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Ethel Nicholson Browne

THE GENUS SPIROCHAETA.

Submitted in partial fulfilment of the requirements for the degree of Master of Arts, in the Faculty of Pure Science, Columbia University.

Approved
Edmund B. Wilson
May 28th, 1907.

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Spirochaeta.

Though most of the work on Spirochaeta has been done within the last two or three years, the organism was described and pictured as early as 1833 by Ehrenberg. Both Spirochaeta and Spirillum he classed as animals, together with desmids, volvox and other low plant forms. He described Spirochaeta and Spirillum as belonging to the family Vibrionia, characterized by an imperfect spontaneous division, the products of which remain together forming a spiral. However, he drew a distinction between the two genera by describing Spirochaeta as flexible, Spirillum as rigid. Later, the two genera were put into different kingdoms, the Spirochaeta being considered as Protozoa, the Spirilla as Bacteria. As each new spiral organism was discovered, a discussion arose as to whether it should be considered a Protozoon or a Bacterium. This raises the question as to the distinction between Protozoa and Bacteria. Bacteria were formerly believed to have no nucleus, but Shaudinn has shown that the nucleus is present in the form of distributed chromatin granules, which divide equally when the cell divides. This is also true for the nucleus of the lower protozoa, for example Tetramitus, which has the nucleus in the form of scattered granules. We cannot therefore distinguish Bacteria and Protozoa on the morphological structure of the nucleus. It has also been commonly held that the two can be distinguished by the method of division, a transverse division taking place in the Bacteria, a longitudinal division in the Protozoa. This, however, does not always hold, for there are some simple flagellates in which the division is transverse

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as it is in the Bacteria. Another criterion has been suggested with regard to cultivation on artificial media, that Protozoa cannot be cultivated, while Bacteria can. But this does not hold, for there are many Bacteria which cannot be cultivated, and the Protozoon Trypanosoma can be cultivated. There is then no hard and fast line between Protozoa and Bacteria; an organism is referred to the one or the other group rather on general considerations than because of any single characteristic.

Spirochaetae are placed in the simplest group of flagellate Protozoa, or Mastigophora. They may be described in general as thread-like, flexible, spirally-wound forms. The type of the genus is the one discovered by Ehrenberg, Sp. plicatilis, a free-living form which was found in water that had stood over winter. According to Schaudinn, this organism has an elongated body with a periplast wound spirally around it in the form of an undulating membrane. The ends are bluntly round off and there are no flagella. The body shows a small number of irregular spirals which are not fixed and may disappear and reappear from time to time. The nucleus appears as a long thread-shaped structure running along the axis of the organism, representing the kinetic nucleus of a trypanosome; scattered about this are chromatin granules which represent the vegetative nucleus.

The Spirochaeta that most resembles Sp. plicatilis is the organism described by Certes in 1882 as Trypanosoma balbianii, which Shaudinn believes belongs rather to the Spirochaeta. This is a parasite in the crystalline style

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and gut of oysters and other bivalves. Sp.balbianii is quite large being from 5 to 150 microns in length and 1 to 3 microns wide, the spiral having three to eight turns which are inconstant. It has no flagella and may or may not have an undulating membrane which, if present, is wound spirally around the animal leaving the rounded ends of the body free. The two latest accounts have been given by Laveran and Mesnil, and Perrin, and are somewhat at variance What Perrin regards as a true undulating membrane, Laveran and Mesnil think is merely a wide periplasmic sheath within which the body of the organism is more or less free. The nucleus, according to both accounts, consists of numerous small chromatic masses in the form of rodlets which are equidistant from one another. Perrin describes these rodlets as arranged in a single row upon a delicate spirally wound thread of achromatic material on the undulating membrane, connected at one end of the nuclear spiral by a delicate thread. This nucleus Perrin homologizes with the kinetonucleus of a trypanosome, but Woodcock denies this homology. The process of longitudinal fission, as Perrin describes it, begins with the division of the undulating membrane. The rod — like chromatic masses along the spiral thread fuse to form a rod of chromatin extending the length of the body. This rod then fragments giving rise to small bacilliform rods which become dumb-bell shape; the dumb-bells by transverse division produce chromosomes, about sixty-four in number, lying in a double row. The split which starts along the undulating membrane continues rapidly till it reaches the outer extremity, when the two organ-

