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RESEARCH IN SOME ASPECTS OF DISEASE ASSOCIATED WITH THE FIELDS OF ZOOLOGY, ENTOMOLOGY AND PARASITOLOGY[1]

IN no other domain of medical science has such progress been accomplished within the past two decades as in that of medical zoology, entomology and parasitology, and it is significant that the great majority of the discoveries upon which this progress is based have been made in connection with the elucidation of the etiology and methods of transmission of the tropical infectious diseases. For, the abolition and prevention of a number of these diseases, obviously based upon such discoveries, has constituted one of the great triumphs of the science of medicine. Discoveries of the relation of insects to disease have in some instances revolutionized our attempts to prevent and control some of the most devastating infections. In fact, it is very largely through the discoveries of the cause and methods of spread of the serious epidemic diseases that preventive medicine has progressed from a blundering art of forty years ago to its present important position among the medical sciences.

However, while very great progress of this nature had been made in earlier years by the recognition of the value of vaccination against smallpox by Jenner in 1796, and of the discovery of the causative organisms of tuberculosis, typhoid fever and Asiatic cholera from 1880-1883, scientific advances of an entomological nature in connection with disease materialized only later and did not begin to be made for nearly another decade. Thus, although man in his conquest and discovery of new territory made through various explorations both by sea and land during the fourteenth to the nineteenth centuries gradually acquired through his conflict with devastating disease consider-

[1] Read at the annual meeting of the American Association for the Advancement of Science, December, 1922.

able epidemiological evidence, and became familiar with certain popular beliefs and even sage suggestions intimating that several infectious diseases might be spread through the agency of insects, it was not until 1879 that Manson demonstrated that the mosquito *(Culex fatigans)* served as a host for the parasite *Filaria bancrofti,* and that this parasite underwent at least a portion of its life-cycle therein. In the following year Laveran discovered the parasite of malaria, and in 1893 Theobald Smith demonstrated that the disease Texas fever was transmitted by the cattle tick *(Boophilus bovis).* Following these discoveries there came a series of brilliant investigations which have led to much of the remarkable progress recently made in preventive medicine. In spite of the great importance and far-reaching application of many of these discoveries which have related to practically all of the important and widely prevalent tropical infections, lack of time will permit only a very brief reference to some of those which are of greater significance to-day.

Obviously, there are many interesting problems relating to even those fields of medical zoology and entomology already well trodden that still await solution. Considering for a moment the aspect of diseases caused by protozoa, it is of interest to recall that while the parasite causing visceral leishmaniasis or kala azar was discovered in 1903, and there has been published since that time much experimental evidence in favor of the transmission of the disease in India by *Cimex rotundatus,* and on the shores of the Mediterranean by *Pulex cerraticeps,* the most recent work has thrown considerable doubt upon the transmission of leishmaniasis by either of these insects. Particularly from the investigations of Patton we know that the parasites ingested from the peripheral blood of cases of kala azar develop into a flagellate in the stomach of *Cimex rotundatus,* and may persist for as long as forty-one days, very much as Rogers has shown that they may in the blood agar culture tube. Patton suggests that by the crushing of the infected bedbug upon the skin human infection occurs. However, it would appear that we are to-day no nearer the final solution of the problem of the transmission of visceral leishmaniasis than we were ten years ago. Also, the relationship between the human disease and the similar associated canine leishmaniasis which occurs particularly in the Mediterranean and Caspian Sea regions still requires an explanation in view of the absence of the latter from other important endemic centers such, for example, as India. A serum test for the diagnosis of kala azar, known as the "formol gel" test has recently been described. The test is performed by the addition of several drops of commercial formalin to about 1 c.c. of clear serum. In a case of kala azar the mixture becomes viscid immediately and within a few minutes jellied and opaque like the white of a boiled egg, while with other diseases, such as malaria and syphilis, the reaction is delayed for a considerably longer period, up to twenty-four hours. A wider application of the test and of its significance is, however, desirable.

Recent experiments have demonstrated that *Phlebotomus paptasii* can harbor for at least three days the virus of Oriental sore, and that such flies when crushed and rubbed into a scarified area of the skin will after a proper incubation period produce a lesion in which leishmania are demonstrable. However, it is only by further investigations that the origin of the virus in this fly, and whether it is derived from a reservoir or is a natural flagellate infection of the fly, as well as the exact mechanism of infection in nature, can be determined. There is little definite evidence that the gecko, *Tarentola mauritanica,* may act as the reservoir for the virus of Oriental sore, although this has been suggested on the ground that this lizard is ravenously fed upon by sand flies, and that in certain regions fifteen per cent. of the geckos contain a flagellate of the leptomonas type.

Very interesting are the observations which have recently been made regarding the occurrence of flagellates of plants, chiefly euphorbias, and the suggestions that they may be a natural source of insect infection. Laveran and Franchini have reported the successful inoculation of healthy euphorbias of two species with pure cultures of *Herpotomas stenocephali* var. *Chattoni,* and have also infected white mice with latex of *Euphorbia nereifolia* naturally infected with leptomonas, subsequently finding leishmaniform parasites in the blood of the mice that survived the experiment. Franca previously had failed to infect animals with *Leptomonas davidi* from latex of euphorbias, but he did not experiment with the insect-borne strain of the parasite.

