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SPIROCHÆTES *

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TO-DAY a spirally shaped micro-organism may be called either spirochæta, spirillum, treponema, spironema, cristispira or saprospira, according to the characteristics of the organism. The choice of the generic name for a given variety is still very much dependent upon the individual views held by different investigators, and this has led to a somewhat chaotic state of affairs in the nomenclature of this group of organisms. This is found to be the case more especially in the medical literature where these minute spiral organisms play an important part as causative agents of certain diseases. Nevertheless, thus far but little attention has been paid to the systematic position occupied by them. Since Ehrenberg [1] in 1838 introduced a new generic term "Spirochæta" to designate a free living spiral organism which he found in a swamp near Berlin, it remained practically unnoticed until 1904, when Schaudinn [2] stated as his view that certain spirochætes constitute a phase of the life cycle of trypanosomes; hence, that they are of protozoan origin instead of being plants. It may here be mentioned that Ehrenberg, Migula, and other systematists classified Spirochæta under bacteria, which classification was accepted for nearly seventy years. Indeed, it was not uncommon among medical authorities to employ the terms Spirochæta and Spirillum interchangeably. Medical men may consider the causative agent of relapsing fever as being either a Spirillum or a Spirochæta, according to their inclination. This sort of indiscriminate use of terms has gradually extended to other spiral organisms, such as the causative agent of syphilis. According to the old school it was of very little importance whether a spiral organism had one or two polar

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flagella or a tuft of flagella, so long as both Spirochæta and Spirillum belonged to the same family. On the other hand, Schaudinn and his school maintained that the difference between Spirochæta and Spirillum is no longer so easily disposed of, since one is of plant and the other of animal origin. The revolutionary view of Schaudinn was based chiefly upon his observations on a protozoan, Leukocytozoon ziemanni, found in the blood of the Little Owl (Athene noctua), and regarded by Schaudinn as a trypanosome, which is said to undergo a spirochætal stage while passing through an intermediary host (Culex pipiens). While the accuracy of Schaudinn's observations has been questioned by later investigators,[3, 4, 5] the great impetus which his theory occasioned has had a far-reaching effect upon the development of our present knowledge concerning the organisms generally known as "spirochætes." It was soon after announcing his views that Schaudinn made his famous discovery of the occurrence of Spirochæta pallida in syphilis. In their first publication Schaudinn and Hoffmann[6] gave the name Spirochæta pallida to the spiral organism found in syphilitic lesions because of its resemblance to spirochætes in general, but within a year Schaudinn recognized certain features (preformed cylindrical spiral filament, difficulty in staining, regularity of curves, etc.) which he considered distinctive enough to classify it apart from the usual spirochætes (changeable curves, taking on of a violet component of Giemsa, no preformed spiral, ribbon form, etc.).[7,8] Thereupon he replaced the generic name Spirochæta with a new term Treponema.[9] This all occurred in 1905. But before Schaudinn had had time to decide upon a new generic name for his organism, Vuillemin[10] (1905) proposed that it be called Spironema. In the meanwhile some authors, particularly in France, commenced to use the term "spirilla." There were also some newer generic names created by still later systematists; for example, Microspironema[11] (Stiles and Pfender*), Borrelia[12] (Swellengrebel), Spiroschaudinnia[13] (Sambon), and Spiro-

* The statement of some authors (Gross and Gonder) that this antedated Schaudinn is erroneous. Schaudinn published his note on Oct. 19, and Stiles and Pfender on Dec. 2 of the same year.

soma [14] (Schilling), but these are of no importance to-day. The only difficulty in choosing the generic name for "Spirochæta pallida" lies in the fact that although Schaudinn corrected his error within several months after his discovery another suggestion had meanwhile been made to answer the same purpose, and according to the international code of nomenclature Vuillemin's Spironema would have had to receive preference over Schaudinn's own Treponema, had it not been for the fact that the term Spironema as proposed by Vuillemin is not acceptable to those who maintain, like Schaudinn, that the organism of syphilis belongs to the Protozoa, because in 1892 it was used by Klebs as a genus of Flagellate.[15] The same name had also been used by Meek in 1864 for a fossil snail. Of course, "Spironema" may be available for any one who holds that "spirochætes" do not belong to Protozoa.* Thus, Gross [16] (1910) used this term to include various spirochætes allied to the spirochætes of relapsing fevers, syphilis, etc., with the specification that he believed these to be of a bacterial nature. It may be mentioned that the term "Spirochæta," as taken up by Schaudinn in 1905 in the sense of protozoan organism, had already been used by Michael Sars in 1856 for an annilid genus. It seems that the creation by Schaudinn of the genus Treponema was perfectly justified, although not all the characteristics attributed by him to this genus are found to be distinctive from those of other "spirochætes." Schaudinn did not live long enough to witness the gradual modification which the Spirochæta question went through. As a result of the works of various systematists and zoologists, we are brought to realize that the original S. plicatilis, described by Ehrenberg in 1838, is an entirely distinct organism and bears little relation to the other organisms which we now call "spirochætes." We also know that the latter should no longer be designated as spirochætes, and that the spiral organisms found in the crystalline style of various mussels are neither trypanosomes, as held by Perrin [17] nor typical

* The use of two identical terms, one in the animal and the other in the plant kingdom, has been known to occur and is permissible. For example, "Bacillus" and "Coccus" are found in zoölogical as well as in botanical genera.

spirochætes, but form another group which may be seen to possess one or more genera. These facts were revealed after the death of Schaudinn by the careful studies of Novy and Knapp,[18] Schellack,[19] Gross,[20, 21] Zuelzer,[22] Gonder,[23] Dobell,[24, 25, 26] Hoelling,[27, 28] Fantham,[29, 30] Swellengrebel,[31] Bosanquet,[32, 33] and others. Although much light has been thrown upon the structure of these organisms, no definite conclusion has yet been reached as to the affinity of the "spirochætes" in the system of natural history. While there are still some who consider "spirochætes" as allied to bacteria and others who regard them as of a protozoan nature, there now appear to be certain authors who are inclined to set them apart both from bacteria or protozoa and to place them in the domain of the Protista, *i.e.*, organisms belonging to neither plant nor animal. Dobell [24] represents this view, and Doflein [34] compromises by calling them Proflagellates, and placing them between Bacteria and Protozoa. Zuelzer [22] holds a somewhat similar opinion to that of Dobell. In order to bring up some of the more important data relative to the question of classification, we shall now review the present situation.

As remarked at the beginning of this paper the Spirochæta of Ehrenberg was regarded as a genus of the family of Spirillacea and no question was raised in regard to its possible affinity with the Protozoa until the publication of Schaudinn's fascinating observations on Leukocytozoön ziemanni. Since that time there have appeared numerous partisans of Schaudinn's view that so-called spirochætes are of protozoan nature. Their main contentions are based on the following characteristics: (1) Longitudinal division as the mode of multiplication; (2) presence of an undulating membrane; (3) high degree of bodily flexibility; (4) absence of cell membrane; (5) absence of a motor organ such as the flagella; (6) presence of a periplastic process; (7) peculiar nuclear arrangements; (8) band-like bodies; (9) encystation or resistant form; (10) a certain periodicity in their pathogenic activity in the infected hosts; and, (11) effect of certain chemicals such as sodium taurocholate, saponin, etc., which bring about the dissolution of these spiral organisms and thus offer a contrast to the great resistance shown by bacteria (especially spirillum) to these substances. The foregoing characteristics tended to place the spirochætes in the Flagellate group, but subsequent studies by different investigators, especially those who have employed a more recent and approved cytological technic, seem to indicate that many of the above criteria were based upon erroneous or insufficient observations. According to the observations of Dobell,[24]

Gross,[16, 20, 21] Zuelzer,[22] Swellengrebel,[30] Novy and Knapp,[18] and others, the following features are characteristic of "sphirochætes."

1. In the case of the majority of "spirochætes" transverse division is the only mode of multiplication (Koch, Levaditi, Fraenkel, Novy and Knapp, Borrel, Gross, Zuelzer, Swellengrebel, Schellack, etc.). Only in certain pathogenic small varieties has the occurrence of longitudinal division been reported.[7, 35]

2. No undulating membrane has been definitely demonstrated in any spirochæta. The alleged undulating membrane depicted by Perrin[17] and Schaudinn[36] in the dried preparations of certain mussel spirochætes is an artefact brought about by improper fixation, namely by the torn crista of a cristispira.[20]

3. The alleged chromatin rods and spirals described by Perrin in the case of certain mussel spirochætes known as Spirochæta balbianii (Cristispira) are now said to be nothing but a distorted arrangement of volutin substance or chromidial granules which under optimum fixation gather themselves along the walls of the chambered structure of the cell body.

4. The absence or presence of cell membrane seems to depend upon the variety of "spirochætes." Thus, the original type organism of Ehrenberg was described as being devoid of a membrane and is still so regarded by all who have studied this organism. On the other hand the mussel spirochætes and various small parasitic species are now said to be provided with a thin but elastic membrane which cannot be differentiated from the cell body by means of staining reactions. The presence of a membrane would suggest a close affinity with Spirillum, but the latter has a stiff non-elastic membrane.[23]

5. In regard to the motor organ no generalization can be made. The original type organism of Spirochæta and all mussel spirochætes are devoid of any motor apparatus. On the other hand, a terminal process, consisting of a delicate, elastic filament with minute, regularly set curves, may in the case of various small parasitic spirochætes be found to project from one or both ends of the body. Borrel[37] and Zettnow[38] obtained some preparations in which the Spirochæta of fowl spirillosis and relapsing fever appeared to possess peritrichial flagella, but this must have been a case of artefact formation as no one has since been able to confirm their findings. Schaudinn considered the terminal process to be identical with the periplastic appendage of a flagellate.