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isms may remain united for sometime before separating; this giving the impression of a transverse division. In this condition of the body, the chromosomes fuse together and the nucleus is reconstituted. Besides the organism just described, which Perrin calls the "indifferent form", he also describes male and female gametes. The male gamete is formed from the indifferent type; a refractive swelling appears in the middle of the body, half of the chromatin is given off to it, and it splits off as a sort of polar body. A thin organism results with a reduced number of chromosomes, thirty-two. The female gamete is thicker than the male; its development has, however, not been observed. Conjugation between the two gametes was observed in very rare cases when the crystalline style was melting in the gut of the oyster. These reproductive forms of Perrin are regarded by Mesnil as involution forms. Encystment of the "indifferent" forms has been described by Perrin. There is no cyst membrane, but the periplast bursts and the protoplasm flows out and hardens to form a cyst-covering, while the organism becomes compact within. This Perrin considers intermediate between the spore-formation of the Bacteria where the protoplasm is cut off within the cell to form reproductive bodies, and the Protozoan encystment where the whole cell surrounds itself with a protecting membrane. The cyst here is extremely small although the organism may be as large as a paramoecium. If the smaller spirochaetes, such as Sp. obermeieri or Sp. pallida encyst and the cysts are relatively the same size, they are absolutely invisible. An encystment of female forms has

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also been described by Perrin. Here the nucleus is said to break up into several parts, all but two of which degenerate; these two fuse, suggesting parthenogenesis. The existence of cysts, however, has been denied by Mesnil who considers the so-called cyst forms as involution phases.

As to the position of Sp. balbianii there are several different views. Shaudinn considers it very similar to Sp. plicatilis in the presence of an undulating membrane, absence of flagella and the form of the nucleus, and would therefore class it with the true Spirochaeta. Perrin who was working in Shaudinn's laboratory and seems to have been permeated with the life-history of the trypanosomes is convinced of the trypanosome character of the parasite, finding in it the ancestor of the trypanosomes and the connecting link between these and the Bacteria; it resembles Bacteria, he says, in the form of the nucleus and in spore-formation. Laveran and Mesnil together with Léger, who has recently studied the organism, think that Sp. balbianii is not a Protozoon but a Bacterium.

Several other free-living or non-pathogenic parasitic spirochaetes have been found, one form much like Sp. plicatilis but living in salt water, another form resembling Sp. balbianii, parasitic in the crystalline style of the fresh-water mussel and called Sp. Anodonta. However, most of the spirochaetes seem to be pathogenic. Sp. dentium which lives in the human mouth was thought to be harmless, but Miller has recently claimed that it has pathogenic properties. Another parasitic found in the mouth is Sp. buccalis described by von Prowazek. Spirochaetes have also

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been found in the human intestine and in ulcers in differ-ent parts of the body. Among animals ,too,they seem to be quite wide-spread. Theiler described a form,Sp.Theileri, parasitic on cattle and transmitted by the tick,also a form which he called Sp.ovis,parasitic in the blood of the sheep,which may,however,be the same as the one in cattle. A third spirochaete he found in the horse's blood,and called it Sp.equi. The parasites have also been found in bats(Sp.vespertilionis),and recently in insects, in the intestine of the mosquito Anopheles maculipennis , and in Tsetse flies(Sp. glossinae).Sp.Anserinum,parasitic in geese,was discovered in 1890 by Sacharoff in the Caucasus. The disease produced by the organism is fatal, death occuring some days after the parasites disappear from the blood. It can be transmitted to geese by inject-ing into them blood containing the organisms;ducks and chickens can also be infected,but they recover more easily. The natural mode of infection is unknown,though it is supposed to be by an insect.

Perhaps the most carefully studied spirochaete disease occurring in animals is Spirochaetosis or Spirillosis of the fowl,Sp.gallinarum,the cause of the disease was discovered by Marchoux and Salimbein at Brazil in 1903 and was found to be transmitted by the bite of a tick, Argas miniatus,when the insect is kept at a temperature of 30° to 35° but not when kept at 15° to 18°. The disease is a relapsing fever,an alternation of high fever with normal temperature occuring,and it is often fatal. The spiro-chaetes have been found especially in the blood ~~of~~ ves-

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sels, the liver and the spleen. The organisms lie among the cells, but not within the cytoplasm, being distinctly intercellular not intracellular. They have however been found in all stages of degeneration in the leucocyte cells, indicating their destruction by these cells. Especially are they found in great numbers in the phagocytic cells at the end of the fever, when they disappear completely from the circulation. Some of the parasites evidently escape destruction at the crisis and reappear to give rise to a recurrence of the fever. The organisms were found by Levaditi to be present not only in the liver and spleen, but also in the ovary and within the ova; he suggested that they might be carried there by phagocytes which he found present in the ovary. Borrel, by experiments, showed that he could infect chick embryos with spirillosis by introducing into the fertilized egg a certain quantity of blood from a hen containing spirochaetes, even in eggs at the beginning of incubation; these embryos died within six or eight days, of spirillosis. With the unfertilized egg, he obtained no results. Levaditi confirmed the work of Borrel and by further experiments he reached the following conclusions. (1) The spirillosis does not end by a disappearance of spirochaetes from the circulation in embryos as it does in adults. This is due, he thinks, to the imperfect means of defense developed in the embryo. Thus the disease is much more severe in embryos than in adults, as is the case in congenital syphilis. (2) The liver is the organ first to be infected, by means of the umbilical circulation (3) Spirillosis is not hereditary; this is due either to the eggs containing the parasites being killed by them before they develop,or to the inability of these infected eggs to be fertilized. It has also been shown that chickens can be immunized against the action of the spirochaetes,and that the blood of such immune birds possesses marked preventative properties,even a slight curative action;in a mixture of immune serum and spirochaetal blood the organisms become immobile and agglutinate.

