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THE RELATIONSHIP OF CERTAIN "FREE-LIVING" AND SAPROPHYTIC MICROORGANISMS TO DISEASE[1]

IT seems profitable on this occasion to endeavor to take in review the progress of our knowledge of the broader outlines of biological development in respect to some of those microorganisms which at least during one stage of their existence may be referred to as free-living, saprophytic or symbiotic, and of their potentiality in a different environment to assume a parasitic existence and produce disease. A considerable growth of our knowledge of several of the microorganisms to be discussed in this connection has very recently occurred. Obviously a consideration of this nature should demand much insight into the fundamental problems which relate to the microorganisms in question, and a careful investigation not only of their morphological details but, as well, of those of a physiological and pathological nature, and of the effects of changed environment and of evolution on the microorganism. It is only through such careful observations of form, function and reaction, and by series of experimental inoculative procedures in artificial culture media, or in different animals or plants in which the microorganism is given a new environment, that we can be brought to appreciate such evolutionary changes as occur, and the relationships which exist between certain allied species morphologically indistinguishable.

Evidently the accurate determination and recognition of the species under investigation in the different environments is a primary requisite of such an investigation. This, however, particularly in the case of some of the protozoan or protistan microorganisms to be discussed, is beset with many difficulties.

There has been some difference of opinion as to whether or not some of those spirochætes which have been supposed to be purely saprophytic and to lead a free existence might, under other conditions, acquire pathogenic properties. It has been suggested that by mutation and adaptation they may in some instances be able to become transformed from harmless saprophytes into highly pathogenic parasites. Neumann[2] believes that non-parasitic saprophytic spirochætes

[1] Address of the retiring vice-president, Section N (Medical Sciences). Read at the annual meeting of the American Association for the Advancement of Science, December, 1924.

[2] Neumann, *Central. f. Bakt.*, 1923, XC, Heft 2, p. 100.

can rather quickly change in character and acquire pathogenic properties. He calls attention to the fact that two of his experimental rabbits acquired genital spirochætosis through contamination by manure which contained the microorganisms. Other observers have objected to these conclusions and believe that in Neumann's experiments in rabbits, the possibility of infection of the wounds with spirochætes from the intestine was not excluded. Moreover, Warthin, Buffington and Wanstrom[3] have recently emphasized the fact that infection with a form of spontaneous venereal spirochætosis in rabbits may occur and spread by both contact with other infected rabbits or by coition. Worms[4] has also claimed to have induced typical genital spirochætosis in rabbits by inoculating them with *Spirochæta dentium* of the normal human mouth, but it has also been objected that no proof is given that the spirochætes which persisted in the lesions were actually pathogenic to the animals, and that they might merely have survived as saprophytes.

In this connection some studies have recently been carried out with the presumably saprophytic species of spirochætes found in the human intestine. Werner, in 1909, described two types of spirochætes found in his own stool after typhoid fever. One of these he named *Spirochæta eurygyrata,* which was loosely coiled, very active and flexible, with rarely more than two spirilla, mostly as "S" forms. The other type, called *Spirochæta stenogyrata,* was tightly coiled, not so active and less flexible. It is well recognized that in apparently healthy persons spirochætes may be sometimes found in small numbers in the feces, and that in other individuals suffering with some form of colitis enormous numbers of spirochætes are sometimes encountered. In the latter instance, *Spirochæta eurygyrata* has often been regarded as the excitant factor. Le Dantec, Luger and others have believed that the intestinal spirochætes may give rise to a form of dysentery. However, the investigations of Muhlens, Macfic, Pons and others suggest that at times at least this spirochæte may exist in the intestine as a harmless saprophyte. Delamare[5] has raised the question of the intensity of infection with spirochætes in the human intestine which indicates the border-line between health and disease. From his investigations he regards from six to ten spirochætes per microscopic field as the standard to adopt. He believes that the spirochætes multiply rapidly when the intestine is in a condition favorable to their growth, such for example as exists in cholera or in amoebic dysentery.

[3] Warthin, Buffington and Wanstrom, *Jour. Infect. Dis.,* 1923, XXXII, 315.

[4] Worms, *Klin. Woch.,* 1923, II, 836.

[5] Delamare, *Bull. et Mem. Soc. Med. Hopit. de Paris,* 1924, XLVIII, 725.

Without attributing any pathogenic properties to the spirochætes in this locality, he believes their presence in large numbers is nevertheless an indication that the intestine and especially the colon is in an abnormal condition. Parr[6] has found that spirochætes can be demonstrated in about one third of the healthy persons about Chicago, though the intensity of the infection is slight. The spirochætes were localized in the cecum and ascending colon and in many cases did not appear in the feces.

Attempts to infect successfully animals with *Spirochæta eurygyrata* and similar intestinal spirochætes have not been conclusive.

Blanchard[7] is reported to have introduced the exudate from the false membrane of a case of Vincent's angina into the digestive tube of a dog and produced a dysenteriform state in the animal in which both spirochætes and fusiform bacilli were recovered from the stools. However, Tanon, who studied the effects of subcutaneous, intravenous and intraperitoneal injections of spirochætal intestinal material into guinea pigs, rabbits and monkeys, only obtained negative results.

Teissier and Richet[8] also fed rabbits and guinea pigs fecal suspensions rich in spirochætes and obtained only negative results. When the material was injected subcutaneously, abscesses were formed, but they contained no spirochætes. When intraperitoneal injections were made into guinea pigs, the peritoneal cavity was found to be subsequently rich in spirochætes, but when some of the fluid was injected into a second series of guinea pigs, no spirochætes were obtained.

Hassenforder[9] reported positive results only when the fecal suspensions containing spirochætes were associated with virulent amoebae and injected intrarectally into cats.

Hogue[10] fed three cats 6 cc of a culture of *Spirochæta eurygyrata,* but no spirochætes were subsequently found in the stools.

Parr[11] injected feces containing spirochætes intraperitoneally into six guinea pigs, and in no case was an abdominal exudate found containing spirochætes. Intratesticular injections in rabbits were also negative. The exact nature of the material injected and the approximate number of spirochætes in it is not stated. Apparently the fusiform bacillus was not present.