6. Certain spirochætes such as S. balanitidis and S. buccalis, and others, were said by Schaudinn,[36] Hoffmann and Prowazek,[39] to have a flattened, ribbon-formed body. Later investigations hold that the body is cylindrical and round on section.

7. Encystment, or the resting stage, such as observed in protozoan organisms, has been suggested[37, 40] as existing, but never satisfactorily proved.

It will be seen that the findings of later investigators deduct much of the foundation upon which the protozoan theory of "spirochætes" had been based. Not only do they separate the spirochætes from the Protozoa, but they also bring out certain new facts which make it difficult to include them among the Bacteria as was formerly done by those who opposed the view of their protozoan nature. As has been briefly remarked, the spiral organisms called spirochætes are not of uniform structure, but, according to recent investigations, fall under several great divisions. It was owing to the imperfection of the methods of study that the free living forms and numerous parasitic varieties were at one time all held to belong to the same genus. Since the introduction of dark-field microscopy [41] many points which could not be satisfactorily determined with stained specimens have been carefully checked up and the entrance into the field of certain excellent cytologists has helped to clear up many points relating to the systematic grouping of these organisms. These cytologists made extensive series of comparative studies, at the same time carefully examining the structure of bacteria, spirilla, spirulina, and oscillaria.

As has been pointed out by Bütschli, [42] [43] bacteria are composed of a central body and a plasmatic layer. The former contains volutin granules and some chromidial elements. The spirillum has a series of chambers, each of which is constructed like a single bacterial cell. Both are covered with a stiff cell membrane. The structure of Spirulina is similar to that of Spirillum, differing from the latter by the highly flexible character of the membrane. Now, a very similar structure was demonstrated by Gross [20] in the body of mussel spirochætes and speedily confirmed by Dobell, [21] Zuelzer [22] and others. Gross, Dobell and Zuelzer all agree that the original free-living Spirochæta described by Ehrenberg is a unicellular organism which bears no relation either to the mussel spirochætes or to the small parasitic varieties. This fact implies the dissociation of the long used term "Spirochæta" from those organisms which in reality were commonly known as spirochætes. Odd as it may seem, the true Spirochæta has been but rarely studied, even by biologists, and certainly not to any great extent by medical men who have so much to do with the so-called "spirochætes."

Gross [23] was the first person who proposed to distinguish the true spirochæta from the other varieties of spirochætes by creating new genera for the latter which, according to his studies, could not be classified with spirochæta in the strict sense of the term. Thus for the latter type he created the name Cristispira (those with Crista), for the large parasitic spirochætes in fresh shell fish, saprospira (those without Crista), and the small parasitic varieties, including all pathogenic species, he designated as Spironema. Gross maintains that Cristispira, Saprospira and Spironema belong to the bacteria and places them under the family name of Spironemaceæ. Gross and Bosanquet recorded a few instances in which certain mussel spirochætes went into sporulation comparable to the true bacterial feature.

Dobell and Zuelzer both admit the striking resemblance between the chambered structure of Spirillum and Cristispira, but cautiously avoid accepting the bacterial theory of Gross on the ground that the last-named organisms have a more elastic and flexible membrane and that they are not necessarily bacteria. Dobell, as has been stated, has proposed a new family name Spirochætoidea which should include not only Gross's Spironemaceæ but also Spirochæta. The writer does not accept Gross's Spironema as it was applied to a flagellate in 1892 (Klebs), but retains Schaudinn's Treponema to designate all small parasitic and pathogenic varieties. He does not consider that there is a sufficiently essential difference between them to warrant two genera. Zuelzer regards the affinity between the mussel spirochætes and spironema (one of the Cyanophyceæ genera) as being much closer than that between these types and spirillum. On the other hand, Gonder accepts the classification of Gross more completely. He does not, however, share Gross's view that these organisms are definitely of a plant nature, holding that certain features indicate their partial affinity to the protozoa. He also differs from Gross in retaining Schaudinn's term Treponema for the organisms of syphilis and yaws and such affections, while accepting Gross's term Spironema for other varieties such as the spirochætes of relapsing fevers, tick fever, etc. The situation is still confused.

CLASSIFICATION AFTER GONDER (1912)

SPIRONEMACEA (GROSS, 1910)

Spirochæta (Ehrenberg, 1838)	Type: Spirochæta plicatilis, etc., all free living.
Cristispira (Gross, 1910)	Type: Cristispira balbianii, and other varieties found in mussels.
Spironema (Vuillemin, 1905)	Type: Spironema recurrentis, and other parasitic and pathogenic varieties living in blood.
Treponema (Schaudinn, 1905)	Type Treponema pallidum, Treponema pertenue, and other varieties with closely set spirals.

CLASSIFICATION AFTER GROSS (1912)

Spirochæta (Ehrenberg)	Type: Spirochæta plicatilis. Unicellular organism without a membrane or flagellum, highly flexible. Free living. Transverse division.

SPIRONEMACEA (GROSS, 1912)

Cristispira (Gross, 1910–11)	Including different varieties living in certain mussels. C. balbianii, C. anodontæ, C. pectinis, etc. All possess a crista. Chambered structure of the body. Sporulation. Transverse division.

Saprospira (Gross, 1911)	Similar to the foregoing except that there is no crista. Found in foraminiferous sand. Sporulation. Transverse division.
Spironema (Vuillemin, 1905)	Including small parasitic varieties: S. pallidum, S. pertenue, S. recurrentis, S. gallinarum, etc. Probably multicellular (or chambered). Transverse division. Flagella or terminal thread present.

Classification After Dobell

spirochætoidea (dobell) 1910–1911

Spirochæta (Ehrenberg, 1838)	Free living forms, fresh water or marine. Spirochæta plicatilis (Ehrenberg) Sp. gigantea.
Treponema (Schaudinn, 1905)	Parasitic in animals, vertebrates and invertebrates. T. pallidum (Schaudinn), T. recurrentis, T. dentium, etc.
Cristispira (Gross, 1910)	Parasite in Lamellibranchiata (mussels). C. balbianii certes, C. anodontæ, C. pectinis, C. veneris.

Classification After Migula (1897)

Bacteria	Coccaceæ, Bacteriaceæ, Spirillaceæ, Chlamydobacteriaceæ and Beggiatoaceæ.

spirillaceæ

Spirosoma	Rigid; no organ of motion.
Microspira	Rigid; one seldom two or three, polar wavy flagella.
Spirillum	Rigid; polar tufts of 5–20 flagella, mostly semicircular or wavy.
Spirochæta	Flexuous, motion organ unknown, probably an undulating membrane.

Classification After Swellengrebel

Bacteria	Spirochætaceæ	More or less distinct properties of protozoa but not much more so than the bacteria capable of forming S or Fe in contrast to those producing nitrification. Plasmolysable like the Spirilla.
	Spirillaceæ	
	Coccaceæ	

Classification After Levaditi (1912)

spirillaceæ (migula)

1. Sub-family, Spirillacea (Swellengrebel). Not flexible.
   - 1. Genus, Spirillum.
   - 2. Genus, Vibrio.

2. Sub-family, Spirochætacea. Spirally flexible, surrounded by periplastic cover, with or without flagellum-like appendage.
   - 1. Genus, Spirochæta. No preformed spiral; loss of spiral form at rest.
     - a. No flagellum with undulating membrane.
     - b. Flagellum, but no undulating membrane.
       - One or two terminal flagella.
         - S. buccalis.
         - S. balanitidis.
         - S. refringens.
       - Peritrichial flagella.
         - S. gallinarum.
         - S. duttoni.
         - S. obermeieri (?)
   - 2. Genus, Treponema. Preformed spiral; no visible undulating membrane.
     - a. Flagellum at one end.
       - T. pallidum.
       - T. frænboesia.
     - b. Flagellum at each end.
       - S. dentium.

Let us consider each of the groups in some detail on the basis of a newer classification.

Spirochæta.—According to Schaudinn [36] the type organism of Spirochæta possesses certain features which are also found in trypanosomes—an undulating membrane, periplastic fibrillar process, longitudinal division, etc. But this apparent resemblance has been shown to be erroneous. Thus, according to the latest contributions made by Zuelzer,[22] the original type organism, Spirochæta plicatilis, has no chambered structure, but is provided with a straight fibrillar axial filament surrounded by a plasmatic spiral layer which covers it unequally in different places. The organism consists of a single cell. Volutin granules which can be demonstrated by certain microchemical reactions are regularly disposed within the plasmatic layer. During motion the plasmatic layer at a given position becomes thickened or reduced in volume according to the current of the substance. The spirals of the plasmatic layer surrounding the straight axial filament occur regularly and closely, while the whole body shows several irregular undulations. There is no flagellum or periplastic terminal process, and no membrane has been demonstrated. It measures 100–200μ on an average, sometimes attaining a length of 500μ, whereas it is only 0.5–0.75μ in width. Unlike the other spiral organisms bearing the name of spirochæta (undoubtedly indiscriminately applied) the members of this group of real Spirochætes do not swim, but their locomotion is effected by a creeping movement along the surface of a supporting object. Multiplication is brought about by transverse division which is effected by a thickening of a certain part of the axial filament where a cross fissure takes place, followed by the strangulation of the plasmatic layer at the corresponding spot. Since Ehrenberg described the first species, four more species have been added, one by Cantacuzène in 1910,[46] and three by Zuelzer in 1912.[22] They are all free living and are not known to be responsible for any pathological conditions in either human beings or animals.