There are conflicting accounts of the morphology of Sp.gallinarium. Borrel claims that it has peritrichous flagella,that it has no undulating membrane,and that it divides by transverse fission. From Borrel's account,Novy and Knapp conclude that Sp.gallinarum is not a Protozoon but a Bacterium. Levaditi gives no description of the organism,but believes with Borrel that transverse fission occurs,indicated by the presence of many spirals united end to end,and the absence of Y-shaped spirals such as would indicate longitudinal division. Von Prowazek has more recently given quite a different account. He describes a deeply staining elastic band running from one end of the animal to the other which he compares to the undulating membrane of a trypanosome. Division,he says,is longitudinal beginning with this undulating membrane and continuing until the two organisms lie in a straight line but with a thin place in the spiral which Borrel took as evidence of transverse division. Von Prowazek has found no flagella. From these facts and from the fact that plasmolysis characteristic of bacteria is not produced,he concludes that the organism is a Protozoon.

Similar to Spirochaetosis of the fowl is the human

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disease,relapsing fever,likewise caused by a spirochaete. The organism was discovered by Obermeier in 1868,but owing to the fact that the fever occurs mainly in Russia and the Balkan peninsula,it has been little studied. The relation of Sp.obermeieri to the disease was demonstrated by inoculating man,monkeys,rats and mice with blood containing the spirochaetes. As to the natural mode of transmission nothing is definitely known,though the bed-bug Acanthia lectularia is commonly credited with being the carrier of the infection. The organisms are supposed to remain alive in these insects for a month or more;it is unknown whether they multiply there or not. According to Novy and Knapp,the length of Sp.obermeieri is from 7 to 17 microns or more. Short forms,7 to 9 microns long,about the length of a red blood corpuscle occur;these they believe represent ~~the size of~~ single cells which are united to form the long organisms The actual units may,however,be shorter ~~Spirochaeti~~S-forms into which the spiral breaks up in disintegration within ~~to~~ the phagocytes. The whole organism would thus be a catenoid colony of these ~~spirochaete~~ spirochaete forms strung together. If the blood of patients in which these forms alone were found was mixed with normal blood,the forms were found to grow out into typical cork-screws. The width of the spirochaete is.25 microns ,the number of turns in the short spiral (7-9 microns) two or three,the increase to four being preliminary to division. The turns in short forms are 2-2.7 microns apart;in the larger forms 1.5 microns from crest to crest,and the extreme width averages about 1 micron. The spirochaete has a long flagellum at one end. Novy and Knapp describe no internal structure, but others have shown granules to be present, which seem by the stain and by analogy with other organisms to be chromatin granules. The spirochaete is extremely mobile; it moves forward and backward and rotates along its long axis making a screw motion. It reproduces by transverse fission. Novy and Knapp have carried out extensive experiments on relapsing fever with the following results. A characteristic feature of the infection is the rapid disappearance of the spirochaetes in the circulation and their reappearance in a day or two, causing the alternation of fevers with crises. This is so in the case of man, monkeys and mice but there is no relapse in rats and consequently no reappearance of the organism. By examination of the blood of rats, it was found that the spirochaetes decrease in number as the disease goes on. This decrease is believed to be due to a germicidal substance which forms as a result of the multiplication of the spirochaetes. As evidence that such a substance exists, blood in which the organisms had been present was mixed with blood rich with spirochaetes; the spirochaetes were found to die in a very short time. An immunizing body distinct from the germicidal agent is also believed to be present, for blood with very little germicidal action was shown to prevent infection. Active immunity follows recovery from the infection; passive immunity can be imparted by injections of recovered blood. In relapses, a temporary immunity is thought to take place, due to the destruction of the organisms by the germicidal agent and, secondly, to the presence of a special immune body. Each relapse has the effect of an injection of spirochaete blood. Some organisms escape and are hidden in some part of the body, probably

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in extravascular places, till the antibodies have decreased, when they multiply and reappear in the blood causing another relapse. Hereditary immunity can be obtained and is probably due to infection "in utero". Preventative innoculations can be made successfully in rats, mice and monkeys, and a cure can be obtained by injection of immunized blood.

The organism of relapsing fever has usually been regarded as a Bacterium, though recently its protozoon nature has been suggested. Novy and Knapp are convinced that it is a Spirillum; they give the following reasons--

(1) Structural characteristics; there is no undulating membrane, and no nuclear structure is visible.

(2) A flagellum of wavy character is present at one end, similar to cholera spirillum. The flagella of the Protozoa are coarse and thick and have no regular wavy bends.

(3) Division is transverse, not longitudinal as it should be for Protozoa.