[6] Parr, *Jour. Infect. Dis.,* 1923, XXXIII, 369.

[7] Blanchard, cited by Hassenforder, Thesis, Lyons, 1914.

[8] Teissier and Richet, *Bull. et Mem. Soc. Med. Hopit. de Paris,* 1911, XXXI, 775.

[9] Hassenforder, Thesis, Lyons, 1914.

[10] Hogue, *Jour. Exper. Med.,* 1922, XXXVI, 617.

[11] Parr, *Jour. Infect. Dis.,* 1923, XXXIII, 379.

Broughton-Alcock,[12] however, attaches a definite etiological significance to *Spirochæta eurygyrata* in certain chronic and intermittent cases of dysentery, and he found that the organism occurs in great numbers in the mucus passed in the acute and subacute stage of such cases when no other microorganism is present to account for the pathological condition. His experience has led him to believe that *Spirochæta eurygyrata* can produce in human beings a catarrhal condition of the intestine with the passage of mucus containing shed degenerated epithelial cells, occasionally red blood cells, and, rarely, typical dysenteric symptoms. He adds that there is always the argument that a primary agent has produced a vulnerable surface over which the organism acts symbiotically. The idea of the spirochæte having acquired pathogenic properties or an increased virulence in this condition is not suggested, but he believes that the spirochæte of somewhat similar but not identical form found in the normal feces is *Spirochæta stenogyrata,* which is non-pathogenic, *Spirochæta eurygyrata* being found only in the mucus and not in the feces. Attempts to infect mice with *Spirochæta eurygyrata* were unsuccessful. Davis and Pilot[13] believe that some cases of gangrenous appendicitis appear to arise from the fusiform bacilli and spirochætes of the intestine which have acquired a new virulence.

The relationship to bronchial spirochætosis of *Spirochæta bronchialis* (morphologically similar to *Spirochæta vincenti, Spirochæta schaudinni,* and *Spirochæta refringens*) raises the question of whether there is an acquired pathogenesis for this species in this condition. The recent investigations of Pons[14] further substantiate the view that the spirochætes in the sputum in bronchial spirochætosis are really similar to those described as occurring in the human mouth by many observers and that possibly they may have found in this pathological condition in the bronchi a suitable medium for further development. Perhaps such a favorable medium also occurs in tuberculosis and in certain other pathological conditions of the lung. Trocello has expressed the view that the oral spirochætes can extend directly to the bronchi. Pons studied nine cases of bronchial spirochætosis and came to the conclusion that it was impossible to confirm deMello's[15] observations that the bronchial spirochætes are distinct from those of the mouth. He was not able to differentiate *Spirochæta buccalis* from *Spirochæta bronchialis.* He was also not able to confirm such statements as those which affirm that the oral forms are less motile and retain their motility longer than the bronchial forms, and do not produce the coccoid bodies to the same extent. He however did observe a rapid loss of motility in the bronchial forms. Attempts to reproduce the disease bronchial spirochætosis by intratracheal inoculation of normal rabbits failed. He however does not believe that the spirochætes encountered in pathological conditions of the lung are purely saprophytic but rather that they afford evidence of an abnormal condition due to varying causes, and that they are able to give to these conditions characteristics such as chronicity and ulceration which one is accustomed to associate with the occurrence of spirochætes in a lesion.

Obviously, still further investigation is desirable upon the question of the pathogenicity under some circumstances and under the influence of some possibly symbiotic microorganism or bacteriophage, of *Spirochæta bronchialis* and the morphologically similar forms.

In the mouth and from the genital organs of some individuals spirochætes have been observed living apparently as harmless saprophytes which are morphologically indistinguishable from some of the well-recognized pathogenic species. These organisms have been found in association with fusiform gram-negative bacilli not only in these situations but particularly in the lesions about carious teeth and in gangrenous putrid infections about the mouth. Broughton-Alcock[16] has found spirochætes with other bacteria in a catarrhal exudate from the antrum, and Tunnicliff has observed them in a frontal sinus. Davis and Pilot[13] have recently emphasized the importance of the occurrence of spirochætes and fusiform bacilli not only in Vincent's angina but in ulceromembranous stomatitis, noma, putrid otitis media, putrid bronchitis and gangrenous pneumonia. They believe that such conditions are usually caused by these organisms which presumably come from the mouth or tonsils or both. They also conclude that the several gangrenous processes that occur at times about the male and female genitals presumably result from invasion by these organisms that occur normally there. They inoculated material containing fusiform bacilli and spirochætes and pyogenic cocci from teeth, tonsils, smegma and putrid sputum, both intrapleurally and subcutaneously into rabbits, and obtained putrid and gangrenous lesions containing these organisms. The cocci, especially streptococci, were found to be the most aggressive organisms and sometimes alone invaded the adjacent cavities and the

[12] Broughton-Alcock, Proc. Roy. Soc. Med., 1923, XVI, Pt. 3, p. 46.

[13] Davis and Pilot, Collected Studies from the Dept. of Pathology and Bacteriology, Univ. of Illinois, Chicago, 1922–1923, p. 27.

[14] Pons, *Bull. Soc. Path. Exot.,* 1924, XVII, 170.

[15] deMello, *Bol. Geral. Med. e Farmacia,* Bastora, 1924, Feb. 9th series, p. 46.

[16] Broughton-Alcock, Trans. Roy. Soc. Trop. Med. & Hyg., 1923, XVII, 337.

blood stream. The fusiform and spirochætal organisms tended to remain more locally, causing necrosis and gangrene in the already invaded tissues. Predisposing factors are considered usually as of first importance in determining the development of the microorganisms, and they believe that at times they may, like bacteria, develop a degree of virulence sufficient to enable them to gain a foothold in the normal tissue.