Since the essential characteristics of the group of true Spirochæta do not agree with those of various other species hitherto unreservedly called spirochætes, the necessity of reclassification

became apparent as soon as these facts were known about 1910, whereupon Gross, Dobell and others undertook special studies in this connection. As has been mentioned, Gross, Dobell, and Gonder all possess their individual ways of classification, but all agree on one point, *i.e.*, that the majority of the organisms known as spirochætes are not spirochætes in the strict systematic sense and must, therefore, be differently designated. Gross was the first to do this and he was followed by Dobell and Gonder, who introduced some modifications, but it seems that the family term Spironemacea of Gross has found a wider acceptance than Dobell's Spirochætoidea, although both include practically the same constituent organisms under a slightly different generic name. Thus Dobell accepts Gross's generic names Cristispira and Saprospira (provided that this genus can be recognized by other investigators) to cover the varieties found in shellfish, while preferring to use Treponema instead of Spironema as proposed by Gross. Dobell's family Spirochætoidea comprehends, besides all the constituents of Gross's family Spironemaceæ, the genus of the true Spirochæta. Whether the segregation of Spirochæta from the other genera composing Spironemacea is justified or not seems still debatable, inasmuch as the differences between the genus Cristispira and the genus Spironema are, I believe, no less striking, and induced Gross to separate the Spirochæta from them. According to personal observations on small "spirochætes" there seem to exist more affinities in the structure of true Spirochæta and the small parasitic varieties than are assumed by Gross and other investigators. For the present I will dwell upon different groups of organisms which those investigators have classified as separate genera, and in order to give a basis for further development of the subject, I propose to employ the new generic names proposed by Gross without, however, committing myself to his views.

Cristispira.—This genus was created by Gross in 1910 for the large saprophytic commensal spiral organisms found in the alimentary canal of certain varieties of shellfish. They are chiefly found in the crystalline stile which is a jelly-like projection in the stomach. The most unique feature of the genus is the presence

of a crista or ridge which extends spirally along the whole length of the body, whence the name Cristispira. Certes [47] considered the type organism of the genus Cristispira balbianii to be a trypanosome on account of the presence of an undulating membrane (later recognized by Gross as a ridge) and it has been called Trypanosoma or Spirochæta indifferently. Laveran and Mesnil [48] in 1901 regarded it as allied to the bacteria. Perrin [17] in 1905–1906 took up the subject and arrived at the conclusion that it has many features in common with the trypanosomes. This he observed from stained preparations in which he found an undulating membrane, a spirally arranged nuclear rod, as well as various mitotic figures and longitudinal division. Perrin's observations were in part confirmed by Keysselitz, [49, 50] Swellengrebel,[31] Hoelling,[27, 28] Gonder,[45] and Fantham,[29] but a later investigation of Schellack [19] brought out an entirely different set of facts. According to Schellack the undulating membrane and spiral nuclear rod or alleged karyokinetic figures are an artefact caused by improper fixation (dry method). In properly fixed preparations the cell-body is composed of an alveolar protoplasm and contains a number of transverse walls. In their later works Gonder [45] and Fantham [29] confirmed Schellack's observations. Zuelzer [22] and Dobell found chromatin (and volutin) granules to be deposited along the surface of the transverse septa, while Gross [20] failed to see any chromatin granules in Cristispira. On the other hand, Hoelling thinks that the entire cell-body is saturated with diffuse chromatin substance. The chambered structure of the cell-body is regarded by Gross as a sign of the multicellular nature of the organism, but many authors hesitate to accept this view, maintaining that it is a single organism with numerous cross septa. Gross, Zuelzer and Dobell all agree that the cell-body is surrounded by a strong membrane similar to that found in bacteria, although Zuelzer distinguishes it from the latter by its high flexibility. They found that the membrane had a double contour and protected the cell-body from the solvent action of various substances, such as saponin, as well as from acids and alkalies, a fact explained by Gonder as not necessarily due to the presence of a membrane but to the more concentrated external

fibrillar layer on the cell surface. In fact, Gonder described a fibrillar appearance of the external layer of the cell-body after the organism had been acted upon for some time by certain chemicals.[45]

Opinions still vary as to the origin of the ridge or crista. Earlier workers viewed it as an undulating membrane.[17] Gross, Zuelzer and Dobell hold that it is a superposed structure having no direct connection with the cell-body, while Schellack regards it as a true periplast traversed by numerous fibrils. He believes that the so-called undulating membrane of the authors of the term Cristispira is an artefact produced by defective technic. Hoelling as well as Fantham and Porter [35] entertain a view similar to that of Schellack, and the presence of a myoneme in the periplast was even maintained by Fantham and Porter. Mackinon [51] and Vlès [52] were unable to demonstrate any myoneme in the periplast, although Borrel and Cernovedeanu [53] assume that there exists a myoneme in the membrane which enables it to flatten or fold the ridge. When the organism is subjected to macerating or solvent agents (saponin, acid, alkali, etc.), the membrane is first attacked. The delicate fibrils become quite distinct in the course of dissolution, but the whole structure finally disappears completely, showing the plasmatic nature of the membrane. The cell-body is much more resistant.

Division is exclusively transverse according to the investigations of Schellack, Gross, Zuelzer, Dobell, and Laveran and Mesnil, while earlier investigators (Perrin, Keysselitz, Gonder, etc.) considered it longitudinal. Fantham and Porter [35] working with S. obermeieri and S. duttoni found both modes of division to occur. It is possible that a peculiar mode of division, described by Gross [20, 54] as an *incurvation*, might have been the cause of mistaking it for longitudinal division. Incurvation is a phase of the transverse division of Cristispira, whose body first doubles up (incurvates) at the segment where the fission is to take place and then after some time completes the process. During the incurvation both halves of the organism intertwine and simulate a stage of longitudinal division.

Sporulation was described by Gross [44] who saw a Cristispira

produce a series of somewhat smaller, highly refractile, oval bodies out of the square chambered structure of the cell body. These oval bodies were seen to separate into individuals, but no new cristispira could be made to sprout out of these bodies (or so-called spores). Bosanquet [32] made a similar observation. The question of sporulation is still open to further confirmation and is very important in view of the divided opinion regarding the affinity of this group in the system.

The cell-body is highly flexible, round on section, wavy or spirally wound, possessing not more than three or four curves. There are neither flagella nor terminal projections, except in one small species, C. spiculifera, which Schellack described as having a terminal filament.

There are about 18 known species which inhabit different varieties of shellfish belonging to nearly twelve different genera of Lamellibranchs, including common oysters and fresh water mussels. These genera are Ostrea, Anodonta, Chama, Pinna, Mactra, Pecten, Modiola, Lima, Gastrochæna, Saxicava, Tapes and Umo. Cristispira balbianii and C. anodontæ are the largest species and measure 100–130μ in length and 3–5μ in width, while the smallest representative of the genus, C. papillosum, measures but 18.5–20μ by 1.1–1.4μ.

Saprospira.—Gross [20] proposed to introduce this genus in order to group together a new species of mussel "spirochætes" which distinguished themselves from Cristispira by the absence of a crista. Their habitat and other cytological features are the same as those noted in the Cristispiræ. According to this investigator, Saprospira grandis and S. nana undergo multiple transverse division and bear a more distinctly bacterial aspect.

Spironema and Treponema.—Under Spironema Gross classified all the pathogenic and small saprophytic varieties. Dobell [24] substituted Spironema for Schaudinn's Treponema on the basis that the former term was applied to a flagellate (Spironema multiciliatum,[15] by Klebs, in 1892; for he did not consider it necessary to create two genera out of these organisms. Gonder still hesitates to drop the distinction between the group of "blood-spirochætes" and that of "tissue-spirochætes," the latter con-

produce a series of somewhat smaller, highly refractile, oval bodies out of the square chambered structure of the cell body. These oval bodies were seen to separate into individuals, but no new cristispira could be made to sprout out of these bodies (or so-called spores). Bosanquet [32] made a similar observation. The question of sporulation is still open to further confirmation and is very important in view of the divided opinion regarding the affinity of this group in the system.

The cell-body is highly flexible, round on section, wavy or spirally wound, possessing not more than three or four curves. There are neither flagella nor terminal projections, except in one small species, C. spiculifera, which Schellack described as having a terminal filament.

There are about 18 known species which inhabit different varieties of shellfish belonging to nearly twelve different genera of Lamellibranchs, including common oysters and fresh water mussels. These genera are Ostrea, Anodonta, Chama, Pinna, Mactra, Pecten, Modiola, Lima, Gastrochæna, Saxicava, Tapes and Umo. Cristispira balbianii and C. anodontæ are the largest species and measure 100–130μ in length and 3–5μ in width, while the smallest representative of the genus, C. papillosum, measures but 18.5–20μ by 1.1–1.4μ.

Saprospira.—Gross [20] proposed to introduce this genus in order to group together a new species of mussel "spirochætes" which distinguished themselves from Cristispira by the absence of a crista. Their habitat and other cytological features are the same as those noted in the Cristispiræ. According to this investigator, Saprospira grandis and S. nana undergo multiple transverse division and bear a more distinctly bacterial aspect.