A very great advance has been made in the discovery of the value of tartar emetic in the treatment of leishmaniasis. Formerly the death rate from kala azar in India in untreated cases varied from seventy to ninety-eight per cent., while through the use of repeated intravenous injections of from two to ten c.c. of a sterile one per cent. solution of tartar emetic the mortality has been very greatly reduced. Thus Dodds-Price has reported during the past year a series of over two thousand injections of tartar emetic in the treatment of cases of kala azar with sixty-seven per cent. rate of recoveries. However, this drug is very toxic, and its use in human beings is attended with some danger. A number of reliable investigators have reported deaths due to poisoning in the doses recommended. In some of the fatal cases fatty degeneration of the heart, liver and kidneys was noted. Much that we should know in regard to tartar emetic is still undetermined. The formula of it is even not certainly known. No satisfactory method of preparing a suitable stable solution for injection has been discovered, and it is not known exactly what chemical change occurs when the drug is submitted to sterilization in the autoclave. It is so irritating locally that it can not be used advantageously except by intravenous injection. Its method of excretion from the body is also not certainly known, and whether it remains as antimony potassium tartrate in the circulating blood, or whether it combines with a constituent in the body of the patient before destroying the parasite, has also not been ascertained. Since tartar emetic has proved to be not only of so great value in this disease but also in the treatment of bilharziasis, trypanosomiasis and granuloma inguinale, it is highly important that we should have more accurate pharmacological knowledge regarding it. Weiss and Hatcher have recently shown that tartar emetic (antimony and potassium tartrate) induces vomiting reflexly through local irritation after its introduction into the stomach or duodenum, and that it does not cause emesis in the cat or dog when it is applied directly to the vomiting center described by Thumas and which lies in the floor of the fourth ventricle. It is particularly on account of the powerful destructive and irritant action upon the gastro-intestinal tract that the drug can not be satisfactorily given by the mouth in the treatment of infectious processes. Intravenous injections of tartar emetic induce vomiting after varying intervals of time largely dependent on the size of the dose. This emesis is not prevented by the removal of the gastro-intestinal tract, or by the removal of the celiac plexus and simultaneous cutting of the vagi below the diaphragm, but it is profoundly influenced by cutting these nerves in the neck or by paralyzing the vagus endings with atropin. It is also apparently abolished by severing all nervous connection between the heart and centers, by removal of the stellate ganglia and cutting the vagi in the neck in the cat.

Of the recent compounds of antimony, p-acetyl-amino-phenyl-stibinate (stibacetin) appears in some ways to be the most favorable for use, and Brahmachari has found that urea stibamine is a much safer antimonid for use in the treatment of kala azar than tartar emetic or other antimonyl tartrates. There are many difficulties in employing aromatic compounds of antimony which are with a few exceptions non-crystallin and the construction of an efficient less toxic and stable preparation of antimony still remains one of the most urgent problems of tropical medicine. Further work upon this subject is being pursued in my department at the Harvard Medical School.

With reference to malaria, while approximately forty years have passed since the species of plasmodia infecting man were described, there is still no complete agreement as to whether there are really three or only one polymorphic human species. Both Grassi and Plehn believe and support the idea that there is but one parasitic species, the form of which changes with the season. While the existence of double or even in some instances of triple infections with the three species might explain many of the apparent transitions in form from one to another, there is still considerable difference of opinion on the subject. It is generally admitted from the classical experiments of Grassi, Bignami and Bastianelli performed in 1898 that only certain species of anophelinæ may transmit human malaria, and also that *culex* and *stegomyia* are not carriers of malaria, but there has been no satisfactory scientific explanation of why this is so. These facts can not be explained upon the ground of geographical distribution, numerical prevalence, zone of flight, length of life, food supply or other different habits of these mosquitoes. In this connection it is interesting to note that the different species of anopheles capable of carrying malaria and playing a large rôle in nature in this respect come from the most divergent groups of the sub-family. In Europe and northern America, according to the classification of James, the relatively primitive protoanophelines are the active agents. In the new world an offshoot of this the true cellia group are the carriers. In the old world the totally distinct deutero-anophelines furnish practically all the carriers, while in Australia and New Guinea the neo-anophelines, an offshoot of the same stock, come into prominence. Many species of *anopheles, culex* and of *stegomyia* will transmit *Filaria bancrofti,* and Ross and others have demonstrated the full development of *Proteosoma precox* in species of *culex,* particularly *fatigans.*

To-day we know that *Hæmoproteus noctuæ* (Celli and San Felice) and *H. Syrnii* (Mauer), parasites of avian anemia, are also transmitted by *Culex pipiens* (Linnæus) and *Culisetta annulata Schrank* (Theoboldi). However, no one has demonstrated the entire development of the human malarial parasite in *culex* and it is generally believed that there is no evidence of the existence of malaria without some of the anophelinæ. Daniels is inclined to suspect that the ædinæ found in jungles may transmit malaria in such localities, but this has not yet been determined by experiment.

This contraction of malaria in uninhabited jungles has again raised the question of whether some other animal than man may not also act as an intermediate host for the human malarial parasite.

Donovan, having found *Plasmodium pithici* in the orangoutang and *Plasmodium kochi* in several species of monkeys, made the attempt to determine whether the infection in human beings in the jungle might not be attributable to infection from monkeys. He, however, found no human plasmodia in seventy-six *Macacus sinicus* or in ten *Presbytis priamus* from the forests near Madras, but he detected a parasite very like *Plasmodium vivax* in the Malabar squirrel, *Ratufa indica malabarica.* In this connection one may recall that all specific human parasites must have had an origin outside of the human species, and probably pursued their evolution independent of man. Nevertheless, if one finds a protozoan parasite in an animal even extremely similar to one affecting man, it would be precipitous to conclude that the two parasites are identical or had the same origin without the additional proof that the animal may be infected with the human species. Manson suggested that cave-dwelling bats may have been the original source of human malaria. However, attempts to infect flying foxes or fruit-eating bats of the species *Cynonycteris straminea* with *Plasmodium falciparum* and *P. malariæ,* both by specific inoculation and by exposing them to the bites of *anopheles,* have failed. A number of investigators have observed *plasmodia* in the higher apes, and Mesnil and Roubaut have succeeded in infecting chimpanzees with both benign and with malignant tertian malaria by direct inoculation, but not with bites of *Anopheles maculipennis.* Both infections died out shortly and naturally.

Reichenow believes that he has found in the gorilla and chimpanzee in West Africa all the forms of *plasmodia* which infest man, and he confidently asserts that the anthropoid apes are as sure a source of danger to Europeans living in those parts as are negroes. Still more recently Blackloch and Adler in a chimpanzee in West Africa have also found both small rings and crescents morphologically indistinguishable from *Plasmodium falciparum* and apparently identical with the parasite described by Reichenow. Further confirmation of this work is desirable.

Some work of considerable significance has recently been performed which may help to explain the condition known as dormant or latent malaria and its relation to relapse.