The free-living spirochætes of water have generally been considered as saprophytic organisms. They have been found in fresh and in marine water, often more particularly when the water is stagnant and when through the decomposition of protein in the presence of ammonia, nitrites and nitrates, hydrogen sulphide gas is freely generated. They are also often found on the surface of filters, about the apertures of water taps, and on the undersurface of metal closure caps of certain bottled drinking waters in northern Brazil. A number of these spirochætes correspond morphologically with the parasitic species, such as *Treponema pallidum* of syphilis, *Leptospira icterochæmorrhagiae* of Weil's disease, *Spirillum obermeieri* of relapsing fever, and *Spirochæta hebdomadis* of seven-day fever. We have referred to the fact that in the mouth and in the genitals of some healthy human beings there occur spirochætes which are morphologically indistinguishable from certain of the well-known pathogenic species such, for example, as some of those just enumerated, but several of these spirochætes from the mouth and genitals are also morphologically indistinguishable from some of the spirochætes found recently in water. Evidently, therefore, morphological resemblances alone are entirely insufficient for us longer to attempt to differentiate spirochætes generally into different species, nor are they obviously sufficient for us to establish the identity of a number of these water spirochætes with some of the species known to cause disease. Animal inoculations in some instances, however, have furnished additional evidence in this respect.

Spirochæta schaudinni, morphologically identical with *Spirochæta vincenti* and *Spirochæta refringens,* has been encountered by many observers in open ulcers of the skin.

Very often the spirochætes in such lesions have been found associated with fusiform bacillary forms. These organisms in certain localities and lesions are practically always associated. By some observers they are regarded as distinct organisms, perhaps living symbiotically, while by others the fusiform bacilli and spirochætes are believed to be merely different forms in the life-cycle of one organism.

Ruth Tunnicliff[17] holds the latter view. She admits, however, that there are several different strains of fusiform bacilli.

In portions of northern and central Brazil, chronic ulcerative processes of the skin are exceedingly common, and in one form of tropical ulcer, *Spirochæta vincenti* and fusiform bacilli are invariably present and apparently constitute the most important etiological factor in this particular form of ulcer. The spirochætes and fusiform bacilli are found in abundance not only on the surface of the lesions, but usually extending for at least several millimeters into the tissue surrounding the ulcer. Numerous cocci and other bacilli are also usually encountered in the exudate upon the surface of the ulcers. Small pieces of tissue were removed from a number of these ulcers, and after they had been thoroughly rinsed in sterile normal saline solution were ground up in a mortar, resuspended in other saline solution, and the suspension injected subcutaneously into monkeys and also intratesticularly, after injury, into rabbits. Suppurative and ulcerative lesions were produced in these animals thereby, in which both spirochætes and fusiform bacilli as well as cocci were found present.[18]

It seems probable that *Spirochæta vincenti* can not usually establish itself in healthy skin or even in many aseptic wounds, but if the integument is bruised, burned or otherwise injured and the circulation interfered with and the vitality of the tissues otherwise impaired, it may often assume pathogenic properties and a phagedenic ulceration result. The recent observations of Van Nitsen[19] are also in accord with this view.

In view of the fact that there are in some waters spirochætes which are morphologically indistinct, or very similar to *Spirochæta vincenti,* experiments were undertaken to see if these spirochætes were also pathogenic for animals. In Manaos various samples of stagnant water and scrapings from the surface of filters and apertures of water taps and under-surface of caps of mineral water bottles were suspended in saline solution, were centrifuged and the sediments which contained spirochætes resuspended in saline solution and injected subcutaneously and intraperitoneally into mice and guinea pigs and subcutaneously into monkeys.[18]

Only negative results were obtained. The animals remained healthy, and we were entirely unable to produce any lesions such as had been done with the material obtained from the tropical ulcers. Our experiments with the spirochætes from tropical waters,

[17] Tunnicliff, *Jour. Infect. Dis.*, 1923, XXXIII, 147.

[18] These experiments were undertaken in conjunction with Dr. George C. Shattuck and Mr. Ralph Wheeler, and will be reported upon in detail elsewhere.

[19] Van Nitsen, *Annales Soc. Belge de Med. Trop.*, 1924, III, 317.

however, are far from being complete and are not conclusive, since the spirochætes employed in our inoculations were never obtained and injected in large numbers. On my return to this country I found that Noguchi[20] had studied some of the spirochætes which he had isolated from more or less stagnant ponds, swamps and ditches in the northern United States, and had previously reported on this study. He obtained growth of the water leptospiras in impure culture on his regular leptospira media, though with considerable difficulty. Inoculations of the leptospira water samples into guinea pigs, white rats and mice were repeatedly made, but no infection could be induced in the animals. His injections of cultures likewise proved to be harmless. The kidneys and liver of the inoculated rats were removed after three weeks and suspensions of these organs injected into guinea pigs with the hope that passage through rats might have enhanced the virulence of the organisms, but no positive results were obtained. Noguchi has concluded that the water leptospiras which he studied appeared to be non-pathogenic for guinea pigs as well as rats.

On the other hand, Uhlenhuth and Zuelzer[21] have isolated by culture from aqueduct water a spirochæte which subsequently acquired distinctly pathogenic properties for animals. This spirochæte in doses of 2 to 4 cc of the culture, when injected intraperitoneally, produced in guinea pigs a disease which after four to eight days caused death. The entire appearance in the animals so infected corresponded with that of Weil's disease. This water spirochæte was also pathogenic for mice. Zuelzer regards this spirochæte as identical with the one which produces human Weil's disease. The cultures were made in sterile tap water to which was added 15 to 20 per cent. of rabbit serum. Before being inoculated the tubes were warmed for an hour at from 55° C. to 60° C., in order to inactivate the serum. By frequent inoculation from the surface of such culture the spirochætal growth was increased. The culture of the spirochæte obtained was not pure, but was mixed with a coccus, but the coccus when injected alone into the animal produced no pathogenic changes. It is reported that with two of the water strains isolated, a potent immune serum against *Spirochæta icterohaemorrhagiae* was made.

From these experiments and others of a similar nature, Zuelzer and Oba[22] conclude that non-parasitic, saprophytic spirochætes may become under certain conditions pathogenic, but that such changes come about very slowly. Thus the strain of *Leptospira icterohaemorrhagiae,* which was isolated from water, it is stated only became pathogenic for guinea pigs after it had been cultivated in serum media for one and one fourth years. Elaborate serological and biological experiments were carried out which demonstrated the identity of this strain with the natural pathogenic strain of *Leptospira icterohaemorrhagiae* of Weil's disease.[23] This is the most striking example reported of a free-living, saprophytic spirochæte which has gradually acquired definite pathogenic properties.