(4) Rapid multiplication agrees with the Bacteria and is at variance with trypanosome and plasmodial infections.

(5) Plasmolytic changes. When dialyzed in distilled water, Protozoa such as trypanosomes change their form and are killed in an hour or so: Bacteria come to rest without change of form and can be revivified. Sp. obermeieri acts like a Bacterium.

(6) Heat action--Trypanosomes (Ty. lewisi) exposed for 30 minutes at 45° are killed and disappear. Bacteria are still alive under similar circumstances and undergo no change when dead. Sp. obermeieri acts like a Bacterium in resist-

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ance to heat.

(7)Persistance of form- Protozoa tend to round up,Bacteria and Sp. obermeieri keep their form under adverse circumstances.

(8)Active immunity--Bacteria form powerful anti-bodies so that the serum acts like an active germ icidal agent. This is not true for Protozoa,though it is for Sp.obermeieri.

(9)Absence of Aërotropism--If a bubble forms under the cover glass,trypanosomes group around it;this is not so for Bacteria nor does Sp.obermeieri show any such tendency.

Many of these arguments are based o n too little evidence,generalizations being formulated for which there are not sufficient grounds. As against these arguments we may urge:-

(1)Transverse division does occur in Protozoa.
(2)Some Bacteria do plasmolyze.
(3) By analogy with other diseases,it seems almost certain that there is an intermediate host. No Bacterial organism is so transmitted.

Tick fever which is prevalent in many parts of equatorial Africa was shown to be due to a spirochaete in November 1904. Ross and Milne made the discovery in Uganda,and Dutton and Todd independently at the same time in Easterm Congo. The fever caused by the organism is much like relapsing fever,the patient having a high fever of 104° or 105° for three or four days,then an intermission occurs of about sixteen days during which he feels well,then the fever recurs. The symptoms correspond so closely to those of relapsing fever that Koch as well as Dutton and Todd believed it to be an African form of the European fever.Novy,however, is convinced that the two organisms are different. As experimental evidence,he has found that this spirochaete,which

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he calls Sp.duttoni after Dutton who lost his life in studying the disease,is usually fatal to monkeys while they recover from relapsing fever. In innoculated rats,the organisms were present from three to nine days after innoculation,which is not the case with Sp.obermeieri. Moreover, Tick fever is characterized by shorter attacks,and there are much fewer spirochaetes found in the blood than in the case of relapsing fever. Brenl and Kingnom have pointed out that rats and mice innoculated with Sp.duttoni usually die while Sp.obermeieri is not fatal;and that in rats,the former causes relapses while the latter does not.Morphological evidence as w ell as experimental is given to p rove to the distinction between the two organisms. The length of Sp.duttoni is 16 microns;of the long,dividing forms 32 microns; that is,twice th e length of Sp.obermeieri. There are but two or three turns in both spirochaetes though one is twice the length of the other. Sp.duttoni measures 4 or 5 microns between the turns,Sp.obermeieri only half that distance. The width of the whole spiral is 2 to 2.7 microns in Sp.duttoni,1 micron in Sp.obermeieri. Other differences are the rarity of the spirochaetes in Tick fever in comparison with their frequency in relapsing fever;the tendency of Sp.duttoni to curl up into figure-8 forms or circles which is absent in Sp.obermeieri;the presence of diffuse flagella,a terminal one corresponding to that of Sp.obermeieri and besides many all along the organism. There are several divisional zones in Sp.duttoni,and there is the same tendency as in Sp.obermeieri for the organism to break up into S-shaped fragments. The nucleus consists as in the

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other case of scattered chromatin granules,and in both cases division is transverse. The organism is transferred by the bite of the tick,Ornithodorus Moubata. Dutton and Todd found that young ticks hatched in the laboratory were capable of transmitting the disease,that is,the young ticks inherit this power from the mother. Koch,moreover,showed that spirochaetes were present in the eggs of the ticks, and that a fourth or fifth part of the eggs were infected. On examination of ticks from different localities it was found that 11% to as many as 50% contained spirochaetes. There is,however,no sign of multiplication in the tick; it seems that the parasite is merely harbored in the tick until transmission to a human host.

As in the case of Sp.obermeieri,Novy and Knapp are convinced that the parasite is a Bacterium,and for practically the same reasons,transverse division,absence of a definite nucleus and undulating membrane, and presence of flagella. The presence of diffuse flagella does seem to point to the bacterial nature of the organism,but it has been questioned whether they are true flagella,and moreover the fact that the parasite exists in an intermediate host would indicate its protozoon nature.