Spironema and Treponema.—Under Spironema Gross classified all the pathogenic and small saprophytic varieties. Dobell [24] substituted Spironema for Schaudinn's Treponema on the basis that the former term was applied to a flagellate (Spironema multiciliatum,[15] by Klebs, in 1892; for he did not consider it necessary to create two genera out of these organisms. Gonder still hesitates to drop the distinction between the group of "blood-spirochætes" and that of "tissue-spirochætes," the latter con-

taining Treponema pallidum as type organism. While Schaudinn's original criteria for Treponema are no longer valid as regards several points, Gonder proposes to retain the term Treponema for the pallidum group and to accept Spironema for the more irregularly curved, wavy varieties to which most of the "blood-spirochætes" and saprophytic parasites belong. From personal observations I believe the differences between the two groups to be differences of degree, not of quality. They should belong to one and the same genus, as may be seen from the characteristics enumerated below. Spironema and Treponema have a slender, cylindrical, spirally wound, highly flexible cell-body, which exhibits serpentine, cork-screw-like, and sometimes lashing movements. The spiral curves are partially stretched and drawn together with a certain rhythm, so that an actively motile organism resembles a spiral spring which is alternately drawn out and relaxed. When reduced in motility the organism may rotate along its axis in one and then in another direction without changing its curves. In certain species a lateral bending or swinging motion of one-half of the body may be seen. It seems to be the general rule that the more active and energetic an organism is, the less rigid are its curves. On the whole the pallidum group (Treponema) exhibits a less energetic motility than the heavier group (Spironema) which it relinquishes much sooner than the latter. Therefore, it is only in perfectly fresh material (such as that obtained from an experimental syphilitic lesion in animals at the moment of examination) that the stretching of the curves as in the case of so-called Spironema can be recognized. This point can be clearly demonstrated in a section of a syphiloma in a rabbit's testicle fixed immediately after removal from the animal. Here we find the organisms to show most striking irregularity of curves very unlike the accustomed picture of regularly curved specimens found in a section obtained from postmortem material such as a tissue from macerated congenitally syphilitic fœtus (Flexner[55]) or from a preparation made after the organism has become sluggish. The reverse is also true. A spironema from a case of relapsing fever is always wavy and irregularly curved in a stained preparation, but

it is much more regular when observed under the dark-field microscope, and becomes completely regular when nearing death as a result of being exposed to progressively unfavorable conditions. In a culture where the motility is somewhat less active the organism appears just as regularly curved as a treponema. The sudden death of these organisms leaves them in a state of motion, hence their irregular curves.

The cell-body of Spironema is much heavier than that of Treponema and in relation to different dyes it may be stated that the former takes on a more bluish component of Giemsa's solution than the latter, which usually takes on the red. In regard to the structure of the cell-body, the minuteness of these organisms precludes the possibility of obtaining much information by means of our present methods of differentiation. Many authors assume the presence of a membrane analogous to the periplast of a flagellate and believe that it can be demonstrated by means of maceration. In one species of Spironema, Prowazek [40] assumed a central axial filament surrounded by a layer of cytoplasm. The active motility exhibited by these organisms led some investigators to suggest the existence of contractile fibrils or a myoneme in the cell-body. My observations on fresh specimens obtained from pure cultures of these organisms support the view that the spironemata are provided with an axial spiral filament covered with a layer of protoplasm. On the surface of the cell-body there is a thin membrane which can be detected when the organism undergoes degeneration. At this stage the cytoplasm becomes so rarefied, *i.e.*, it escapes from the space which it occupied, that the axial filament and the membrane can be easily recognized. In a subsequent phase the membrane also disappears, leaving the axial filament denuded. This is a common phenomenon in the cultivation of this group of organisms. Schellack [19] maintains that the external layer of the cell-body stains red with iron hæmatoxylin eosin, while the inner layer takes on a dark bluish tint, hence the former is of ectoplasmatic and the latter of endoplasmatic origin. Gonder [56] describes an ectoplasmatic layer in Spironema vesperuginis. Fantham and Porter,[35] as well as Prowazek,[40] mention the existence in Spironemata of an undulat-

ing membrane, as was originally suggested by Schaudinn [36] owing to a wavy movement which he observed to travel through the body of a resting spironema. Gross and Zuelzer failed to demonstrate any such particular structure. Another important feature of Spironema and Treponema is the presence of a terminal appendage projecting from the end of the cell-body. The bodies of spironemata and treponemata taper at both extremities, from which is sent out a very fine terminal thread, at one or both ends. The length of the terminal appendage may reach ⅓ to ½ of the body and is immeasurably thin. In old cultures, especially when grown in a fluid medium, these terminal appendages are much heavier and more easily recognized than in a specimen derived direct from the natural habitat. The terminal filament is provided throughout its length with numerous, closely set, regular curves.[57] It is rigidly joined at the pointed ends of the body or sometimes in such a loose manner as to permit the joint to bend at any angle to the long axis of the organism. No proper motility can be discerned in the appendage, which is elastic. In certain specimens an active swinging or jerking movement can be seen to be transmitted by the organism, which is able to do this by means of its contractile element (myoneme?) contained within the body. In several instances in which the cultivated Spironema recurrentis had been exposed to the solvent action of certain chemicals (saponin, sodium taurocholate, etc.), I have observed many denuded axial filaments (their cytoplasmic layer having been dissolved) to which the terminal filaments were also attached. Suddenly I saw some of the terminal projections commence active jerking and swinging motions. The skeletal axial filaments still remained. By means of careful examination it was found that there were a pair of highly refractile, round bodies attached to the skeletal filaments near both extremities. These bodies, which measured about 0.5μ in diameter, appeared to have some contractility as suggested by the alternate change in the degree of the refraction of light. Whether or not these bodies represent some sort of myonematous elements cannot be definitely stated, but it is significant that similar nodules, if not in pairs, can be seen to travel from one point to

another in an actively motile spironema. Prowazek [58] once called attention to the phenomenon of plasmatic condensation in the body of Spironema gallinarum.

The nature of the terminal appendage is not known. Many authors (Hoffmann, Prowazek, etc., on S. buccalis and S. balanitidis; Novy and Knapp on S. recurrentis) view it as a prolongation of the periplastic fibrils which are in connection with the periplast. Others regard it simply as a drawn-out part of the cytoplasm produced at the line of division. I am inclined to think that the terminal projection with regularly set curves is a separate part not directly connected with the membrane, nor existing as a prolongation of the axial filament. It is connected with the cell extremity by means of a tendinous substance. It resembles the flagellum of certain bacteria, inasmuch as it is similarly elastic, finely set with regular curves, and visible under the dark-field microscope. On the other hand a great many of the bacterial flagella cannot be demonstrated in a fresh preparation even by means of a dark-field illumination. Zettnow, Borrel and Fraenkel [59] obtained preparations of S. recurrentis, S. gallinarum and S. duttoni in which peritrichal "flagella" were shown by means of flagella staining methods, but these flagella-like fibrils are now regarded as fibrils which have become detached from the external layer of the organisms through maceration. By means of the lucidol method of Szécsi,[60] Gonder [45] succeeded in staining one fine terminal projection at each end of S. recurrentis, as did also Wolbach by the adoption of Casares-Gil's [61] method.

There are several views regarding the mode of multiplication. The theory most generally accepted is that these spironemata undergo transverse division like bacteria, differing from the latter, however, in not forming a wall at the point of division. The division is effected by means of a thinning-out process of the protoplasma which for a time bridges the two newly-formed daughter cells. Finally they separate by the severance of the connecting thread. Novy and Knapp [18] described a cleft formation at the point of division. The view of the transverse division is held by Koch, Novy and Knapp, Metschnikoff, C. Fraenkel, Borrel, Laveran, Sobernheim, Gross, Thesing, Schellack, Nakano [62]

and others. On the other hand Schaudinn, Hoffmann, Hartmann, Keysselitz, Herxheimer, Prowazek, Gonder, Fantham and Porter support the theory of a longitudinal division as in the flagellates. Indeed, Krysztalowicz and Siedlecki [63] in 1905 went so far as to propose the term "Spiroflagellata" under Mastigophora. I have also observed instances in which the phenomena could only be explained by longitudinal division. Thus, in pure cultures of various spironemata and treponemata we find forms in which a longitudinal cleft can be traced in the somewhat heavier specimens. The cleft may run but a short distance or one-third, one-half or almost the entire length of the body. In some specimens the cleft widens up and causes one-half of the body to be split into two limbs (two daughter cells in half separation). Observed under the dark-field microscope the process is seen to be slow. It may be added that it is tedious to actually follow up the entire process of any mode of division under the microscope, no matter whether this be transverse or longitudinal. As may easily be conceived, those who held the theory of transverse division argue that the forms held by their opponents to be a stage of longitudinal division are formed by two entwined spironemata which, having been produced by transverse division, are still connected by a delicate plasmatic bridge. This argument, however, can also be used in the reverse sense in favor of longitudinal division, as it is also possible that the two daughter cells which have just undergone cell-division can remain united at their ends, thus bearing the appearance of representing a stage of transverse division. A strong support in favor of the transverse mode of multiplication lies in the formation of a very long thread consisting of several sections united by means of a delicate bridge between them. This phenomenon is of common occurrence in any spironema or treponema culture. It is highly probable that the usual mode of division in cultures is transverse, although the possibility of longitudinal division cannot be excluded. Recently Meirowsky [64] advanced the view that Spironema and Treponema besides multiplying transversely also do so by means of a process of fructification (Doldenbildung) and budding (Knospbildung) similar to that observed in some

lower plant organisms. His ideas were chiefly based upon phenomena observed by means of various methods of vital staining in a culture of Treponema pallidum (furnished by Sowade). He describes numerous granules collected in a group at one point or another along the body of the pallidum and also branching out of sprouts from some of the specimens. There are many factors to be taken into consideration in such an experimental arrangement which will make it difficult to properly estimate the value of the observations. Those made under the microscope on a preparation containing the organisms, consisting of semi-coagulated horse serum, solution of precipitable aniline dyes (effected particularly through a change of reaction in the medium) are of a disputable character when we consider the absence of strict aseptic precautions as well as the comparatively long period of observation (many days and weeks) during which a preparation had been kept for observation. It is possible that under these unfavorable conditions various forms of involution result which do not appear under normal cultural conditions. Certainly it is not convincing to admit that this so-called fructification or budding also occurs in the body of infected hosts.