The subjects of symbiosis and mutualism and their relationship to parasitism because of their broad biological interest should appeal not only to the physiologist and pathologist, but to the zoologist as well. These phenomena which have so frequently been observed among both animals and plants may affect profoundly their structure and behavior, or even their existence. The term "symbiosis," which has sometimes been misapplied in medical literature, signifies a condition of conjoint life existing between different organisms of varied or even the same species, both organisms being benefited by the partnership. Obviously a condition of perfect symbiosis with different species is very rarely if ever realized either in plants or animals, since such a condition would require that each of the organisms should render to the other an exact equivalent of what it received, and that the organisms in question should be entirely adapted to a life in common. This ideal balance, even if attained, becomes frequently disturbed, and in some groups of animals sooner or later one organism becomes more dependent upon the other and the symbiotic relationship passes into one of parasitism, in which the degree of adaptation may vary greatly. Thus, the new condition may approach that of symbiosis and a mild parasitism result, or it may pass to the other extreme, one organism assuming highly pathogenic properties for the other and perhaps causing its destruction. Again, certain forms of parasites do not nourish themselves on any part of the host. They belong to the group of commensals or more correctly, according to Fantham and Stephens, of "space parasites" which simply dwell within their host and do not even take a portion of the superfluity of its food. However, the presence of such "space parasites" may be regarded as constituting the first stage of commensalism which, in

[20] Noguchi, *New York State Jour. Med.,* 1922, XXII, 426.

[21] Uhlenhuth and Zuelzer, *Central. f. Bakt. u. Parasit.,* 1922–23, Orig., LXXXIX, 171.

[22] Zuelzer and Oba, *Central. f. Bakt. Abt. I,* Orig., 1923–24, XCI, 95.

[23] Buchanan has since isolated a similar microorganism from mud and slime. See *British Med. Jour.,* Nov. 29, 1924, p. 990.

turn, may again be regarded as a sort of transition stage to true parasitism.

On the other hand, in some instances it is conceivable that the symbiosis originates through a preliminary stage of parasitism on the part of one of the organisms, the struggle for supremacy ending in mutual adaptation. In either instance it is evident that there is no definite boundary between symbiosis and parasitism. As Nuttall[24] has pointed out, the factors governing immunity from symbionts or parasites are essentially the same. The condition of life defined as symbiosis may then be regarded as balancing between two extremities, complete immunity on the one hand and deadly infective disease on the other.

Among insects there are found innumerable examples of such progressive adaptations toward an association with microorganisms of different type. Nuttall has classified symbiosis in insects into four groups: (1) the utilization by insects of microorganisms cultivated by them outside their bodies; (2) symbiotic organisms developing in the lumen of the intestine and its adnexa; (3) intestinal symbionts situated in the epithelial cells of the digestive apparatus; and (4) intracellular symbionts of deep tissues. The symbionts may be bacteria, yeasts, saccharomycetes, protozoa or rickettsia. Various hypotheses have been advanced to explain the possible function of the symbionts, but our knowledge of this subject is still very meager. Even the classification of these microorganisms is sometimes difficult. Thus Buchner[25] has found symbionts in *Cimex lectularius* which live in large cells called mycetocytes or within a new symbiotic organ, the mycetome. These microorganisms he regards as bacteria. On the other hand, Arkwright, Atkin and Bacot[26] have described a similar microorganism in *Cimex* under the term *Rickettsia lectularia.* Also Jungmann[27] and Arkwright and Bacot[28] have described in the sheep louse, as stages of *Rickettsiae,* certain forms which are regarded by Hertig and Wolbach[29] as yeasts or "yeast-like" organisms.

The recent observations of Hertig and Wolbach (*loc. cit.*) only serve to emphasize how much confusion there still exists regarding the forms of life so widely distributed throughout the arthropods and which have recently been referred to under this term of *"Rickettsiae."* They also demonstrate the great difficulties in the recognition of *Rickettsia,* some observers regarding the forms described under this term as yeasts, bacteria, symbionts or even as degenerated cell products or flagella of protozoa. While the cultivation of some of the pathogenic species of *Rickettsia* has been described, until more satisfactory methods of culture are discovered and direct inoculations made into man or susceptible animals with these pure cultures, even the etiological relationship of at least most of these forms to disease must still remain somewhat obscure.

As the writer pointed out some years ago[30] certain of the *Rickettsia* of *Pediculi* which are harmless to man, as was demonstrated by experiments in which such *Pediculi* were fed on healthy human beings, can not be distinguished morphologically or by any other known means from the *Rickettsia* believed by some observers to be the cause of disease (trench fever). However, there is no evidence of any of these symbionts being pathogenic for the arthropod, and among the *Rickettsiae* only *Rickettsia prowazeki* exhibits moderate pathogenicity for its insect host, while the *Rickettsia* described as the cause of Rocky Mountain spotted fever would appear to be a harmless parasite rather than a symbiont to its insect host, the tick (*Dermacentor venustus*). Wolbach believes that this *Rickettsia* is probably parasitic in process of adaptation to the tick. The view recently expressed by Weigl[31] that *Rickettsia prowazeki* is merely the form assumed by *Bacillus proteus X* in the louse seems hardly tenable.

As no one has succeeded yet in cultivating on artificial media the symbionts of blood-sucking insects, their true nature is also still undetermined. Florence[32] has recently studied the symbiont in the hog louse, *Haematopinus suis,* and has been able to obtain some evidence in favor of the view that it is connected with the physiology of digestion in the insect in that the location of the mycetocytes containing the symbiont is in the mid-intestine where digestion takes place; that the mechanical control of the increase of the symbiont is through the rupture of the mycetocytes; that there is careful provision for transmission of the symbiont to the next generation and an inability to raise a second generation of it when the lice are removed from their natural host and fed on man.