At the suggestion of Professor Köhler,President of the Imperial Board of Health,Shaudinn,working with Hoffman, undertook investigation to find out whether micro-organisms were present in syphilitic lesions. In the first report April 10,1905,he states that both in the living subject and in stained preparations,he found organisms which must be referred to the protozoan genus Spirochaeta . The organ-

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isms. were found not only on the surface of syphilitic papules and primary lesions, but also deep within the tissues and also within specifically infected inguinal glands. In another report, May 17, Shaudinn announces the discovery of spirochaetes in secondary syphilitic papules. Soon after, Metchnikoff and Wechselmann also found typical spirochaetes in secondary papules in which the surface was quite intact, and which were far removed from the genital organs, on the breast and back; these observations were confirmed by Hoffman and Shaudinn. On May 5, Shaudinn found Spirochaetes in blood drawn by puncture from the spleen of a syphilitic patient, the day before the appearance of the rash. Drs. Buschke and Fischer about the same time discovered spirochaetes in a case of congenital syphilis; in such cases they were also found by Levaditi, Shaudinn and Salmon soon after. Metchnikoff made the important discovery of the same spirochaetes in primary lesions of monkeys which had been innoculated with syphilis. Finally, only very recently, they have been found in tertiary lesions. After the first discovery of Spirochaetes by Shaudinn, there were confirmations by various workers; from all sides came reports that spirochaets had been found in syphilitic material; in fact in almost every case of syphilis ~~xxtxxixi~~ whether congenital, primary or secondary, spirochaetes have been found to be present. The spirochaete which Shaudinn first discovered and which was found subsequently in all cases, he called Sp. pallida. In the living state, this organism is extremely delicate, weakly refractile, has steep and narrow spirals. It is very difficult to stain, Giemsa's azur-eosin stain being the only one

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which Shaudinn used with success. Further,Sp.pallida is characterized by the possession of a single flagellum at each end; Shaudinn has been unable to demonstrate the presence of an undulating membrane,though he believes that it is present. The length of the organism,Shaudinn gives as varying between 4 and 14 microns,the width almost unmeasureable and at most .25 micron. The number of turns varies between six and fourteen;the distance between the nodes is 1.25 to 1.5 microns. The movements during life are characteristically of three kinds,rotation about the long axis,gliding movments forwards and backwards,and movements of flexion of the whole body.

In some cases,Shaudinn and others found another Spirochaeta present in the syphilitic lesions together with Sp. pallida and of about the same size as it. At first Shaudinn was uncertain whether the two were distinct species or not, but he became more and more convinced that they were. The second one,to which he gave the name Sp.refringens,is much like the type spirochaete,Sp.plicatilis Ehrenberg. He distinguishes it from the Sp.pallida by being more refractile in the living state,having a coarser form,having wider and flatter spirals,and being deeply staining with any ordinary Spirochaete stain,gentian violet,carbol fuchsin,or Romanowsky. The extremities of Sp. refringens are blunt,without flagella, and there is a distinct undulating membrane present. The two organisms are therefore quite distinct morphologically; they may occur side by side in the same lesion,or one may occur alone. The question arises whether both species are responsible for syphilis or only one. Since Sp.refringens

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has been found in complicated syphilis and in non-syphilitic diseases, whereas Sp.pallida occurs alone in uncomplicated syphilis and in congenital syphilis, and together with the other variety in cases of syphilis complicated with other diseases, it is probably Sp.pallida and not Sp .refringens that is concerned with syphilis. Other workers, however, Krzysztalowicz and Siedlecki, although they agree with Shaudinn in finding the two varieties, believe that the distinction between the two breaks down at certain periods in the life-history of Sp.pallida. When Sp.pallida contracts, they say, it takes on an appearance like Sp.refringens; it refracts the light, stains deeply and becomes coarser. Moreover the flagella which most of all distinguish Sp.pallida from Sp.refringens, these workers believe to be merely the ends of the body drawn out into a thread. Still other observers, for example Sh ennan, have shown that there are many intermediate forms between typical Sp.pallida and Sp.refringens, so that it is often impossible to refer forms to the one or the other group.

With regard to the nucleus of ~~the~~ Sp.pallida, owing to the small size of the organism, there have been conflicting statements. Shaudinn does not describe a nucleus; Krzysztalowicz and Siedlecki, however, assert that in every spirochaete they find a clear space near the ce nter of the organism; this round empty space is, according to their interpretation, a cellular nucleus. The empty appearance is caused by the very small amount of chromatin present. Just as in Coccidia, the chromatin is not present in the nucleus itself

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but in the nuclear membrane and karyosome, so here, they believe, the ch romatin is not all in the nucleus, but in small deeply staining bodies co mparable to karyosomes. Woodcock, however, thinks that what these workers take for a nucleas recalls the cytoplasmic vacuole of typanosomes, and is very unlike the typanosome nucleus. Wechselmann and Löwenthal claim to have seen nuclei in Sp.pallida with the aid of an ultramicroscope. Herxheimer describes small scattered chromatin granules which he compares to the blepharoplast of typanosomes, and larger chromatin granules which he thought represented the vegetative nucleus. The statements conflict, and we may say that nothing is really known about the nucleus of Sp.pallida. As with the nucleus, so in the matter of division workers do not agree. Löwenthal describes areas in the spirochaete where there are no stains, and where it breaks apart into two or more organisms; those areas occur at the nodes, so that here as in the case of Sp. obermeieri and Sp. duttoni, these S~~pirochaete~~-shape segments may represent the units of the organism; reproduction is therefore accomplished b y transverse division. Goldhorn also believes that transverse division takes place. Shaudinn, on the contrary, found in his preparations, spirochaetes w ith two flagella at one end, which he interpreted as a stage in longitudinal division. Krzysztalowicz and Siedlecki describe all stages in the longitudinal division. It commences, they say, at the pointed extremity, proceeds through the body until the two daughter spirochaetes are united end to end. S o they interpret the colonies of several spirochaetes in a single line as stages in division, before the daughter individuals completely break away from each other. This is the same