Grassi and Schaudinn attribute the origin of relapse to pathogenesis of macrogametes, and Ross to the persistence of a few schizonts. Whitmore in a study of bird malaria has shown that the blood of infected canaries remains infective by injection for at least twenty-nine months after parasites have ceased to be discoverable in the blood by a microscopic examination.

E. Sargent had also observed persistent infection after apparent disappearance (as determined by microscopical examination) of the parasite from the bird's blood, by demonstrating that its blood is still infective for *culex*.

Until very recently comparatively little was definitely known regarding the time that *anopheles* might remain infective after biting a malarial patient. A number of observers had claimed that *anopheles* generally lose their power of infection after hibernating, and that those which have hibernated during the winter are not able to produce infection in the spring unless they themselves become reinfected.

Wenyon, however, has recently shown that if infected *anopheles* were placed in an ice chest between 9° and 12° C., oocysts already formed became degenerated in every case. If, however, the temperature was only lowered to that of the laboratory, development of the oocysts could be temporarily arrested for as long a period as three weeks and then revived again when the mosquitoes were placed in the incubator.

Mayer infected *culex* with the *proteosoma* of birds and found five weeks later that not only the insects' salivary glands but also the muscles of the body and appendages of the palpi contained numerous sporozoites. Fifty-two days after the infective feed, isolated sporozoites could be detected in the muscles. This suggests that under certain conditions the sporozoites may persist for longer periods in these locations than in the salivary glands.

Finally, Mayne has shown that *plasmodia* of malaria were detected in the salivary glands of five specimens of *Anopheles punctipennis,* kept at a temperature of 44° to 78° F., from sixty-eight to ninety-two days after infection, and that *plasmodia* of malaria were proved to be viable by inoculation into a human host by the bite of a mosquito which had been infected fifty-five days previously. Thus under certain conditions the carriage of malaria through the winter by the mosquito would seem to be a possibility.

It is almost certain that hereditary infection of the mosquito does not occur. Schaudinn claimed to have observed sporozoites of the benign tertian parasite in the ovaries, but this observation has not been substantiated. Mayer and Muhlens both found sporozoites in the vicinity of the ovaries in infected *culex* and *anopheles,* but not within these organs.

Great confusion still exists in regard to the subject of immunity in malaria. Complete natural immunity for man depending on the presence in the blood of substances which prevent any development of the naturally inoculated virulent human malarial parasites in considerable number is doubtful, and the relationship of partial natural immunity to latent infection in which symptoms are long delayed beyond the usual incubation period of the disease has not been satisfactorily explained. On the other hand, partial immunity or tolerance of the infection without evident symptoms of disease is apparently generally acquired gradually by frequent infections and reinfections repeated continuously during a number of years. Such infections include the malarial carriers. Whether the acquired immunity ever becomes sufficient by the production of specific antibodies unaided by drugs to destroy all the parasites, and to sterilize the patient, is still questionable. The brothers Sergent, in studying the immunity of canaries to avian malaria, found that the normal immunity rate in 965 canaries was 0.72 per cent. By injecting blood taken from a canary during the incubation period after an experimental infection, the immunity rate in canaries could be raised to 21.3 per cent. in 61 canaries. Finally, by the injection of sporozoites kept in vitro for twelve to forty-eight hours, the immunity rate in 24 canaries could be raised to about 30 per cent. Further extension of these interesting experiments is also highly desirable. Cremonese believes with others that as mosquito infection with malaria is greatest in the autumn, consequently primary infection must in the main be then acquired. He further believes that the first evidence of symptoms of the malarial attack occurring in the spring is due to the effect of climate acting upon humoral antibodies, in cases of long latent malarial infection. Primary latency is held to be the rule in malaria and natural immunity to depend upon the parasites acting as antigen, but only as long as they are extracorpuscular. Once the parasite becomes intracorpuscular, complement is held to be powerless to unite with antibody. In connection with this subject the influence of sunlight in the spring as well as of ultraviolet rays upon the development of latent malarial infection and relapse has recently been emphasized by Whitmore.

With reference to quinin, still the most specific drug in the treatment of malaria, the brothers Sergent found in experiments in which *Plasmodium relictum* was injected into canaries, and daily injections of quinin given, in only one case was there evidence of the development of a strain resistant to quinin, this character being retained in full during its passage through two other canaries and in lessened degree through a third. It appeared in a canary kept quininized for nine months without symptoms, these, then, appearing in spite of the continuation of the quinin. From twelve experiments they conclude that to administer prophylactic quinin before there is risk of infection is useless. Macht has recently shown that the toxicity of quinin sulphate is much greater when injected into frogs if these animals are exposed to sunlight than it is when they are kept in the dark. Further experiments revealed that it was the light waves from the violet end of the spectrum that were the most effective in increasing the toxicity of quinin and quinidin. In other experiments the rays of an electric arc lamp were utilized instead of sunlight, and the same potentiation in toxicity was qualitatively noted. It would be of interest to ascertain whether this increased toxic action is associated with the property of fluorescence.

The relationship of the wild game to human sleeping sickness and the controversy as to whether human trypanosomiasis is communicated to man by *Glossina morsitans* that has been infected with trypanosomes from feeding on wild animals, is far from being settled. However, the recent experiments of Taute and Huber in which Taute inoculated himself and Huber and one hundred and twenty-nine natives with blood containing virulent *Trypanosoma brucei* from naturally infected animals without obtaining a single human infection go far indeed towards demonstrating that *Trypanosoma brucei* of Nagana and *Trypanosoma rhodesiense* of human trypanosomiasis are not identical.