24 Nuttall, Rep. Brit. Assoc. for Adv. Sc., 1923, p. 197.

25 Buchner, "Tier. u. Pflange in intracellular Symbiose," Berlin, 1921, and *Arch. f. Protist.*, 1923, XLVI, 225.

26 Arkwright, Atkin and Bacot, *Parasitology,* 1921, XIII, 27.

27 Jungmann, *Deut. med. Woch.*, 1918, XLIV, 1346.

28 Arkwright and Bacot, Trans. Roy. Soc. Trop. Med. & Hyg., 1921–22, XV, 146.

29 Hertig and Wolbach, *Jour. Med. Research,* 1924, XLIV, 329.

30 Strong, Contributions to Med. and Biol. Research dedicated to Sir Wm. Osler, 1919, p. 1205.

31 Weigl, *Zeit. f. Hyg. u. Infekt.*, 1923, XCIX, 3, p. 308; *Klin. Wochen.*, 1924, Aug. 26, III, No. 35, p. 1590.

32 Florence, *Amer. Jour. Trop. Med.*, 1924, IV, 397.

Wheeler[33] has recently emphasized the importance of the study of the phenomenon of symbiosis and parasitism in many species of ants and termites, and of the importance in the lives of certain termites of the existence of the numerous intestinal infusoria or flagellates. These occur only in the soldiers and workers of the termites and have been variously interpreted as parasites, commensals and symbionts. The recent investigations of Imms[34] and of Cleveland[35] are confirmatory of the idea that these protozoa of termites are true symbionts which break down the particles of wood ingested for food and render them more easily assimilable by the termites. The termites themselves, as Cleveland has shown, die in ten to twenty days if fed on wood after the protozoa have been removed from them because they can not digest their food. On the other hand, the termites do not die but live indefinitely when fed digested wood or when reinfected with protozoa and fed wood. Hence he believes that it is highly probable that the termites are dependent on the protozoa to digest thir food for them.

Buscalioni and Comes state that *Trichonympha agilis,* harbored by *Reticulitermes lucifugus,* when treated with iodine dissolved in iodide of potassium, gives a characteristic glycogen reaction in a region near the nucleus, and that this reacting region is sharply defined from the rest of the body. Cutler[36] could not locate a definite glycogen reacting area in *Pseudotrichonympha pristina,* harbored by *Archotermopsis wroughtoni,* but, on the contrary, found that the glycogen reaction was diffused through the entire organism. According to Certes glycogen is present in the protoplasm of the infusoria and the latter perform a special rôle in the digestion process of certain ruminants.

In the study of parasites of termites in Brazil, numerous gregarines, flagellates and spirochætes were found in great abundance in the intestine. While no information was obtained as to whether these microorganisms lived symbiotically within their host, none of them seemed to affect unfavorably the life of the termites and apparently they led therein a saprophytic existence. The spirochætes observed were particularly of two types: In the first type, the organism measured from 65 to 75 μ in length and from 1 to 1½ μ in thickness. The ends were rounded. They showed seven to eight spiral turns. The second type measured from about 40 to 50 μ in length, and about 1 μ in thickness, the extremities being tapering and pointed. These spirochætes seemed more motile than those of the first type. They also had from seven to eight spiral turns. These two types observed in termites in Brazil probably correspond to *Treponema termitis* (Leidy) and *Treponema minei* (Prowazek) which have recently been described particularly by Hollande.[37]

In continuance of our experiments with saprophytic spirochætes, inoculations of suspensions in normal saline solution of portions of the intestinal contents of termites containing spirochætes were also made into white mice and guinea pigs.[38] The inoculations were made both subcutaneously and intraperitoneally.

In the experiments in which the inoculations were made intraperitoneally into guinea pigs, drops of fluid were often withdrawn from the abdominal cavity by means of a capillary glass pipette about two hours after the inoculation, and examined under the microscope and with the dark field illumination, but no living spirochætes were found and no evidence of the pathogenicity of these spirochætes was obtained from either the intraperitoneal or subcutaneous inoculations into mice and guinea pigs.

Turning to more highly organized forms of protozoa, the majority of the recent experiments reported, in which subcutaneous or intraperitoneal inoculation of laboratory animals such as mice and guinea pigs have been made with the flagellates which are found in insects, and which live an apparently saprophytic existence in them, have generally resulted negatively. Laveran and Franchini[39] reported the successful infection of mice by causing them to ingest material containing *Herpetomonas ctenocephali* or by inoculating them subcutaneously with this flagellate, and Fantham and Porter[40] reported that young mice may be inoculated subcutaneously or fed with *Herpetomonas jaculum* from the gut of the hemipteron *Nepa cinerea,* the so-called water scorpion, with fatal results. On the other hand, Hoare[41] was unable to infect fifteen mice by intraperitoneal inoculation of *Herpetomonas jaculum.* Chatton[42] also inoculated a suspension of thirty dog fleas containing *Herpetomonas ctenocephali* intraperitoneally into two mice, but the results were negative. Noller[43] likewise only obtained negative results in the inoculation of mice with pure

[33] Wheeler, "Social Life among Insects," New York, 1923.

[34] Imms, Proc. Roy. Soc., London, 1919, 209B, p. 75.

[35] Cleveland, *Biol. Bull.,* 1924, XLVI, 179.

[36] Cutler, *Quarterly Jour. Micro. Sci.,* 1921, LXV, 247.

[37] Hollande, *Arc. Zool. Exp. et. Gen.,* 1922, LXI, 23.

[38] These experiments were undertaken in conjunction with Dr. Joseph Bequaert and Mr. Ralph Wheeler.

[39] Laveran and Franchini, *Bull. Soc. Path. Exot.,* 1914, VII, 605.

[40] Fantham and Porter, Proc. Camb. Philosophical Soc., 1915, XVIII, 39.

[41] Hoare, *Parasitology,* 1921, XIII, 67.

[42] Chatton, *Bull. Soc. Path. Exot.,* 1919, XII, 313.