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explanation as was given by Perrin for the colonies of Sp. balbianii and by von Prowazek for Sp.gallinarum. Schultz says that he has been unable to find any trace of transverse fission in his preparations,while on the contrary he often finds two organisms closely united for a half or a third of the length of the body,which he interprets as a phase of longitudinal division. Besides this asexual reproduction by means of fission,Krzystalowicz and Siedlecki claim that sexual reproduction also takes place. Some Sp.pallida,they say,enlarge and pass into the typanosome stage;these are much like the contracted spirals but are wider and more fusiform. The body is drawn out as a long filament at one end,while the other end is short and conical. Between this extremity and the middle of the body lies the large ovoid nucleus. At one side of the nucleus is a deeply-staining corpuscle. Sometimes a deeply-staining filament can be recognized,starting at the corpuscle and running superficially in a sinuous course. This is regarded as comparable with the flagellar border of the undulating membrane of a trypanosome,the corpuscle representing the kinetonucleus These forms Krzysztalowicz and Siedlecki call "Trypanosoma luis." and they say that they play the part of female gametes or macrogametes;they may divide longitudinally just as the ordinary spirochaete. These macrogametes result from the growth of an ordinary individual,the contracted spirals representing a transition stage between the two. Probably, they think,all the organs,nucleus,undulating membrane etc. which are visible in the trypanosome phase are also to be found in the spirochaete form,but here they are too small to be discerned. Other individuals were also found,

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somewhat elongated and having several nuclei;besides these were other very minute individuals,3 microns long,with a single nucleus;these they believe come by fragmentation of the multinuclear forms. These small ones become still further reduced by repeated longitudinal division. They are regarded as micro-gametes or male gametes. Only in one case where Krzysztalowicz and Siedlecki able to find stages which resembled the process of conjugation,in a preparation from a large primary ulceration which was commencing to cicatrise. A male gamete becomes attached to a female by one extremity and the two gradually join together laterally and at length fuse completely. The subsequent development was not followed. The authors believe that a resting period ensues in which the parasite becomes encysted. Various enigmatical,rounded, and kidney-shaped bodies were observed which might have to do with cyst forms. Thus these workers claim a complete cycle for Sp.pallida such as is found in other Protozoa;a non-sexual,sexual and resting stage following each other in succession. Schultz objects to this work of Krzysztalowicz and Siedlecki on the ground that the smears were,for the most part, derived from ulcerated and secondarily infected lesions ;the cyst forms, he considers, stages in involution. Similarily Schultz attempts to establish a life cycle. He found Sp. pallida and Cytoryctes luis Siegel in syphilis and thought them to be stages of the same organism;further,he suggested that Sp.refringens might be a macrogamete stage,and Sp.pallida a microgamete. But this theory is invalidated,as Schultz points out,by the occurrence of Sp.pallida in all cases of syphilis and in syphilis only,while Sp.refringens is present in many non-specific lesions. Each worker gives to Sp.pallida a life cycle analogous to that of the organism to which he thinks it most nearly related. In reality we know nothing definitely of a sexual reproduction or the life cycle of Sp.pallida. In fact about all we do know of it is that it is a spiral organism with a fine flagellum at either end, which probably reproduces by longitudinal division.

Is Sp.pallida the cause of syphilis? Such would seem to be the case from the occurrence of the organism in all cases of syphilis, primary and secondary lesions, congenital syphilis and in innoculated monkeys, and its absence in other conditions. The demonstrations of the spirochaetes, however, in smear preparations seemed insufficient to establish their etiological significance; it was deemed necessary to stain and examine the organisms in the tissues. After many stains had been tried in vain, silver nitrate was suggested by Bertarelli, Volpino and Bovero, and this was used with success. Finally, Levaditi announced a method which has proved most successful in all spirochaete material. It consisted essentially of impregnating formalin-hardened material with nitrate of silver and reducing with pyrogallic acid; Giemsa is used as a counter stain. With this method the nuclei are stained blue, the spirochaetes black. By means of the Levaditi method preparations of different tissues were made. Levaditi himself and Shhultz examined especially cases of congenital syphilis in children still-born or dead two or three days after birth. Various organs were examined for spirochaetes and the organisms were found to be present in varying numbers. Levaditi found them in greatest numbers