A large amount of research has been recently performed regarding the chemotherapy of trypanosomiasis. Pearce has recently called attention to the good results obtained with tryparsamide and exceedingly favorable reports have been given of the efficacy of a new preparation known as "Bayer 205" in the treatment of trypanosomiasis both in man and animals. The preparation is stated to be much more efficient for this purpose than atoxyl and antimony, and much less toxic. However, it is not harmless for man since it has an effect upon the blood and the kidneys, in moderately large doses causing destruction of erythrocytes, anemia and albuminuria. Albuminuria has also been observed in man sometimes after quite small doses. It usually does not appear until some days after the injection of the drug. The substance circulates in the blood for weeks and even months, so repeated doses must have a cumulative effect. For man a ten per cent. solution has been recommended, 0.5 gram being given at the first dose, and, if this is well borne, twenty-four to forty-eight hours later one gram intravenously, up to three grams within a week. After this there should be at least a fourteen-day interval with a careful examination of the urine. Many of the cured animals remain immune against subsequent infection for weeks and months. Pfeiler has treated horses suffering with dourine with this preparation. Some of the animals have apparently been cured, but in other cases the treatment has failed. The serum of healthy rabbits previously injected with this drug is therapeutically active, and it is possible to cure rats and mice infected with *Trypanosoma brucei* with such rabbit serum removed up to fifty-one days after the injection of "Bayer 205." The drug perhaps combines with some element in the serum before directly killing the trypanosomes. If this is substantiated it would appear that "205" is a protoplasmic poison which acts in this connection in a somewhat similar manner to several other chemotherapeutic preparations recently investigated. Thus it has been suggested that an organic compound containing arsenic, as salvarsan for example, does not itself directly kill the spirochætes, but that the cells of the patient must in some way cooperate, perhaps by oxidizing this substance. A somewhat similar action has been suggested for emetin. Dale and Dobell found that when emetin is applied to fresh amœbæ taken from the dysenteric lesions, it has a surprisingly weak action on these organisms, and that they were still motile after contact for two hours in a one per cent. solution of emetin hydrochlorid. Dale then prepared certain derivatives of emetin and found that di-methoxy-emetin was ten times as poisonous for the amœbæ and not nearly as poisonous for the animals (cats) as emetin, but it had no therapeutic effect whatever. Hence, it was concluded that the curative action of these alkaloids was proportional not to their direct poisonous action on the amœbæ, but to their poisonous action on the patient, and it is suggested that the body of the patient must play an essential and perhaps a primary part in the killing of the parasite. However, the experimental difficulties in connection with the chemotherapeutic study of emetin in cats infected with amœbæ are considerable, and it is exceedingly desirable that the problem should be approached in other ways. The further study of the nature of the reaction between these chemotherapeutic agents and the cells of the patient and the final action upon the parasite is exceedingly important.

A vast amount of research has in recent years been carried on in relation to the spirochætal diseases and their methods of transmission. The exact systemic position of these microorganisms has been productive of considerable controversy since Schaudinn in 1905 discovered the species specific for syphilis and named it *Spirochæta pallida.* However, their zoological position has not yet been satisfactorily determined and there is still considerable dispute as to whether the spirochætæ should be classified with the protozoa or with the bacteria, or in an intermediate kingdom, the protista. Recent investigations show that they possess a firm but flexible periplast and multiply by both longitudinal and transverse division. By differential staining they may be seen to possess bars or rods which stain deeply and assume a chromatin tint, while some species show a non-undulatory membrane. According to Balfour, Leishman and others, a resting or resistant stage, the granular stage, constitutes an important phase in the life-cycle of the spirochætes, and is supposed sometimes to explain the infection of the offspring of the intermediate hosts, ticks or lice, and also the human relapses when the blood stream is flooded with parasites, developed apparently from the granules located in the internal organs. While the nature of the chromatinic granules produced by spirochætes has been much discussed, there is still not unanimity of opinion regarding them. Marchoux and Couvy, Wolbach and Betances consider the granules or coccoid bodies to be degeneration products. Leishman, Bosanquet and Fantham, however, do not coincide with this view. Leishman believes that he has seen young spirochætes apparently attached to a granule clump suggesting what might be interpreted as growth of a spirochæte from a granule.

Sergent and Foley have recently shown that monkeys can be infected with human blood containing no visible spirochætes, and they believe that the virus of recurrent fever may exist in the blood of man and also in the louse in a very minute form. The existence of this cycle or form, they declare, is an argument in favor of the relationship of the spirochætes of relapsing fever to the protozoa.

Todd believes that it is only by definitely seeing granules grow to comma forms, and commas to spirochætes, that the development of spirochætes from granules can be demonstrated. Balfour has more recently observed a development of some of the granules from the malpighian tubes in ticks into spirochætal-like forms, but he has been unable to trace a complete cycle of development in the tick. Possibly by the continuous observation of these organisms kept at favorable temperatures in a moist chamber, and examined by direct illumination and also with the darkfield, further information regarding this question may be obtained.

Since Schaudinn's discovery of *Spirochæta pallida,* five other important species of *spirochætæ* causing disease in man have been described: *S. pertenuis* by Castellani in 1905 as the cause of yaws, *S. bronchialis* by Castellani in 1907 as the cause of bronchial spirochætosis, *Leptospira icterohæmorrhagiæ* by Inado and Ido in 1914 as the cause of hemorrhagic jaundice, *Spirochæta morsus murium* in 1915 by Futaki as the cause of rat-bite fever, and *Leptospira icteroides* by Noguchi in 1919 as the cause of yellow fever. *Spirochæta schaudinni,* which has been described as the cause of ulcus tropicum, can hardly be differentiated morphologically from *Spirochæta refringens* discovered by Schaudinn and found by him in syphilitic lesions of the external genitalia and in open ulcers of the skin. While there are some suggestions in the literature indicating the pathogenicity of *Spirochæta eurygyrata* Werner in the intestine in man, the recent investigations would indicate that further studies on this subject are desirable. Hogue did not find the coccoid bodies described by Porter as a means of transmitting this organism, but she was able to cultivate this spirochæte in a medium consisting of 15 c.c. of 0.85 sodium chlorid solution and 0.3 c.c.. of sterile serum water, with a p_{H7} reaction, the tubes being covered with paraffin oil.

There are a number of recent reports in the literature calling attention to the prevalence of the condition known as bronchial spirochætosis. *Spirochæta bronchialis* can hardly be distinguished structurally from *Spirochæta refringens.* There is considerable evidence which shows that the spirochætes in the sputum in this condition are really oral spirochætes which have found a suitable medium in the bronchi, particularly in tuberculosis and other pathological conditions of the lung, and there is not general agreement regarding the acceptance of bronchial spirochætosis as a distinct disease.