Balfour [65, 66] noticed the appearance of certain granules within some of the erythrocytes of fowls which had just stood the first attack of the Sudanese fowl spironematosis and thought that these granules gave rise to a new generation of the spiral forms of the organism which reappear at the second attack. That is to say, that a Spironema found by Balfour in a Sudan epizootia possesses a spiral and a granular phase of life. Leishman [67] Blanc,[68] Fantham,[69] Nuttall [70] and Hindle [70] also entertain the belief that Spironema duttoni and Spironema gallinarum adopt a granular form under certain conditions, and that a spiral form can sprout out when the conditions become favorable. Thus, in the body of infected ticks these spiral organisms undergo segmentation, and numerous granules are produced, a process analogous to sporulation. These granules were called by the authors coccoid bodies, infective granules or spores. This view was supported by the histological studies of Hindle, who secured a series of

preparations in which these granules can be demonstrated in the body of the tick. According to Hindle these granules become spiral when the infected tick is incubated at 37° C. for a certain time. In contradistinction to the above findings Marchoux and Couvy,[71] Gleitmann,[72] Gonder,[45] Todd, and Wolbach [73] maintained that in an infected tick some motile spironemata can always be demonstrated and that the granules described by Hindle and others are not specific for the infected ticks, but can also be found in the control specimens. Fantham [69] points out, however, that the granules of normal ticks are not identical with the coccoid bodies of Spironema found in the infected ticks.

Schaudinn, Prowazek and others noticed that certain species formed nodules under adverse conditions and suggested that these may represent a resting stage (or resistant form); but Schellack and Wolbach regard them as a depression phenomenon which can also be induced by prolonged treatment of the organisms with a saline solution. Besides, there is a peculiar, highly refractile, round body which is very often found attached somewhere along the side of the body of the organism. There may be one or more such bodies in a specimen. The significance of this body is still obscure but it may possibly be caused through a disturbance of the osmotic equivalence existing between the cytoplasm of the organism and the medium, not unlike the phenomenon known as plasmaptysis. I have demonstrated its occurrence in the cultivated specimens of various species of Spironema and Treponema. The body is more frequently present in an old culture in which innumerable granules are also found. In certain culture tubes these minute granules are mostly of varying size. By making a transplant of such a culture into a new medium it was found that, when examined several days later, the new culture contained many short spiral forms which were in one manner or another intimately connected with the granules. This phenomenon suggested the possibility of representing the sprouting of the spiral forms from the granules.

Pathogenicity.—Spironemata and treponemata are parasitic, and some varieties are responsible for various diseases in man and

animals. Various forms of acute febrile diseases, as well as chronic pathological conditions, are caused by the invasion of the blood or tissues by this group of organisms. It may be mentioned that the spironemata are almost always transmitted from a sick individual to a normal person through the intermediary of certain blood-sucking insects and invade the blood principally, whereas the pathogenic treponemata are carried from man to man by direct contact and show a predilection for various organs and tissues. As a rule, the phase of the spironemal infection is acute and brief and that of the treponemal invasion runs a chronic course, as instanced in the former case by the type of relapsing and tick fevers and in the latter by syphilis and yaws.

Besides the pathogenic species there are a large number of saprophytic varieties belonging to these two genera (or one according to certain classifications) which are common inhabitants of the oral cavity, genitalia and alimentary tract of man and animals. Some forms are frequently associated with certain pathological conditions, but their etiologic significance has not been definitely determined. Such is the case with S. balanitidis in Ulcus erosiva circinata, S. vincenti in an acute angina, S. schaudinni in Ulcus tropicus and Treponema mucosum in pyorrhœa alveolaris, etc. It may be that some of these play the rôle of a secondary invader and aggravate the conditions.

In the following table I have enumerated the different species of Spironema and Treponema which have hitherto been observed by various investigators throughout the animal kingdom. It will be seen that the search has been more thorough in the case of the warm-blooded vertebrates than the cold-blooded orders, while even mosquitoes, ants, mites and fleas are found to harbor certain species of these organisms.

Spirochætæ (Large Free-Living Forms)

S. plicatilis.* Fresh water. 100–200μ, 500μmax. $\times$0.5–0.75μ Ehrenberg, 1838
S. plicatilis marina * ..... Zuelzer, 1912.
S. plicatilis eurystrepta * ..... Zuelzer, 1912.
S. stenostrepta * ..... Zuelzer, 1912.
S. daxensis†..... Hot spring. 30–100μ $\times$0.5–0.75μ Cantacuzene. 1910.

* Zuelzer cultivated these varieties in a suitable medium and proved each of them to be different from the others; hence he made subspecies. Schaudinn considered them to represent male and female forms.

† In the water of hot springs of Dax (52°–56° C.).

Cristispiræ and Saprospiræ (Large Saprophytic and Commensal Forms in the Alimentary Canals of Shellfish)

Cristispira balbianii‡..... Ostrea angulata
O. edulis..... 100–120μ $\times$3–5μ. Certes, 1882.
C. anodontæ..... Fresh water mussel,
A. cygnea; also
A. mutabilis. 130μ $\times$3–4μ..... Keysselitz, 1906.
C. spiculifera§..... " " .28–36μ $\times$0.7–1.1μ. Schellack, 1909.

C. primæ *..... Prima squamosa,
P. nobilis..... 10–60μ $\times$0.5–3μ.. Gonder, 1908.
C. mactræ..... Mactra sulcataria 45–70μ $\times$0.8–1.0μ Prowazek, 1910.
C. pectinis..... Pecten jacobæus..72μ $\times$1.5μ.... Gross, 1910.
C. interrogationis..... 25μ $\times$0.5μ.... Gross, 1910.
C. veneris..... Venus casta..... Dobell, 1910.
Saprospira grandis..... Gross, 1912.
S. nana..... Gross, 1912.

* Gonder once described a blepharoplast near one blunt end; nucleus in single rod or irregular masses. Specimens with rod-formed nucleus may be male elements, since they are highly active, the others female or indifferent elements. A concentration of all the chromatin into one rounded mass was sometimes observed. Encystment also occurs. Gonder no longer upholds his above-cited interpretations, explaining them on the ground of faulty technical handling of the preparation.

‡ Once considered to be trypanosome or spirochæta.

§ Bosanquet doubts its being a separate species from C. anodontæ.

(Schellack)		Length		Breadth		Ends
		Average	Extremes	Average	Extremes	
C. balbianii	Ostrea edulis	39	35–42	1.3	1.1–1.5	Rounded, no t. ap.
C. ostræ	Ostrea edulis	41.5	38–42.5	1.1	1.0–1.3	Sharp, no t. ap.
C. chamæ	Chama gryphoides / Ch. sinistrorsa	45.6	45–46.5	1.4	1.3–1.5	Rounded, no t. ap.
C. anodontæ	Anodonta mutabilis	46	39–50.5	1.0	0.9–1.2	Rounded, no t. ap.
C. spiculifera	Anodonta	33	28–36.5	0.9	0.7–1.1	Pointed, t. filam.
C. modiolæ	M. barbato	37.5	36–40	0.8	0.7–0.9	Rounded, no t. ap.
C. primæ	P. nobilis	30.4	29–31	1.0	0.8–1.1	Rounded, no t. ap.
C. limæ	L. inflato, L. hiano	37	35–41	1.4	1.0–1.8	Rounded, no t. ap.
C. cardii papillosæ	C. papillosum	19.1	18.5–20	1.2	1.1–1.4	Rounded, no t. ap.
C. tapetos	T. decussata	34.5	29–35	1.3	1.1–1.4	Rounded, occasional t. ap.
C. acuminata	Tapes læta	47	43.5–49.5	1.0	0.9–1.1	Pointed, no t. ap.
C. saxicavæ	Sax. arctica	31	30–32	1.7	1.6–1.8	Rounded, no t. ap.
C. gastrochænæ	G. dubia	29	constant	1.2	1.1–1.3	One end blunt, one sharp, no t. ap.
S. pusella *	Anodonta, Unio, Lima, Tapes, etc.	13	12–14	...	0.3–0.4	Sharp pointed.

* Bosanquet found a spirochæta 10–12μ in length which he thinks may be identical with Spirochæta hartmanni of Gonder or with S. pusella of Schellack. No crista?