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ranged in decreasing order: liver, lung, suprarenal capsules and the skin. And these are the organs

in the following organs, in the same order, that he found by histological examination to be most attacked by syphilis; the brain and kidney which are c omparatively free from syphilitic attack have few spirochaetes. He concludes that, since this isthe case and since many spirochaetes are found in still-born infants ~~the~~, Sp.pallida is directly connected with syphilis. The infection, he believes, in cases in which the disease comes fromthe mother, is by way of the placenta. This is made probably by the fact that the liver which is the first organ to receive the blood from the mother contains the most parasites, just as was the case in spirochaetosis of embryo chicks(Borrel and Levaditi). Althoug h the blood is the path by which the spirochaetes are carried from the mother to the foetus and from one organ of the foetus t o another, this is not the place where the spirochaetes choose to develop, for they are rarely found in the circulation; especially rare are they in the placenta. On the contrary, as in the case of Sp.gallinarum, they leave the vessels and penetrate their walls, thus reaching the connective tissue where they multiply. The parasites are found in the connective tissue of all organs but show marked preference for glandular epithelium, the cells of which they penetrate. Sp. pallida in contradistinction to all other spirochaetes, with the exception of the cancer spirochaete, is intra-cellular. Schultz points out that their presence in the cytoplasm of epithelial cells cannot be considered a phenomenon of phagocytosis, for the organisms are in a perfect condition and stain well. That phagocytosis, however, does occur has been shown by Levaditi; in congenital syphilis, this is very active. He has found them in all stages of degeneration, and the fact is further proved by the rarity of them in the spleen and the large amount in the liver. As to the position of Sp.pallida, most workers consider it a Protozoan though a few hold to its bacterial nature. Schaudinn concluded that it was a Protozoon for the following reasons;--(1) It has a single polar flagellum at each end instead of a group of flagella; (2) the spirals are numerous and very flexible; (3) There are undulatory movements over the whole body and the body may bend or lash, so that there are not stiff longitudinal axes as in Spirillum. Moreover the organism is cell-penetrating, a feature not found among Bacteria. Schaudinn, however, recognized the differences between Sp.pallida and the type Sp.plicatilis Ehr. The two characteristics of the Sp.plicatilis are the absence of flagella and the presence of an undulating membrane, features absent from Sp.pallida. He therefore took Sp.pallida out of the Spirochaeta group and accepted the name proposed by Vuillémin, Spironema, which was later changed to Treponema. Sp.refringens, on the other hand, agrees closely with the type Sp.plicatilis and is generally considered as a true spirochaete.

Although cancer is so prevalent that 11,000 deaths were caused by it in New York alone in the past year, its cause has always been a puzzle. All tumors are characterized by a great proliferation of cells in a certain region of the body. In contradistinction to a non-malignant tumor, the cells of a malignant tumor, or cancer, proliferate at the expense of the neighboring tissues; they penetrate the tissues and organs and send cells to other parts of the body through the

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circulation, giving rise to metastases, or new growths in other regions. Until six years ago carcinoma was known as a purely human disease, but since then it has been found in all vertebrates as low down as the fishes. In 1899, Leo Loeb found a rat with a curious sarcoma; he transplated this from rat to rat for three years when it was lost. In 1902, Jensen of Copenhagen discovered a mouse with cancer, the histolog y of which was identical with that of human carcinoma. With this discovery began the cancer research in many laboratories in all parts of the world, at Buffalo, Paris, Liverpool, Berlin, Heidelberg etc. Attempts have been made to transplant the mouse cancer to other animals but without success. In transplanting it to other mice two methods are used, either the tumor is removed and ground up with about three times its bulk of normal salt solution with a mortar and pestle and this is injected beneath the skin, or in the peritoneum; or else, a small piece of the tumor is cut out and placed under the skin of a normal mouse. The result is the same; in about two weeks a swelling appears which grows rapidly till a large tumor is formed which may be a third or half the size of the mouse. It is supposed that the growth is not on the part of the normal mouse, but that it is a growth of the transplanted cells themselves, caused by the virus injected with them. The Jensen tumor has been transplanted eighty times with about 90% of "takes" in some strains. The amount of tissue that has been proliferated as a result of the first Jensen tumor is enormous; it has been estimated that it would take a ray of light 105 years to penetrate the side of the cube such as this mass of tissue would form, and that