[43] Noller, *Arch. f. Schiffs. u. Tropen.,* 1920, XXIV, 168.

cultures of *Herpetomonas ctenocephali*. Recently Glaser[44] and Becker[45] were also unable to infect animals by subcutaneous or intraperitoneal inoculation of other species of flagellates; namely, *Herpetomonas muscae-domesticae* and *Crithidia gerridis*. Shortt[46] in extensive experiments has, in addition, failed to infect vertebrates with *Herpetomonas ctenocephali* and *Herpetomonas luciliae*. During the past year, however, two successful reports of the inoculation of mice with other flagellates of insects have been made. The first of these results was obtained by Shortt and his coworker Swaminath,[47] whose negative results with other flagellates of insects have just been referred to, and these positive results are therefore more striking. Bedbugs (*Cimex hemiptera*) were fed on cases of kala azar which showed parasites in the peripheral blood. They were dissected nine days later and the contents of the guts were suspended in saline solution and injected intraperitoneally into mice. The minimum period of nine days was chosen, as it was presumed that at the ninth day all the forms present would be in the flagellate condition. Of five mice which were inoculated with the suspension of twelve to twenty of the insects' intestines, one gave a positive result on the 123rd day, a culture of the flagellate being obtained from the spleen. Shortt concludes that it is thus demonstrated that nine days after feeding bedbugs on a kala azar case, the intestine of these insects may contain flagellate of leishmania which are infective to mice.

Gaminara[48] found the Uruguayan *Triatoma* to be naturally infected with intestinal flagellates corresponding morphologically to the developmental forms of *Schizotrypanum cruzi* in the insect. Inoculations of experimental animals with these flagellates resulted in their infection. The infected animals showed in their cardiac and skeletal muscles the typical leishmania-like forms of the parasite.

During the previous year the writer[49] has shown that a flagellate which passes one stage of its life-cycle in *Euphorbiae* and which is not pathogenic for laboratory animals when inoculated from such an environment may, after its passage from the plant through its insect and vertebrate hosts, acquire pathogenic properties for monkeys. This is apparently another striking example of a microorganism which in its adaptation to changed environment gradually acquires pathogenic properties. The effect of environment upon species in all forms of life both in suppressing or bringing inherent potentialities to expression seems unquestionable, and either physiological or morphological changes may be developed in a parasite as a result of exposure to altered environment.

Even with lower forms of life, the bacteria, we are cognizant of examples of somewhat similar phenomenon. Thus Courmont and Rochaix[50] have found, as have other investigators previously, that by frequent subculture of the four types of bacillary dysentery bacilli on the same sugar media, they come in time to acquire the power of splitting that particular carbohydrate. One generation of organisms can be trained to ferment a sugar to which it is unaccustomed. While this property lasted usually only for that generation and could not be transmitted to the next, there were certain exceptions to the rule.

D'Herelle[51] reported that under the influence of his bacteriophage it was possible to produce secondary races from the original Shiga strain of the dysentery bacillus which differed mainly in their power of fermenting certain sugars.

Busson and Ogata[52] have very recently reported experiments, using eleven strains, of Shiga, Strong, Flexner and Schmitz types of dysentery bacilli and three bacteriophages. By injecting rabbits with a mixture of original strains which had been acted upon in a typical maner by the bacteriophage, agglutinating sera were prepared. The sera thus prepared were tested against the original and secondary strains. By these means it was shown that the secondary races were no longer agglutinated by the original serum. The authors believe that the secondary races are divisible into two types: those which permanently retain the newly acquired properties and those which do not.

Fejgin[53] also found that the dysentery bacillus under the influence of its lytic bacteriophage substance produced a new race differing both in serological and biochemical properties from the original strain of Shiga's bacillus.

Reference has been made to the fact that animals even as high in the scale of life as insects find it difficult or impossible to digest crude cellulose. In this connection Imms[54] has pointed out that the symbiosis between the intestinal protozoa and the termites is paralleled by the occurrence of numerous genera of

44 Glaser, *Jour. Parasitol.*, 1922, VII, 99.

45 Becker, *Amer. Jour. Hyg.*, 1923, III, 462.

46 Shortt, *Indian Jour. Med. Res.*, 1923, X, 908.

47 Shortt and Swaminath, *Indian Jour. Med. Res.*, 1924, XI. 965.

48 Gaminara, Annales de la Fac. de Med. Montevideo, 1923, VIII, 311.

49 Strong, *Amer. Jour. Trop. Med.*, 1924, IV, 345.

50 Courmont and Rochaix, *Jour. Physiol. et Path. Generale*, 1924, XXII, 377.

51 D'Herelle, "Le Bactériophage," Monographies de l'Institut Pasteur, 1921.

52 Busson and Ogata, *Wien. klin. Woch.*, 1924, XXXVII, 665.

53 Fejgin, *Compt. Rend. Soc. Biol.*, 1923, LXXXIX, 1381.

54 Imms, Philosophical Tr. Roy. Soc. London, 1919, 209B, pp. 75–180.

infusoria in the stomachs of ruminants, notably of the ox, sheep, goat, camel and reindeer. It is believed that by means of their action upon the vegetable matter consumed by the ruminants these infusoria help to render it capable of being digested by the latter. These infusoria are absent from the stomachs of the young ruminants prior to being weaned from their parents. According to Certes, glycogen is present in the protoplasm of the infusoria and the latter perform a special rôle in the digestive process of the ruminants. Cruby and Delafond maintained that the protoplasm of the infusoria is itself digested and thereby contributes towards the nutrition of the host ruminant. Similarly, the infusoria inhabiting the large intestine of the Equidae, Imms states, are possibly symbiotic in their relations with their host. Whether a true symbiosis exists in a number of these instances seems questionable. It is true that the ciliated infusorium, *Balantidium coli,* lives commonly in the normal intestine of pigs apparently as a harmless commensal. There is no evidence in favor of or contrary to the fact that it lives in symbiosis with its host. Glaessner has reported the isolation of a diastase and a hemolysin from this infusorium, but no proteolytic ferment has been yet obtained. It is also not known that it plays any special rôle in the digestive process of the pig. However, when this infusorium enters and lives for a time in the large intestine of man or in orangutans, under certain conditions which we can not fully explain, it gradually assumes pathogenic properties and invades the tissue, giving rise to an ulcerative form of dysentery[55] which, especially in orangutans, may result fatally.