SPIRONEMA

S. obermeieri*	Man, Europe.	8–16μ×0.25μ	Colin, 1877 ([74]).
S. carteri	Man, India.	.8–16μ×0.2μ.	Mackie, 1907 ([75]).
S. duttoni	Man, West Africa.	.16–30μ. ×0.2μ	Novy and Knapp, 1906, Breinl, 1906.
S. kochi	Man, East Africa		Schellack, 1907 ([77]).
S. berbera	Man, Algiers	12μ.	Sergent, 1908.
S. ægyptica	Man, Egypt	13.5μ.	
S. novyi†	Man, North America.	12μ.	Schellack, 1907 ([77]).
S. ictero-hæmorrhagiæ	Man,	4–9μ×0.3μ, exceptionally 25μ	Inada, 1914–15 ([70]).
S. nodosum	Man		Hübener and Reiter, 1916 ([80]).
S. gallinarum‡	Fowl		Marchoux and Salimbeni, 1903 ([81]).
S. anserina	Goose		Sacharoff, 1890 ([82]).
S. theileri	Cattle.	20–30μ×0.25–0.33μ.	Laveran, 1902.
S. bovis caffris	Cattle		Nuttall, 1910.
S. equi	Horse		Novy and Knapp.
S. equina	Horse		Theiler, 1906 ([83]).
S. ovina	Sheep		Blanchard, 1906.
S. macaci	Inacacus, Ceylon		Castellani and Chalmers, 1908.
S. pitheci	Cercopethicus pates, French Sudan		Thiroux and Dufongéré, 1910.
S. lutræ	Otter		Prowazek, 1907.
S. lovati	Grouse's coecum	16–32.5μ×	Fantham, 1910.
S. vesperuginis	Tunisian bat.	12–18μ×0.25μ	Gonder, 1908.
S. lagopodis	Grouse's blood.	.10–18μ×.	Fantham, 1910.
S. laverani	Mouse.	1.8–3.75μ×0.1–0.2μ.	Breinl and Kinghorn, 1906 ([84]).
S. suis	Pigskin lesion or tumor	6–12μ	Dodd, 1906, Cleland, 1906.
S. muris	Rat	3–7μ×0.2μ.	Wenyon, 1906 ([85]).
S. minor	Rat	5–9μ	Carter, 1887, ([86]).
S. microgyratum	Ulcerated cancers.	.5–11μ× 1.5–2μ 2.5–6μ×0.16–0.25μ.	Löwenthal, 1906 ([87]).

*Synonymous with S. recurrentis, Lebert, 1874.

† The designation of this variety as S. novyi originated in Schellack's article above quoted and it has since gained a wide acceptance. In going over the literature one cannot escape the impression that a better recognition ought to have been accorded Norris, who, with Flournoy and Pappenheimer, was the first to transmit this spirochæta from patients to ordinary laboratory animals and succeeded in securing a transient culture for two successive generations *in vitro*. Of course Novy deserves the credit of differentiating this variety from the closely allied types by immunity relations.

‡There are three subspecies: S. granulosa penetrano, in Sudan; S. Nicollii in Tunis, and S. neveuxi in Senegal.

S. eugyratum....Human intestine, 4.6–7.3μ..Werner, 1906.
S. stenogyratum .Human intestine.3.6–6.7μ .Werner, 1906.
S. gondii.....Rodent Ctenodactylus gondi 16–19μ×0.3μ.....Nicolle, 1907.
S. gadi.....S.W.Fish, Gadus minutus..... 10–16μ×3.5–4μ.....Neumann, 1909.
S. pelanychis S.W. Pelamys sarda....9–10μ× 1–1.9μ.....Neumann, 1909.
S. jonesi.F.W. ..Fish, Clavias angolensin.... 18μ×0.1μ.....Dutton, Todd and Toby, 1906.
S. hartmanni....Prima squamosa, P.nobilis intestine 6–14μ×1μGonder, 1908 [88].
S. bufonis.....Bufo vugaris Rectum 8–10μ×1.5μ.....Dobell, 1908.
S. minei.....Work ants. Termes lucifugus..15–50μ×0.3–1μ Prowazek, 1910.
S. glossinæ.....Tse-tsefly stomach .8–15μ..Novy and Knapp, 1906.
S. culicis.....Gnat. aliment. canal .large..Jaffé, 1907.
S. buccalis*.....12–20μ×0.5–xμ..Cohn, 1877.
S. vincenti.....Pharyngitis.....10–40μ..Blanchard, 1906. [89] [90].
S. gracilis.....Abscess near jaw.....Vesprèmi, 1907 [91].
S. Schaudinni....Tropical ulcer.....Prowazek, 1907 [92].
S. pseudopallidumVarious ulcers.....Mulzer, 1905 [93].
S. bronchialis....Bronchitis in Ceylon 15–30μ Castellani, 1907 [94].
S. phagedenis....Phagedenic ulcer in man...Noguchi, 1912 [95].
S. refringens.....8–12μ×0.33μ..Schaudinn, 1905 [6].
S. balanitidis....Balanitis..... 8–12μ×0.5–0.75μ..Hoffmann and Prowazek, 1906 [39].
S. obtusum.....Yaws lesion.....Castellani, 1905 [96].
S. acuminatum...Yaws.....Castellani, 1905 [96].
S. aboriginalis....Ulcerative granuloma on pudenda....18–20μ....Cleland, 1909 [97].
S. interrogans....Yellow fever...14μ×0.17μ.Stimson, 1909.
S. hyos.....Hog cholera.....King, Hoffmann, Bæslack, 1913 [98] [99] [100].
S. grassi.....Termite in Italy.....Doflein.
S. termitis.....Termite in Ceylon, large...Dobell, 1910.
S. ctenocephali...Dog flea.....Patton.

Lingard described Spironema in the blood of the camel, dog, elephant and horse; James, in an ulcer of the dog's muzzle, and Lucet in gastro-enteritis; Mathis and Leger in the blood of the zebra and antelope; Bell and Ruquet in the stomach of a normal dog; Dobell in Tropidonatus stolatus;

*Subspecies: Undulata and inequalis.

Mühlens and Gleitmann in the boa constrictor; Ed. and St. Sergent in the alimentary tract of anopheles maculipenis larva; Mühlens often found spirochætes resembling S. recurrentis in the Culex mosquitoes.

TREPONEMA.

T. pallidum.....Syphilis.6–14μ×0.2–0.25μ.Schaudinn and Hoffmann, 1905 ([6]).
T. pertenue.....Yaws.....Castellani, 1905 ([101]).
T. microdentium.....3–12μ×0.2–0.25μ..Noguchi, 1912 ([102]).
T. dentium*.....Koch, 1877.
T. macrodentium.....6–18μ×0.3–0.5μ..Noguchi, 1912 ([102]).
T. mucosum.....Pyorrhœa alveolaris.....
3–12μ×0.2–0.25μNoguchi, 1912 ([103]).
T. calligyrum....Condyloma.....Noguchi, 1912 ([104]).

The foregoing list may be classified according to the habitat of the organisms. Thus, when they multiply within the blood circulation of man or animals they may either lead to a grave pathological condition or may produce no appreciable disturbance of the host which harbors them. In the case of certain tissue-invading species, serious pathological consequences may ensue or the host may remain more or less indifferent to the parasite.

1. VARIETIES WHICH INVADE THE BLOOD PRINCIPALLY.

a. Those which cause characteristic fevers known as relapsing or tick fever (pathogenic). There are seven for man, five for mammals and at least two for birds. These are:

For Man.—Spironema obermeieri (in European relapsing fever), S. carteri (in East Indian relapsing fever), S. duttoni (African tick fever), S. kochi (in East African tick fever), S. novyi (in American relapsing fever), S. aegyptica and S. berbera (in Egyptian and North African relapsing fever).

For Mammals.—Spironema theileri (in South African cattle fever), S. equi and S. equina (in horse), S. ovinæ (in sheep), S. macaci and S. pitheci (both in East Indian monkeys).

For Birds.—Spironema gallinarum (in South American and African chicken fevers), S. anserina (in goose fever).

b. Those which do not seem to produce any grave condition, but are incidentally found (non-pathogenic).

For Man.—None.

*Subspecies: S. recta, S. tenue, S. denticola.

For Mammals.—Spironema lutræ (in otter), S. gondii, S. vesperuginus (Tunician bat), S. muris, S. minor (both in rats), S. laverani (in mouse). The organism found by MacNeal [105] may be identical with S. muris.

For Birds.—Spironema lagopidis (in grouse's blood).

For Reptiles.—Spironemata found in Tropidonotus and Boa.

For Fish.—Spiroonema gadi, S. pelamydis, S. jonesi.

2. Varieties Which Invade the Tissue Principally.

a. Those which cause characteristic lesions and symptoms (pathogenic). In this group there are no Spironema, but the only two known varieties belong to Treponema. No pathogenic tissue parasite belonging to Spironemaceæ was found in animals. The two pathogenic treponemata for man are Treponema pallidum (in syphilis) and T. pertenue (in yaws).

b. Those which do not seem to cause any noticeable lesion. To this belongs a Spironema (or Treponema) discovered by Gaylord [106, 107] and Borrel [108] in mouse cancers. Similar organisms were found by Tyzzer,[109] Deetjen [110] and Mezinescu.[111]

3. Varieties Which Invade Both the Blood and the Tissues Indifferently.

Spironema (?) icterohæmorrhagiæ (in Weil's disease prevalent in Japan) and S. nodosum (in Weil's disease prevalent in Germany) is the only one so far known to come under this heading. This organism, first discovered by Inada, is probably identical with S. nodosum of Huebener and Reiter, who also found it independently of Inada a year later. Stokes confirmed the work of Inada on the cases prevalent in Flanders.*

4. Varieties Which May be Associated with Certain Pathological Conditions, and Some of Which are Regarded as Having a More Intimate Relation to the Lesion Than That of Mere Secondary Invaders.