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it would be greater than the diameter of the sun. This great proliferation is only parellelled in Biology by the growth of cells from a fertilized egg and by the process of regeneration, both of which are normal growths, there being no breaking through tissue boundaries. To account for the growth, chemists, botanists and physiologists have proposed various theories. Ehrlich in 1906 working at the Frankfurt laboratory applied his well-worn "side-chain" theory. Farmer, Moore, and Walker, in studying the cytology of carcinoma at the Royal Research Laboratory, held the view that cancer is due to the fertilization of an epithelial cell by a lencocyte, accompanied by the ordinary mitotic process. Prof. Calkins has, however, shown that this so-called fertilization process is rather a degeneration of the lencocytes inside the epithelial cells, giving rise to "cell inclusions". The most satisfactory theory was given in 1882, called the Conheim theory, still held by many people. According to this hypothesis, some of the embryonic cells, instead of developing at the proper time, are secreted in the organism where they may remain inactive for from ten to forty years, when they suddenly become active, giving rise to these abnormal growths. Prof. Calkins has pointed out that this theory is biologically impossible; we cannot conceive how these embryonic cells lie latent for so many years and yet retain such vitality as to give rise to this enormous mass of tissue. According to Beard, the pancreas stops the proliferation of embryonic tissues. Applying this idea to cancer, he treated several mice with amylopsin and trypsin, and on finding that the cancer disappeared, he published an account of this

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as a cure for cancer. As a criticism to his work,it may be said that 40% of transplanted tumors recover spontaneously and even if this extract does cure the disease,it leaves the cause unexplained. Still another suggestion was offered by Ribbert who thought that the growth was caused by the anarchistic development of a cell which had gotten loose from the control of the organism. He has however not shown that such a cell is present,nor how it might give rise to the tumor if it were. Another line of argument was followed by Marchand,who conceived that there were toxins liberated from the organism from some such cause as mal-nutrition, and that these acted on susceptible cells causing them to proliferate. Now,either this toxin is an initial stimulus sufficient to keep the division energy going in this long series of Jensen mice,or the stimulus is recreated in each new mouse. If the former were the case,the stimulus would weaken,but on the contrary,whereas there were only 4 % or 5% of successful transplants in the beginning,there are now 90%. If new stimuli are set up in each new organism,we might suppose that the stimulus reproduces itself. No known toxin, no inorganic thing can do so; it must therefore be a living organism. The parasite theory is in utter disrepute among medical men,for the symptoms of cancer are very different from those of other parasitic diseases. The parasite,however,of the mouse tumor was discovered by means of the Levaditi method by Gaylord. Spirochaetes have been found in all the Jensen tumors examined,in the Buffalo tumor and in a primary tumor which recently developed in a mouse at Columbia University by Prof. Calkins.The rarity of spirochaetes in primary tumors may be related with the great difficulty in transplanting primary cancers, only 2 out of 38 having been done successfully. Spirochaeta microgyrata(Lowenthal) var. Gaylordi, the cancer spirochaete, was first found in an exposed lesion of human cancer. It is characterized by the close approximation of the nodes, of which there are about six but varying from four to thirteen. The thickness of the spiral is .6 micron; the length from node to node is .6 micron: it has blunt ends with no flagella. This spirochaete like Sp.pallida is intra-cellular. This organism was also found in dog tumor. Borrel discovered some other spirochaete in the blood of mice which developed a tumor. Another organism Sp.muris was found by Wenyon in mouse carcinoma, differing from Sp.microgyrata in having the nodes about twice as far apart; this has not been found in other cancers so that it is probably a specific organism. Since the parasite theory has been proposed, it has been suggested that the proliferation of tissue may not be a malignant response to a stimulus by the tissue, but that it may be a protection against foreign invasion, a phagocytic reaction to draw the extraneous matter into its cells. This idea is given weight by the fact that the parasites are found in all stages of degeneration in the lencocytes. Interesting in this connection is the experiment of Dr. Fischer who injected some olive oil mixed with scarlet red into the ear of a rabbit. After a couple of weeks it was found that proliferations of the epithelium extended down toward the oil drops and enclosed them. After a while this epithelioma ceased and the normal skin was left This experiment showed a definite response of the epithelial

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tissue to a foreign substance,a sort of chemotaxis. Now if we apply this to cancer,we find the explanation of the proliferation of tissue,the metastases and the transplanted tumors.The parasite theory is given still more weight by an infection of rats which took place from some cages in which rats with cancer had been kept by Loeb a year or so before. This infection suggests an intermediate host in which the spirochaetes pass part of their life history,just as other spirochaetes have been found to live a while in ticks.

Since Spirochaetae have been so universally found in mouse tumors,it would seem probable that they are present also in human carcinoma,which is so similar to the mouse carcinoma. Material from human cancer has been put up by the Levaditi method,but spirochaetes similar to Sp.microgyrata have not been found. Two possibilities are open,(1)Spirochaetes are not present. In this case either the two cancers are alike,and the spirochaetes found in the mouse tumor have nothing to do with it;or the two cancers are utterly different,and spirochaetes are connected with the mouse tumor but not with the human. Neither of these alternatives seems probable.(2) Spirochaetes are present but have not yet been discovered. This seems very probable when we consider the rarity of the parasites in primary mouse tumors. It is necessary to look through a great deal of material before one can feel sure that the tumor is absolutely free from spirochaetes. Until more material has been examined,the possibility exists that spirochaetes somewhat similar to Sp.microgyrata will be discovered in human carcinoma.

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