplication stages were found.

As far as known, this is the first record of the natural occurrence of a Herpetomonas in the blood and internal organs of fishes. Four Dentex, out of 41 examined, were scantily parasited. Herpetomonads were not found in the digestive tracts of the fish.

The significance of this piscine parasite is important, in view of the numerous experiments carried out by the authors and others on the successful infection of vertebrates with herpetomonads and their relation to Leishmania. The leishmaniases are really herpetomoniases of mammals, wherein herpetomonads—which are natural parasites of invertebrates, such as insects—have been introduced into vertebrates, such as mammals, with pathogenic effects.

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Explanation of Plate III

Fig. 1.—Photomicrograph of the flagellate form of *Herpetomonas denticis* in the blood of *Dentex argyrozona,* obtained by using Zeiss 4 mm. apochromatic objective and Huyghenian 2 ocular

Fig. 2.—Photomicrograph of flagellate *H. denticis,* obtained by using Zeiss 1/12″ oil immersion objective and Huyghenian 2 ocular.

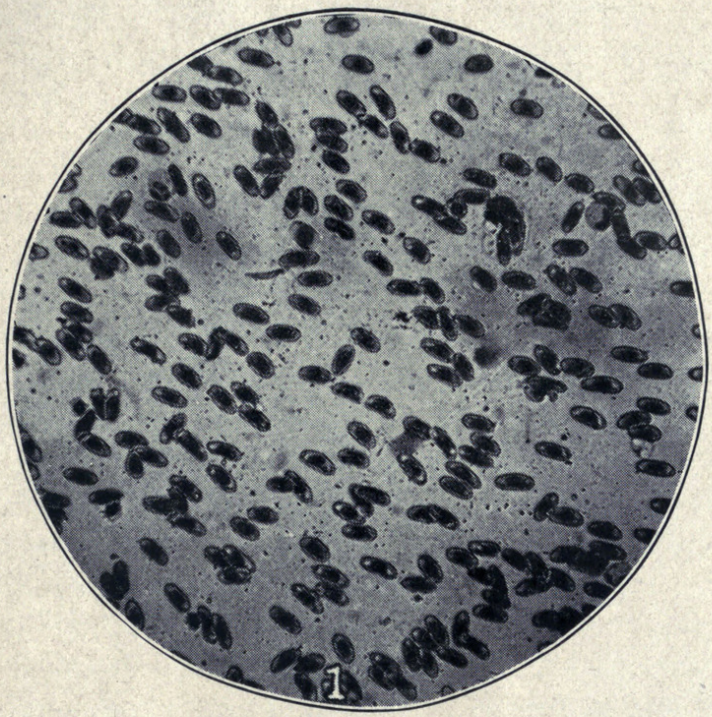

PLATE III

the tissues and eggs of infective ticks, and the undoubted developmental phases present in ticks that were infective and absent in non-infective individuals, together with its occurrence in the lesions characteristic of the disease in man, monkey, rabbit, and guinea pig are evidence of its relation to the disease even though the author was unable to cultivate it. Apart from the morphological data worked out with great care, parasitologists will find of distinct interest the discussions of the relation of the parasite and the disease to Tsutsugamushi disease, discussed in the JOURNAL for December, 1920.

NEW HUMAN PARASITE

Eimeria snijdersi Dobell 1921.—Dr. E. P. Snijders recorded (Parasitol., 12: 427-432, figs. A-D) the discovery at Medan, Sumatra, in the stool of a patient ten years in the tropics oocysts of an Eimeria differing from others previously described from man. Dobell agrees with Snijders that they represent a new species which he describes as follows: Oocyst colorless, spherical, 40-48μ in diameter. Spores fusiform, equally pointed at both ends; length 20-25μ, width in middle 7-8μ. Oocystic residue small, granular. Sporocystic residues in the form of one or two small refractile spheres. No crystalline bodies—like those of *E.oxyspora*—visible at the posterior ends of the sporozoites (Parasitol., 12: 433-436, issued Jan. 10, 1921).

NOTES

Especial attention should be directed to the report of the Committee on the Pedagogics of Medical Zoology and Parasitology published in the Proceedings of the Association of American Medical Colleges (30: 167-176). The Committee was composed of Doctors E. R. Stitt, William H. Park and A. I. Kendall, chairman. The report presents the results of a questionnaire, the analysis and critique of the committee, an ideal program, and the discussion which followed the presentation of the report at the meeting of the Association in Chicago in March, 1920.

Professor von Graff, referred to in the preceding number of the JOURNAL (7: 156), is happily not dead tho he has been compelled to retire from service and is now in a sanitarium.

ERRATA

In THE JOURNAL (September, 1920), Vol. VII, p. 16, line 3 from bottom, for *Leptomonas gracilis* read *Leptomonas bütschlii;* p. 17, line 5 from bottom, for chromated read chromatoid; p. 19, line 13, for *Herpetomonads* read *Herpetomonas;* p. 19, line 29, for artifically read artificially; p. 20, line 5, for *Lacerara* read *Lacerta;* p. 21, line 21, for occur read occurs.