There are about seven spironemata and one treponema which have been recorded in man and may be included in this category. These are: Spironema vincenti (acute pharyngitis), S. schaudinni (in tropical ulcers), S. bronchialis (in pulmonary gan-

* Personal communication from Dr. Adrian Stokes, Captain R. A. M. C.

pig, as the organisms are more abundant in experimental Weil's disease than in human cases. In October, 1915, an opportunity was afforded me to observe a number of cases of this disease occurring in China and, through the co-operation of Dr. Miyajima, some material for experimental studies was collected. One of the patients had had the attack a month previously and was at the convalescent stage. He was anæmic, thin, and moderately jaundiced. The urine (dark, turbid) was collected and inoculated into the peritoneal cavity of the guinea-pig. The animal started to show the typical symptoms (fever, jaundice, epistaxis, petechia, bile pigments in the urine, etc.) within one week and was examined just before death. The heart's blood showed *S. icterohæmorrhagiæ* in moderate numbers. They were motile (their curves were irregular and showed lateral twitching motions or some serpentine movements). Their length varied from 9μ to 12μ and the width was about 0.4μ. More organisms were seen in the emulsions of the liver and kidney. Some of the specimens were as long as 16μ and some as short as 4μ. The number of curves varied from 4 to 10. Inada, Ido, Hoki and others state that the body of the organism seems to be beaded when examined under the dark-field microscope. Like other minute treponemata or spironemata, the unstained Spironema of Weil's disease is invisible under the ordinary microscope. When stained with the Giemsa, carbol fuchsin, gentian violet, or Fontana stains, the organism presents a spiral thread possessing only a few large curves with pointed extremities. There is a certain resemblance to Vincent's spirochæta, although it is somewhat smaller and finer than the latter. A flagellum has not been demonstrated, but in a preparation stained according to the modified Fontana method,* I was able to see a delicate projection drawn out of the pointed end of the organism. Probably there is a terminal thread. It is quite astonishing, however, to find that the organisms stained

* Fix air dried film in (1) a mixture of acetic acid 8 cc., formalin 20 c.c. and distilled water 100 c.c., for a few minutes; rinse off the reagent. Cover the film with a 2a mixture of 20 per cent. tannin plus 1 per cent. phenol and steam it over a flame for one minute; wash the film and then immerse the slide in a 0.25 per cent. silver nitrate solution for a minute or two. After washing cover the film once more with (2) and steam it over a flame; wash and dry.

by the Levaditi method appear to be very heavy, irregular forms with a few tortuous bands and blunt ends. By applying a modified technic [117] the organisms stain much more elegantly and preserve their delicate appearance.

As will be mentioned later, the *Spironema icterohæmorrhagiæ* has been successfully grown on artificial media and the disease reproduced in the guinea-pig by means of the pure culture.

Huebener and Reiter [80] reported early this year (1916) that they were also able to find a spirochæta in the experimental Weil's disease in the guinea-pig. The spirochæta, designated *S. nodosa* by them, seems to be identical with the strains isolated by the Japanese investigators. As briefly referred to, Stokes has just isolated the same organism for the Weil's disease existing in Belgium. He also succeeded in reproducing the typical disease in guinea-pigs in which the organisms were demonstrated in abundance.

The report of Futaki and his associates on the finding of a spironema in the inflamed skin and lymph-glands in two cases of rat-bite fever * is interesting, inasmuch as the clinical feature of this disease had already suggested to Crohn [118] its possible relation to recurrent fever. Hata and others had found an effective therapeutic agent in salvarsan and mercury. These spironemata were found to be actively motile when examined by the dark-field microscope, and were successfully transmitted to the Inus monkey, guinea-pig, and white rat for many generations. The organism discovered by Futaki appears to be allied to the blood spironemata of relapsing fevers. In the meanwhile this will raise an interesting question in regard to the possible existence of a spontaneous spironema infection in rats. So far as I am aware, there is no observation on record of the discovery of any pathogenic Spironema in the rat, notwithstanding the fact that this animal had been much hunted up and examined by health officers for the plague bacilli, thus affording numerous opportunities to make an

* Symptoms: Incubation of 10–27 days, then chills, fever, headache and malaise. Local inflammation at the site of bite: pains in the limbs of the affected side, dark red eruptions and swollen lymph-glands; 3–7 days' fever with an afebrile interval of 2–3 days. Temperature curve similar to that of relapsing fever.

accidental discovery. Perhaps the finding of Futaki may open up a new field wherein to search for a hitherto undiscovered source of disease communicable to man.

Viability.—There are a great deal of experimental data bearing upon the viability of various spiral organisms generally and especially upon the most widely investigated species *Treponema pallidum.* In recording the results it is necessary to make a distinction between experiments made with uncultivated organisms and with those which have already adapted themselves to the artificial cultural conditions, in view of the fact that the latter offer a much greater resistance to certain external influences.

The free-living spirochæta lives for about a week or ten days when taken out of its natural habitat and placed in a vessel without the observance of any special precautions. On the other hand, Zuelzer [22] was able to keep various free-living species of Spirochæta (plicatilis type) alive for an indefinite period of time by keeping them in a hermetically sealed vessel in which a sufficient amount of hydrogen sulphide and certain organic matters derived from stagnant water were supplied from time to time; in other words, in a culture.

The maximum time during which Cristispira can be kept alive is about two days even under favorable conditions. No culture has yet been obtained with any member of the shellfish parasites.

For Spironema it was found that the pathogenic varieties, including *S. recurrentis, S. duttoni, S. novyi, S. gallinarum,* still remain infective after a little more than 40 days when kept in a refrigerator (2°–4° C.).[18] At body temperature (37° C.) complete disintegration of the organism takes place within 48 hours. No accurate data can be found regarding the saprophytic species which, it may be assumed, can remain alive much longer than their pathogenic congeners.

Of the Treponema group, *Treponema pallidum* has received most attention. Authors agree that the syphilis organism quickly becomes sluggish after being removed from the living tissues and that motility can seldom be detected in any specimen which has been maintained at 37° C. for 24 hours. On the other hand, the pallidum contained in a resected tissue (for example, a piece of chancre or rabbit's testicular syphiloma) is still found to be

infective after being kept at room temperature or in a refrigerator for 48 hours or sometimes even 72 hours.* In a culture medium consisting of rabbit's plasma, a piece of rabbit's kidney and ascitic fluid, many pallida introduced in the form of an emulsion of rabbit's testicular syphiloma remain quite active for 3 or 4 days when kept at 37° C. under anærobic conditions. But they do not always multiply to form a real culture. It was found that post-mortem material containing *Treponema pallidum* may still be able to infect a susceptible animal when inoculated within 24 hours.[119] The organism is killed at a temperature between 50° and 55° C. maintained for thirty minutes.

The resistance and viability of cultivated strains of *T. pallidum* are much greater than those of the organisms found in the tissues. Akatsu, working in my laboratory, found that when the pallidum is isolated from a fluid culture and put in a fraction of a c.c. of the same fluid, it invariably dies within 24 hours, no matter whether it be kept at 37°, 15° or 2° C., but it survives for 5 days at 37°; 7 days at 15°; and 10 days at 2° C. when kept in 2 c.c. of the fluid. On the other hand, a small portion of a solid culture set aside in a tube remains capable of transplantation into a new medium for 48 to 72 hours at 15° C. and for 4–5 days at 2° C. In a quantity of about 2 c.c. of the culture the organism remains alive as long as twenty days.

In undisturbed cultures *T. pallidum* remains alive for a considerable length of time. Thus a solid culture, set up according to the original method,[120] will remain transplantable to a new medium for a period of one year uninterruptedly kept at 37° C. At 15° C. it remains alive after standing 4 or 5 months, while in a refrigerator (2° C.) it survives about 2 months. In a fluid medium consisting of ascitic fluid and a piece of fresh rabbit's kidney covered with fluid paraffin, the organism lives about 2 to 3 months and in a double tube method [121] about 4 months at 37° C.

T. calligyrum, T. mucosum, T. microdentium are about the same as the pallidum in regard to their resistance and viability. These organisms resist the action of the sun's rays when exposed directly for several hours (4 hours and 30 minutes) at a tempera-

*Isolated specimens die within 10 hours in a refrigerator (Neisser).

	Treponema Pallidum	Spironema Recurrentis	Cristispira Anodontæ	Spirochæta Plicatilis
Saponin.....	10 per cent. solution: 30 min., immobilized, irregular, paler. 1 hour: mostly broken up. Kills in 1: 75,000 dilution	Like pallidum when treated in 10 per cent. solution	10 per cent. solution: 1–2 hours, crista fibrillar, and then indistinct	10 per cent. solution: still motile in 30 min.; longer contact makes the body shadowy, but no dissolution.
Sodium taurocholate..	10 per cent. solution: like the above; kills in 1:2,500 dilution	10 per cent. solution: immobile in 15 min. The outer layer shrinks into irregular masses, exposing axial filament. Final disintegration	10 per cent. solution: destroyed in 15 minutes	Same as Saponin.
Sodium glycocholate..	Same as sodium taurocholate			
Sodium cholate.....	Same as above, but kills in 1:5,000 dilution.			
Sodium oleate.....	10 per cent. solution: Same as above, kills in 1:70,000 dilution	Almost dissolved in 1 hour, but some may still be motile.		
Cobra lecithid.....	Kills in 1:1,000 dilution.			
Cobra venom.....	Kills in 1:1,000 dilution.			
Pepsin (0.1 in 150 c.c. of 0.3 per cent. HCl)	Cells swell up in 2 hours	.....	Slight change.....	Granules appear in 2 or 3 days at 40° C., but only slight change at lower temperature.