Vincent believes as do a number of others that the lung condition in spirochætal bronchitis is due to invasion of the bronchi by the same organisms which occur in Vincent's angina, and that *S. bronchialis* is identical with *S. vincenti.*

It is interesting to note that with reference to transmission only one species, *Leptospira icteroides,* has been demonstrated to be transmitted by insects, *Stegomyia calopus. Spirochæta bronchialis* is said to be transmitted from man to man by coccoid bodies or granules through droplet infection in coughing, the coccoid granules being more resistant to drying than the spirochætes. With reference to the different species of spirochætes which have been described for relapsing fever in different parts of the world, it seems very doubtful if these various forms really constitute distinct species, since the experimental methods employed for their differentiation and based upon immunity reactions are not entirely reliable for the separation of species of these spirochætes. Nevertheless, in the literature of the past year one finds two more species described as new and different from those hitherto described. Still more recently Blanchard and Lefrou have reported a spirochæte in the blood in blackwater fever, *Spirochæta bilio hemoglobinuriæ,* and Couvy has observed another species in the prefebrile period of dengue fever.

Kasai, by inoculation experiments on rats, mice and guinea pigs, and serological tests involving Pfeiffer's reaction, has concluded that the smaller spirochætal forms obtained from cases of rat-bite fever and the slightly larger forms occurring naturally in rats and mice are identical.

Hoffmann has not accepted the differentiation between *Spirochæta icterohæmorrhagiæ* and *Spirochæta icteroides.* The thorough investigation of the histological and hematological appearances in a large series of guinea pigs infected with *Leptospira icteroides* gave results absolutely identical with those found in another series of animals infected with *Leptospira icterohæmorrhagiæ,* and there were no clinical, pathological and anatomical differences in the two experimental infections, though attempts to differentiate these organisms by the immunity reactions were not included in this study.

Borges in a number of cases of yellow fever occurring in Bahia tested the serum with *Leptospira icteroides* by the Pfeiffer phenomenon and found negative results. However, Noguchi in a still more recent publication believes that these pathogenic *leptospira* are by no means identical, as can be proved not only by their immunological but also by their pathological properties. *Leptospira icteroides* is predominantly icteronephritic, with a marked tendency to produce fatty degeneration of the parenchymatous cells of the liver and kidney. The hemorrhages in mucous membranes are more pronounced in experimental infections with *Leptospira icteroides* than those resulting from *Leptospira icterohæmorrhagiæ.* He believes that the two diseases yellow fever and haemorrhagic jaundice are essentially similar, though caused by two distinct species of the genus *Leptospira,* just as syphilis, yaws and venereal disease of rabbits are three diseases caused by three morphologically indistinguishable species of the genus *Treponema.*

Among the many important advances that have recently been made in our knowledge of medical helminthology, and that have had an important influence in preventive medicine are the recent elucidations of the life-history of the trematodes *Schistosoma japonicum, hæmatobium* and *mansoni, Paragonimus westermanii* and *Clonorchis sinensis;* of *Dibothriocephalus latus* and *Spargonum mansoni;* of *Filaria (Loa) loa,* and of *Ascaris lumbricoides.* The schistosomidæ have been shown to be digenetic trematodes, and certain snails, particularly *bulinus* for *Schistosoma hæmatobium,* of *planorbis* for *Schistosoma mansoni* and *blandfordia* for *Schistosoma japonicum,* have been demonstrated to act as the intermediate hosts. The miracidia of these parasites hatch from the egg in water after entering these snails, develop into cercariæ which, being passed from the snail into the water, may infect man either through the normal skin as when bathing or through the mucous membranes as in drinking infected water. In the case of *paragonimus* there are two intermediate hosts, the first consisting of mollusks of the genus *Melania* in which the cercariæ are developed and from which they pass into the water and then either enter man directly or pass to the second intermediate host (the crustacean) consisting of species of crabs of the genus *Potamon* or *Eriocheir.* The cercariæ may migrate through the tissues of the crab and become discharged again into the water from the gills. Infection of man would appear to depend then not necessarily upon the consumption of uncooked crab or crawfish, but from the free larvæ which, while they can not penetrate normal skin, may enter from the water through the mucous membranes of man or through wounds in the skin. In the life-history of *clonorchis,* the two intermediate hosts have recently been shown to be the molluscan *Bythinia striatula,* from which the cercariæ pass to and encyst in edible fish, particularly *Pseudorasbora parva* and *Leucogobio guntheri,* man becoming infected from the ingestion of these fish. The life-cycle of *Loa loa* has very recently been worked out in two species of *Chrysops* in Nigeria, *Chrysops silacea* and *Chrysops dimidiata,* 358 flies experimentally infected having been examined by A. and S. Connal. Development takes place in the muscular and connective tissue, principally in the abdomen of the fly, but also in the thorax and head. The metamorphosis takes ten to twelve days for completion, and is somewhat similar to that of *Filaria bancrofti.*

Yosida has experimentally infested the dog and cat with larvæ of *Sparganum mansoni* of the species found both in the human body and the frog which it chooses as the final hosts. The larvæ belonging to both species developed into an adult worm, having a completely identical morphology. These parasites have often been found infesting dogs and cats, and ducks and chickens which eat frogs may also become infested. Human infection occurs from drinking water harboring the infected cyclops, or from eating insufficiently cooked infected chickens or ducks. Ransom in relation to the migration of the larvæ of *Ascaris lumbricoides* has called attention to the fact that a more or less serious lung trouble known as thumps is a very prevalent malady among pigs, and is caused primarily by *ascaris* larvæ in the lungs. He believes that pulmonary *ascaris* of a more or less severe form occurs also among human beings.

Of less importance, although of scientific interest, are the applications of serological methods involving the complement fixation test in the diagnosis of a number of trematode infections, particularly those due to *schistosomidæ, clonorchis* and *echinococcus* infection.

The efficacy of intravenous injections of tartar emetic in the treatment of bilharziasis, formerly considered incurable, has already been mentioned. Recently Lasky and Coleman have reported upon 1,000 cases of this disease treated with tartar emetic. Of 500 individuals who persevered with the treatment, which aimed to secure a cumulative effect from about twenty grains of tartar emetic, seventy per cent. were pronounced cured. Reference has also already been made to the toxicity of this drug, and in this series of cases there were ten deaths apparently due to the treatment, though many of the cases were stated to be physical wrecks at the time that the injections were commenced.