The majority of protozoologists who have studied the question have come to believe that the amoebae which are found living in certain of the water supplies of tropical and subtropical countries are saprophytic and of the free-living type.[56] Dobell[57] has insisted that the dysentery amoeba (*Entamoeba histolytica*) can not live and multiply outside of its human host and that it must always live at the expense of its host's tissues.

However, the recent investigations of Cutler[58] and particularly of Boeck,[59] who have both been able to cultivate *Entamoeba histolytica* in artificial media, the latter in one consisting of Ringer's solution, serum and egg media, obviously refute these assertions by Dobell that *Entomoeba histolytica* can not live and multiply outside of the human intestine.

As early as 1904 Musgrave[60] reported experiments in which cultures of saprophytic amoebae from hydrant water which were made upon a media containing merely agar, sodium chloride and extract of beef were fed to monkeys and produced amoebic dysentery in three of these animals. Another culture made on this same medium which was obtained from lettuce after the fourth washing in distilled water was fed to a monkey and produced amoebic dysentery in this animal, while another culture of the same amoeba when injected into the liver of a monkey produced amoebic liver abscess.

Franchini[61] has recently reported the production of liver abscess in two white mice out of a series of nine which were inoculated intraperitoneally with a culture of an amoeba isolated from latex of *Euphorbia.* However, these results have not been confirmed.

The cultures of *Entamoeba histolytica* obtained by Cutler and Boeck (*loc. cit.*) contained amoebae associated with bacteria. Musgrave, Lesage, Gauducheau[62] and others have also cultivated amoebae together with bacteria. All these amoebae were considered by the authors who reported the observations to be pathogenic under certain circumstances. It has often been suggested that bacteria are necessary for the growth and multiplication of amoebae and in addition that a symbiotic relationship exists between the amoebae and associated bacteria. Such a symbiosis, however, has not yet been satisfactorily demonstrated. However, it is well known that amoebae may exist in the intestine of man for long periods and, under certain circumstances, produce no lesions and no symptoms of intestinal disturbance, while under other circumstances, in association with these same amoebae, symptoms of dysentery and ulcerative lesions of the intestine result. Whether such a change in pathogenesis is inherent in or is developed in the amoebae is unknown. Possibly the presence and action of some lytic substance in the amoebae, but perhaps even formed by the host, and either acting primarily upon the amoebae or upon the intestinal walls may give rise to the production of the dysenteric symptoms and ulcerations of the intestine. Could a bacteriophagic agent be responsible for and exert such changes? Gauducheau believes that *Endolymax phagocytoides* is a parasitic species of the intestine of man and constitutes a form of passage between the *Entamoebae*

55 Strong, "The clinical and pathological significance of B. coli." Bureau of Govt. Lab. Bull. No. 26, Manila, Dec. 1904.

56 Strong, Billings—Forchheimer's Therapeusis of Internal Diseases, N. Y., 1924, III, 428.

57 Dobell, "The Amoebae Living in Man," London, 1919.

58 Cutler, *Jour. Path. & Bact.*, 1918, XXII, 22.

59 Boeck, Proc. Amer. Soc. Trop. Med., 1924.

60 Musgrave, Publications of Biol. Lab. Bureau of Sc., 1904.

61 Franchini, *Bull. de la Soc. Path. Exot.*, 1923, XVI, 162.

62 Gauducheau, *Bull. de la Soc. Path. Exot.*, 1922, XV, 229.

which are culturable with difficulty and the free-living amoebae.

Turning for a moment to organisms still more highly organized in the scale of life, we find many striking examples in the helminths of the efforts on the part of nature to perpetuate her species through the establishment of parasitism in which alternating and differently formed generations and cycles of development in intermediate hosts have resulted. From many of these examples it seems natural to conclude that the development of parasitism as well as of a change of host have been gradual transitions. Moniez[63] believes that all entozoa may be traced from saprophytes only a few of which have been able to settle directly in the intestine and there continue their development; these are forms such as *Trichocephalus, Ascaris* and *Oxyuris* which still lack an intermediate host. However, in many other cases the embryos consisted of such saprophytes as were in other respects suitable to become parasites, but were incapable of resisting the mechanical and chemical influences of the intestinal contents. They were, therefore, obliged, if they were to continue to exist, to leave the intestine and they accomplished this by penetrating the intestinal walls and burrowing into the tissues of their animal carriers. Later, by the ingestion for food, by beasts of prey, of some of these carriers, they passively reached the intestine of their new host and there, having become more capable of resistance, attained their maturity. By means of these incidental coincidences of various favorable circumstances these processes of parasitism, according to Moniez, have been gradually established. In the nematodes there are numerous examples of free-living members from which it seems probable that the parasitic species may be descended. Such examples are witnessed in *Leptodera, Rhabdonema* and *Strongyloides*. These mostly, if not exclusively, spend their lives in places where decomposing organic substances are present. Some species attain maturity only in such localities. Should the favorable conditions for feeding be changed, the animals may seek out other localities. It is understandable that such forms are very likely to adopt a parasitic manner of life which at first is facultative, as in *Leptodera* and *Anguillula,* but may be regarded as the transition to true parasitism. In many forms the young stages live free for some time, as in *Strongyloides;* in others, as in the case of *Rhabdonema,* parasitic and free-living generations alternate. A most striking example of a free-living (*Rhabditis*) generation passed in the soil, and a parasitic strongyloid, one which occurs in the intestine of man, is seen in *Strongyloides intestinalis*.[64] Infection of man occurs through penetration of the filariform larvae through the skin, in the manner of hookworm larvae, the embryo migrating through the lungs before becoming parasitic in the intestinal wall.