While the cercariæ of all the species of *schistosomidæ,* which have been demonstrated to be pathogenic for man and animals hitherto described, have been characterized by forked tails and no eye-spots, Tanabe has recently discovered in Boston a new species of *schistosoma, Schistosoma pathlocopticum,* pathogenic for mice, in which the cercariæ, while fork-tailed, have typical eye-spots. The cercariæ of this schistosome were first discovered in species of *Limnæa* collected from the Charles River near Boston.

A new anthelminthic known as butolan has recently been reported to be particularly efficacious in the prompt cure of oxuris infection and to produce no unfavorable symptoms. This substance is the carbamin-acid-ester of p-oxy-diphenylmethane. In the body it breaks up into p-benzylphenol and carbamic acid. The former substance destroys the parasites.

Very interesting are the etiological problems in connection with a number of insect-borne diseases in which the causative organism, while present in the blood, is either of too minute size or in too scanty numbers for its recognition. In this connection I wish to refer briefly to such diseases as typhus fever, dengue fever, pappataci fever, trench fever, African horse sickness, heart-water of cattle, sheep and goats, and tsutsugamushi or kedani disease.

Although the method of transmission of typhus fever by the louse *Pediculus humanus* has been known from the experiments of Nicolle, Rickets, Wilder and others, since 1910, for a number of years the etiology of the disease has been very obscure. It has been possible to transmit the virus by inoculation of the human blood of a typhus patient into monkeys or guinea pigs and to convey it by blood inoculation through long series of these animals. The infection is not fatal in either the monkey or the guinea pig, and the only clinical indications that the infection is transmitted in this way in guinea pigs is by a transient rise in temperature, for these animals usually remain apparently well, continue to eat and act as though in a normal condition. However, if the animal is killed the diagnosis of the infection may be made, as Wolbach and Todd have recently shown, from the histological study of sections of the brain which will reveal the presence of proliferated vascular and perivascular lesions and meningeal infiltrations.

There had not been unanimity of opinion regarding the filterability of the typhus virus. Nicolle, Connor and Conseil inclined to the belief that the virus is filterable because monkeys that had been inoculated with filtrates of blood or of crushed lice that had harbored the virus, while showing no decided temperature reactions, had remained resistant to a subsequent infection of active virus.

Olitsky was unable to filter the virus either from the blood or through Berkefeld V and N candles from the disintegrated tissues of infected guinea pigs. Later, however, he found that in the filtrates of typhus infected tissues of guinea pigs, a substance was occasionally found which produces in these animals thermic reactions, lesions characteristic of experimental typhus, and less frequently immunity to later injections of active virus. However, evidence is given that the filtrates do not contain a living organism.

Recently Rocha-Lima, Topfer and Schuesler, Otto and Dietrich and others have discovered *rickettsia* in lice fed upon cases of typhus fever which occur particularly in the epithelial cells of the intestinal tract of these insects. Wolbach, Todd and Palfrey have also confirmed and greatly extended these investigations. In the meantime, experiments performed by Brumpt and by the writer had demonstrated that lice may be infected with species of *rickettsia* which are not pathogenic for man and that produce no symptoms when such lice are fed upon man. This work has subsequently been confirmed. Thus Noller found *rickettsia* in the louse tick *Melophagus ovinus* of sheep which produced no disease in these animals, and Arkwright, Atkin and Bacot showed that bedbugs, *Cimex lectularius,* heavily and commonly infected with *rickettsia,* produced no symptoms when fed on man.

Besides three species of *rickettsia* in *Pediculus humanus,* two of which have been regarded as pathogenic for man, other species not associated with any disease have been described as occurring in the bedbug *Cimex lectularius,* the mosquito *Culex pipiens,* in the cat flea *Ctenophalus felis,* the mouse flea *Ctenopsylaa musculi,* in the horse louse *Trichodectes pilosus* and the goat louse *Linognathus stenopsis.* Wolbach has also described as the cause of Rocky Mountain spotted fever an organism in the wood tick *Dermacentor venustus* which perhaps should be included in this group. Finally, during the present year, other species of *rickettsia* have been observed in the dove louse *Lipeurus baculus* and in *mallophaga,* the biting lice of the black martin and of the domestic fowl. While it is not entirely clear that all of these represent distinct species, it would nevertheless appear that the *rickettsia* represent a considerable group of microorganisms related to either the protozoa or bacteria, and that there is only evidence that two of these species are associated in any way with disease. In fact, the evidence shows that one species in the louse and one in the bedbug are not pathogenic for man.

Stevenson and Balfour, after studying the pathology of typhus fever, concluded that there were so many different species of *rickettsia* that they doubt as to their exact nature, let alone their pathological significance.

Phear, in reviewing recent work on the relationship of typhus to *rickettsia,* points out that the experimental evidence establishes a strong case in favor of the view that there is some association of a very close character between the virus of typhus and the *rickettsia* bodies, but, in considering the case for the identity of the two, that much depends on the nature of the granular structures which have been described in the vascular endothelium, and that the investigators would doubtless readily admit that confirmatory observations are necessary before the identity of the intracellular granules with the virus of the disease can be regarded as definitely established. Until an infection is actually produced by a pure culture of *rickettsia,* the etiological significance of these organisms will probably not be admitted by all scientific workers, but the recent work of Wolbach and Todd has gone far towards demonstrating the importance of *rickettsia* in human disease.

Several reports of the successful cultivation of *rickettsia* have been made. Kuczynski has cultivated *Rickettsia prowazeki* in modified blood plasma in celloidin capsules in the abdominal cavity of guinea pigs. The *rickettsia* of the sheep louse is said to grow on a relatively simple glucose blood agar medium. Loewe, Ritter and Baehr, during the past year, have reported upon the cultivation of *rickettsia* from the blood of typhus cases and from the brain and kidneys of guinea pigs, reacting to the blood from typhus fever patients. The media employed consisted in a rich ascitic fluid containing a fragment of sterile rabbit's or guinea pig's kidney and a small amount of two per cent. dextrose bouillon, the tubes being sealed with sterile petrolatum. Wolbach has also just announced the cultivation of *rickettsia* by the living tissue method. Bacot and Segal have shown that injections of lice with a concentrated emulsion of platelets obtained by fractional centrifugalization of the blood of a typhus infected guinea pig affords a sure and quick method of obtaining the development of *Rickettsia prowazeki* in these insects, which suggests under these conditions an association between blood platelets and the *rickettsia* bodies.