The genus *Aphelenchus* is of particular interest in that at least six of the species are recognized as pathogenic for plants—some causing serious agricultural pests. The genus comprises between thirty and forty species, the majority of which are free-living forms occurring in association with the roots of plants, in moist humus or in water. The two species known to be of most agricultural importance are *Aphelenchus fragariae,* Ritzema-Bos, 1891, and *Aphelenchus cocophilus,* Cobb, 1919. The former is endoparasitic in the stems of strawberry plants, where it causes hypertrophy and the production of "cauliflower" disease, or it may be ectoparasitic in the buds of the strawberry causing the "red plant disease." The latter (*Aphelenchus cocophilus*) occurs endoparasitically in the stem, leaf, and roots of the cocoanut palm, particularly in the West Indies and portions of Central America. "Red ring disease" of the cocoanut palm, also previously known as "Trinidad root disease," has been studied recently particularly by Nowell,[65] Cobb[66] and Zetek.[67] The diseased trees show a progressive yellowing and browning of the leaves commencing at the leaf-tip; the nuts are shed slightly in advance of the discoloration of the leaves and in a green condition, and this may be the first external evidence of the affection. On section the stem shows a well-marked complete ring of reddish-brown tissue, usually from one to one and a half inches in width and lying about from one to two inches from the periphery of the stalk. The diseased tissue may extend up the stem for several feet and then become broken into longitudinal streaks and irregular small patches. Leaf stalks may also show these same pathological changes. The roots become affected in the cortex, first undergoing yellowish or pinkish discoloration and softening, later becoming brownish red and sometimes dry and flaky. Infection experiments have been conducted by Nowell, Cobb and Zetek in which portions of diseased tissue containing the nematodes have been inserted into healthy palms with the result that the typical diseased conditions have been set up. The adult male and female nematodes occur abundantly in the roots in the areas where the tissues are softened and yellowish to brownish-red in color. The eggs are

[63] Moniez, "Trait de Parasit. Anim. et Veg.," Paris, 1896, 8vo.

[64] Strong, Johns Hopkins Hosp. Reports, 1902, X, No. III.

[65] Nowell, *West Indian Bull.,* 1919, XVII, 189.

[66] Cobb, *ibid.,* p. 203.

[67] Zetek, *U. S. Dept. of Agricul. Bull.,* No. 1232, 1924.

deposited in the tissues of the plant where they hatch out and the larvae invade fresh tissues. The larvae are found not only in the roots but also in enormous numbers throughout the diseased tissues. While the habits of these species outside the host plant have not been thoroughly studied, it seems reasonable to suppose that on the falling of diseased leaves or trunks the nematodes would ultimately find their way to the surface of the soil and the subjacent layers and that infection of the young plants might take place by the migration of the nematodes from the soil to the new plant. It has been shown that the nematodes will live in the soil about an infected palm. Very recently Zetek has made observations which have led him to suggest that the termite, *Coptotermes niger* (Snyder), may be a mechanical carrier of this nematode from the old host to the new plant, he having demonstrated nematodes clinging to the bodies of the termites which were living in a cocoanut palm infected with "red ring."

The writer has been able to study the disease in Panama and particularly in Spanish Honduras. While it would appear that the nematodes in question are certainly concerned in the production of the disease, the lesions produced in the palm consisting of the softening of the tissues, their liquefaction and subsequent necrosis, are not such as are usually attributed to nematodes. Both with the idea of acquiring information regarding the presence of some additional pathogenic agent in the disease and also of ascertaining any pathogenic action of the nematodes not only for the plant, but for animals, inoculations of suspensions of the nematodes in saline solution into mice, guinea pigs and rabbits were undertaken. The guinea pigs were inoculated intraperitoneally, subcutaneously or intraintestinally. The mice were inoculated subcutaneously and the rabbits intravenously or subcutaneously. When the injections were made intraperitoneally into guinea pigs the death of the animals often occurred in which there was a general peritonitis associated with a short bacillus, but no nematodes were found in the peritoneal fluid, the blood or other organs of the animal. In fact, after intraperitoneal inoculation the nematodes were never found alive in drops of fluid withdrawn by a capillary pipette from the abdominal cavity longer than two and one half hours after the time of the inoculation. In the guinea pigs which were inoculated by injecting the fluid containing the nematodes through the peritoneal cavity and walls of the intestines directly into the lumen, the nematodes were not subsequently found in the feces of the animal. Some of the rabbits which were inoculated intravenously also died, in which bacilli were isolated from the blood and liver, but no nematodes were found in these situations. In none of the experiments was any pathogenicity of the nematodes for these laboratory animals demonstrated. Cultures were made from the lesions of "red ring" after burning the surface of the palms and in practically all instances species of fungi or bacilli were cultivated. Much more extended observations must be made before we can conclude that the lesions of "red ring" are produced solely by *Aphelenchus*. Perhaps *Aphelenchus* may also carry mechanically bacteria with it into the tissues of the plant or offer a more favorable portal of entry for other microorganisms. Obviously, termites do not serve as the infective agent in many cases because in many cocoanut palms infected with "red ring" no infestation with termites is present. Therefore, in the case of *Aphelenchus*, so far as our knowledge goes, parasitism has apparently developed for plant rather than for animal life.

In conclusion it is obvious that I have but touched the fringe of this vast subject. However, it is my hope that this brief summary of some of the problems that have been elucidated and of some of those that still await solution may stimulate further research.

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UTILIZATION AND CONSERVATION OF THE TIMBER SUPPLY[1]

IN spite of the confusion of tongues and the tangle of comment, criticism and innuendo which always arise when attempts are made to fix the blame for the destruction of the country's timber supply, the principle that intelligent use of wood is one of the most effective forms of forest conservation has been gradually gaining the recognition it deserves. Acceptance of this principle implies belief in another, *viz.*, that the lumberman, the manufacturer, the retailer and the consumer are corporately responsible for securing from each piece of wood removed from the forest the greatest practicable quantity of service. The economics of good utilization are, however, but vaguely understood even by students of the subject; and the bearing of utilization on conservation is not appreciated at all by the millions most directly concerned.

One who investigates the pathological problems of wood conservation soon comes to realize that there is no such thing as a *normal* life of wood. He finds that fire and flood, wear and tear and breakage, neglect and plain carelessness are destroying an immense amount of wood every day in the year; he finds

[1] From the Office of Investigations in Forest Pathology, Bureau of Plant Industry in cooperation with the Forest Products Laboratory, United States Forest Service, Madison, Wisconsin.