Considerable attention has very recently been attracted to the subject of mitochondria. While there is no evidence which would allow us to assume that they are parasitic, there is also as yet no decisive evidence of their significance and nature.

With reference to other cell inclusions recently described, particularly of the red blood cells of vertebrates, Mayer found in rats and in guinea pigs just recovered from a severe trypanosome infection numerous rods, like, but even smaller than, *bartonella,* and dumb-bell forms reminiscent of *rickettsia,* the blood picture being that of a grave anemia similar to that of Oroya fever. These forms he thought might possibly be regarded as degeneration changes or as an outburst of some latent infection. In the latter case, Mayer proposes under reservation the name of *Bartonella muris* for the inclusions. The question then arises whether in certain forms of pernicious anemia where inclusions of a similar nature occur, there may not be present some similar element of infection or excitation of infection.

Attempts to demonstrate microorganisms causing dengue fever have so far been unsuccessful. In 1903 a piroplasma was described as the cause of the disease. This observation has remained unconfirmed. The virus of the disease is believed to be filterable and the disease has been reproduced in man by the inoculation of filtrates of the blood in several cases. *Stegomyia fasciata* and possibly *Culex fatigans* are known to be the transmitting insects. Pappataci fever is also caused by a filterable virus or organism and is transmitted from man to man by *Phlebotomus papatassii.* Since the discovery by Noguchi that yellow fever is caused by *Leptospira icteroides,* which organism will also pass through a Berkefeld filter, it has been suggested that dengue fever and pappataci fever may also be due to a spirochæte, and in fact Couvy has reported that in an epidemic of this disease in Beirut, Syria, which attacked practically the whole population, direct examination of the blood showed the presence of short, slender spirochætes having two or three turns and fine extremities. These were only present when the blood was taken two or three hours before the onset of the fever, never during the course of the pyrexia or after the temperature had fallen. It is not stated whether the organisms were detected by dark-ground illumination or in fresh blood or in stained specimens. Neither has this observation been confirmed during the past year. The methods which led to the discovery of *rickettsia* in the transmitting insect of typhus fever have not yet been applied in connection with either dengue fever or pappataci fever, and no organism has yet been cultivated from the blood by Noguchi's method or on other media.

Trench fever is a disease which resembles dengue fever in a number of ways and yet is obviously quite a distinct infection. It is transmitted in nature by the louse, *Pediculus humanus.* The virus is present in the blood serum of infected individuals during the febrile stage of the disease, and the infection can be transmitted from man to man by the inoculation of such serum. Attempts to filter the virus through Chamberland filters from the blood have been unsuccessful, but under certain conditions it has been filtered from infected urine, and the disease has been reproduced by the inoculation of such filtrates. Certain experiments have been performed which also suggest that the disease may be caused by species of *rickettsia,* in that lice fed on trench fever cases have sometimes developed *rickettsia,* and that lice infected with *rickettsia* have sometimes given rise to trench fever when fed on healthy men. On the other hand lice not showing *rickettsia* have produced trench fever in man and the question has arisen as to whether the virus of typhus has not sometimes been confused with that of trench fever in certain experiments performed with lice. It has been suggested that trench fever is caused by a spirochæte, but it has not been possible to confirm this suggestion. When the cultivation of the *rickettsia* in vitro has been perfected, the inoculation of pure cultures of the species suggested as the cause of trench fever should shed further light upon the problem. It has been demonstrated that the virus of trench fever is very resistant in nature, and if it is of a similar nature to that of typhus fever there would probably be even less difficulty in cultivating it than the typhus virus.

Tsutsugamushi disease or Japanese river fever is an infection which has been proved to be conveyed by a minute red trombidium, *Leptus akamushi* Brumpt. This insect is an ectoparasite of field mice and freely attacks man in the endemic areas of the disease. Recent studies have shown that the virus is present in the blood and that it is not filterable. Nevertheless, it is suggested that it should be classified with the ultra-microscopic disease-producing organisms. Tsutsugamushi has some analogy to both typhus and Rocky Mountain spotted fever, but animals which have received an injection of the tsutsugamushi virus are still susceptible and react to an inoculation of the typhus virus and *vice versa.* The infection is transmissible to monkeys, in which it produces a febrile reaction, enlargement of the lymph nodes and a leucopœnia. The infection is sometimes fatal for the monkey. Guinea pigs are also said to be susceptible, but in these animals the virus produces only a slight temperature and an enlargement of the lymph nodes.

Miyashima has found small elliptical bodies in the larva and adult trombidium which could be cultivated in ascitic agar and when injected into monkeys produced the typical disease. Nagayo and others also found both in human cases and infected monkeys small organisms, oval or elliptical in shape, with a definite limiting membrane measuring 0.35 to 1 μ. In the endothelial cells of lymph glands their differentiation was difficult.

More recently Hayashi has found minute bodies described as rod, spheroid and ring-shaped in the lymphocytes of lymphnodes and in mononuclear endothelial phagocytes of the spleen and lymph nodes, and in the region of the bite in patients suffering from tsutsugamushi disease. These bodies also occur free in the blood plasma and in severe cases in the red cells. The parasite is said to resemble but show differences from *Bartonella bacilliformis* of Oroya fever and of *Theileria parva* of cattle anemia. For it the tentative name of *Theileria tsutsugamushi* has been proposed. Further confirmation of these observations is extremely desirable.

In more recent work by Kawanura and Yamaguchi no mention is made of the organism described by Hayashi as the causative agent. However, Ishimora and Ogata have found certain coccoid bodies 0.2 μ to 2.15 μ in size in sections made from the lymph glands, spleen and heart of cases of the disease and in the lesions of the testicles of monkeys experimentally infected with blood from human cases. These they were able to repeatedly cultivate in a special ascitic medium and from the cultures to infect monkeys. Megaw in referring to the typhus-like fevers carried by mites in different parts of the world suggests that tsutsugamushi disease is probably related to the sumatra typhus which is transmitted by a tick.

African horse sickness is a disease in which the virus is present in the blood plasma and from which it may be filtered through porcelain filters. It, however, will not pass through collodion sacks or through a layer of one per cent. agar. The virus loses its vitality at 50° C. and it is not destroyed by three per cent. carbolic acid, ten per cent. sodium taurocholate or ten per cent. saponin. A virus mixed with five to ten per cent. solution of lecethin fails to infect but it becomes virulent again when the two are separated. Drying at ordinary temperature destroys it. The virus can be transmitted experimentally by the inoculation of small amounts of the blood. The disease is probably transmitted from animal to animal naturally by the mosquito (*Ochlerotatus*). Ticks can also carry the virus but it has not been demonstrated that the bite of this insect transmits it. *Anopheles* and *Stegomyia* and *Stomoxys calcitrans* have also been shown to transmit the disease at least mechanically. In this connection it may be noted that sometimes the injection of .0001 c.c. of blood into a horse will convey the injection. The disease is not transmitted from animal to animal by contagion, or by feeding, unless the animal is given by the mouth 150 to 200 c.c of blood. Dogs, however, may become infected from eating the meat of infected horses. The disease disappears eight days after frost begins. The virus does not apparently survive in water, although it is fairly resistant. How the infection remains alive for from six to eight months between seasonal outbreaks still remains obscure. Swamp fever or the infectious anemia of horses is probably identical with African horse sickness, but the virus has entirely analogous relations with no other known virus. Whether it undergoes a special life-cycle in the transmitting insect similar to the malarial parasite, for example, is also unknown.

Heart-water disease of cattle, sheep and goats and catarrhal fever of sheep are somewhat analogous diseases, but there is some evidence to show that the viruses causing these diseases are not identical with that of African horse sickness.

Tularæmia has been described recently by Francis as a specific infectious febrile disease due to *Bacterium tularense,* and transmitted from rodents to man by the bite of an infected blood-sucking insect or by handling and dissection of infected rodents. Clinically, the onset of the disease is sudden, with headache, backache, pains in the limbs, lassitude and fever. In cases observed in Utah there was enlargement of the lymph glands which drained the areas surrounding the point of infection and a septic fever lasting from three to six weeks. The site of the insect bite and the adjacent lymph glands became tender and inflamed and they commonly suppurated. The rodents found infected in nature are the California ground squirrel, *Citellus beecheyi,* the ground squirrel of Utah, *Citellus mollis,* the rabbits of southern Indiana and the jack-rabbits of Utah. The insects shown to be capable of transmitting the infection are *Chrysops discalis, Stomoxys calcitrans, Ceratophylis acutus, Cimex lectularius, Polyplax serratus* and *Hæmodipsus ventricosus*. The first four of these insects bite both rodents and man. Infection probably occurs most commonly through the bite of *Chrysops discalis*. The bacillus causing the disease is a very minute organism and can best be cultivated upon egg-yolk media, but no growth is obtained on ordinary nutrient agar. The organism produces a plague-like disease in rodents, and in fatal guinea pig infections the gross lesions can not be distinguished from those produced by *B. pestis*. The pathological histology of the lesions caused by *B. tularense,* as shown by Councilman and the writer, are, however, quite different. In the lymph-nodes, liver and spleen, there are miliary foci formed by accumulation of mononuclear cells followed by necrosis and infiltration with polymorphonuclear leucocytes. There is a general infection of the endothelium of the blood vessels and the organism may be found in these cells in any part of the body. In addition the organisms pass from the endothelium into the cells of the liver, which they gradually destroy and replace, forming large globular masses of bacilli. The infection of the endothelial cells presents a somewhat similar picture to that seen in typhus fever but not in any other disease where these cells are distended with *rickettsia*.

It is hoped that this article will in some degree serve to emphasize the important position which the subjects of medical zoology, entomology and parasitology have recently taken in connection with general progress in medical science, and the fact that they are subjects which have important practical application in preventive medicine; that they constitute adjuncts to hygiene which have been most successfully prosecuted in tropical countries but which can not be wisely neglected in any country; that by the more thorough and fundamental training of medical men in these branches still greater progress in the elucidation of the many and perplexing problems connected with the etiology, prevention and control of the infectious diseases more common in temperate climates may be expected. Finally, it is hoped that it will not only indicate the most important recent progress in the fields of science referred to, but that it will also serve to some extent to stimulate and guide research in a few of those channels in which further knowledge is urgently needed.

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EXTRACTS FROM ESSAYS OF LEONARDO DA VINCI

LOOKING over the translation of "Leonardo da Vinci's Notebooks" by Edward McCurdy (Scribners), I was reminded of the high praise awarded the great artist-engineer in Lyell's "Principles of Geology." Priests and scholars in da Vinci's day were wrangling over the origin of fossils. Were they forms or models produced in the fatty matter (*materia pinguis*) of the earth by the revolution of the stars? Were they relics of Noah's flood; were they, as Voltaire suggested, cockle-shells; were they dropped from pilgrims' hats during the crusade?

In these notebooks, from which I give here a brief extract, Vinci makes the whole matter perfectly clear, for he was a close observer and a skilful engineer, qualities rare in that age of fine painting and loose thinking. If men had listened, it would not have taken 150 years to prove that fossils had once been alive, and another 150 to prove that they were not all buried simultaneously in the great flood.

DAVID STARR JORDAN
STANFORD UNIVERSITY,
APRIL 6, 1923

Defense of Fossil Shells as Once Living Organisms

AS for those who say that the shells are found over a wide area and produced at a distance from the sea by the nature of the locality and the disposition of the heavens which moves and influences the place to such a creation of animal life,—to them it may be answered that, granted such an influence over these animals, they could not happen all in one line, save in the case of those of the same species and age; and not one old and another young, one with an outer covering and another without, one broken and another whole, nor