Trypsin (0.2 in 10 c.c. of 0.5 per cent. Na_2CO_3)	Resist the tryptic digestion for many days	.....	Crista, chambers and contents disappear in 24–48 hours. Membrane resistant	Granules and axial filament made distinct in short contact. At 40° C., for 2 or 3 days, only the axial filament remains. It may break into many pieces corresponding to curves.
H_2SO_4.....	1 per cent. solution: Immobilized immediately, shortened, granular, swollen, indistinct curves. 1 hour the same. 30 per cent. solution: dissolves the organisms	1 per cent. solution: complete immobilization; many appear thinner, but forms well-preserved. 30 per cent. solution: dissolution	30 per cent. solution dissolves them	1 per cent. solution causes immediate stretching of curves, which resume their winding when adding 1 per cent. KOH, or vice versa. This can be repeated many times. 30 per cent. solution dissolves the spirochæta.
KOH.....	10 per cent. solution: rendered indistinct in 30 min.; dissolution in 1 hour	10 per cent. solution: dissolves most of them in 1 hour; more resistant than the pallidum	1 per cent. solution destroys Crista; membrane resists 10 per cent., but dissolved in 30 per cent. with heat	1 per cent. solution dissolves granules, 2–30 per cent. destroy spirochæta, axial filament most resistant. Treatment with absolute alcohol accelerates the dissolving power of KOH.
Na_2CO_3.....	1 per cent. solution: immobilized, but no morphological changes	1 per cent. solution: immobilized, slightly granular, but well preserved	.....	1 per cent. solution: no effect on plasma, dissolves granules.

ture of 30° C. (summer) and 4° C. (winter), although no growth was obtainable with material exposed for 12 hours (Akatsu).

Drying promptly kills them, that is, no growth can be obtained by transplanting the dried cultures into new media.

The thermal death points for *T. pallidum* as tested out with pure cultures are as follows:

	5 min.	10 min.	15 min.	30 min.	60 min.
45° C.	+	+	+	+	+
50° C.	+	+	+	+	+
55° C.	+	+	—	—	—
60° C.	—	—	—	—	—
65° C.	—	—	—	—	—

The above data were obtained by Akatsu and closely agree with those obtained by Bronfenbrenner,[122] who found that the several strains of *T. pallidum* were destroyed at slightly lower temperatures. It must be stated that Bronfenbrenner used isolated organisms suspended in saline or ascitic fluid, while Akatsu subjected them to the action of heat in a thin culture tube.

Microchemical Reaction.—As mentioned elsewhere, a number of substances have been found to exert a dissolving or disintegrating action upon so-called " spirochætes " in general as well as upon certain protozoa. This phenomenon is claimed by certain authors to be decisive enough to place the spirochætes among protozoan organisms as the majority of bacteria (pneumococcus is an exception) remain unaffected, and some can multiply freely in a saponin solution which destroys spirochætes. While a too far-reaching generalization from these observations may be avoided, these reagents nevertheless furnish us with an excellent means of studying the microchemical structure of the organisms.

The preceding table contains a summary of all available data which however are very fragmentary and incomplete.

As will be noticed in the table, certain reagents demonstrate the existence of a resistant membrane in Cristispira, a trypsin resistant axial filament in Spirochæta, and a shadowy sheath (?)

as well as an axial spiral filament in Spironema and Treponema. As in the case of Spirochæta no true dissolution of Spironema (both gallinarum and recurrentis) or Treponema was effected by the saponin, but after several hours' contact they were shrivelled and broken up into irregular pieces.

Resistance to Disinfectant and Chemotherapeutic Agents.— Attempts to determine the resistance of various "spirochætes" are not lacking, but no satisfactory and accurate results were to be expected from the experiments in which their death point had to be determined through the intermediary of susceptible animals. Since the successful cultivation of different "spirochætes" has been effected, it has become possible to determine the effect of different chemicals. The following table shows a summary of the results obtained in two independent series of experiments by the use of common disinfectants.

RESISTANCE TO CHEMICALS

At 37° C.	
Lugol	kills in 1 : 3 dil.; 1 : 5–1 : 10 in 15 min.; 1 : 50 not in 1 hour.
Bichloride of mercury	kills in 1 : 5000 dil.; 1 : 10,000 in 15 min.; 1 : 50,000 in 30 min.; 1 : 100,000 not in 1 hour.
At Room Temperature.	
Phenol	kills in 1 : 200; 1 : 1000 in 30 min.; 1 : 5000 not in 1 hour.
Lysol	kills in 1 : 1000; 1 : 5000 not in 1 hour.
Formalin	kills in 1 : 200; 1 : 500 in 15 min.; 1 : 1000 not in 1 hour.
Potassium permanganate	kills in 1 : 1000; 1 : 5000 in 15 min.; 1 : 10,000 not in 1 hour.

Turning our attention to the chemotherapeutic agents it is scarcely necessary to remark that, thanks to the pioneer work of Ehrlich and his collaborators, especially to his contribution to our chemical treatment of spironematoses and trypanosomiasis,

a new field of scientific research has been inaugurated. Thus Morgenroth initiated a chemotherapy for bacterial diseases by discovering various quinin derivatives as a specific for pneumococcus. Flexner and Clark, the collaborators of Jacobs and Heidelberger,[123] made an extensive series of experiments in order to discover an effective chemical compound to combat poliomyelitis, wherein they obtained some encouraging results. In their early work they had employed numerous new derivatives of urotropin (hexamethylenetetramine) as this substance was known to penetrate into the intrathecal space. The work has since been extended to include various bacterial infections as well [124, 125, 126, 127] as trypanosomiasis and spironematosis (Brown and Pearce) with the use of additional new arsenic and mercurial compounds. While I do not wish to assert that the therapeutic effect of a chemical compound has any direct relation to the latter's disinfecting or sterilizing power against the causative agent *in vitro,* it was nevertheless thought of interest to find out how these new compounds, including various derivatives of urotropin, arsenic and mercury, would behave in relation to the various species of Spironema and Treponema in cultures.

It is a well-known fact that atoxyl arsacetin or arsenophenol, or even salvarsan, attack the trypanosomes and spironemata only after being introduced into the body, where they undergo reduction and produce a highly parasitotropic component. Yet, as will be shown in the following table, salvarsan is by no means inactive *in vitro* against *T. pallidum.* It is a fairly powerful treponemicide. Hence it is not without interest to study these compounds *in vitro* and then, when completed, compare the results with their therapeutic effects *in vivo.* The test tube determination of the germicidal property of these substances should form a part of our knowledge in perfecting chemotherapy. With the co-operation of Dr. Jacobs, who is in charge of the preparation of chemotherapeutic agents at the Rockefeller Institute, the following compounds were tested on cultivated strains of *T. pallidum in vitro* with the results indicated in the tables. A fuller report will be made later by Dr. Akatsu.

Table I gives a general survey of these compounds, while

TABLE I

No.	Preparation	Concentration sufficient to kill T. pallidum	Concentration which no longer kills T. pallidum
9	*p*–Bromobenzylhex. chloride	1 : 1,000	1 : 2,500
16	*o*–Xylylenedi–hex. chloride	1 : 2,500	1 : 5,000
19	2–Nitro–3,4–Dimethoxybenzylhex.chloride	1 : 2,500	1 : 5,000
21	1–(ω–chlorobenzyl)–2–oxy–3–naphthoic methyl ester)+hex	1 : 2,500	1 : 5,000
28	5–Chloromethylvanillin+hex	1 : 750	1 : 1,000
29	5–Chloromethylsalicylic acid+hex	1 : 2,500	1 : 1,500
40	*p*–iodobenzylbromide+hex	1 : 750	1 : 1,000
46	*o*–nitrobenzylchloride+hex	1 : 250	1 : 500
47	*p*–nitrobenzylhex. chloride	1 : 750	1 : 1,000
50	Methylhex. iodide	1 : 100	1 : 250
84	Chloroacetamide+hex	1 : 1,000	1 : 2,500
86	Oxymethylchloroacetamide+hex	1 : 250	1 : 500
90a	Ethyl bromoacetate+hex	1 : 1,000	1 : 2,500
96	Chloroacetylaniline+hex	1 : 1,000	1 : 2,500
97	β–acetoxy–α–chloroacetylnaphthobenzylamine+hex	1 : 1,000	1 : 2,500
102	Chloroacetyl–α–naphthylamine+hex	1 : 500	1 : 750
107	Chloroacetylbenzylamine+hex	1 : 500	1 : 750
109	Chloroacetyl–β–naphthylamine+hex	1 : 1,000	1 : 2,500
111	*o*–Methylchloroacetylbenzylamine+hex	1 : 2,500	1 : 5,000
112	Chloroacetyl–*p*–aminobenzoic ethyl ester +hex	1 : 1,000	1 : 2,500
114	Chloroacetylurea+hex	1 : 1,000	1 : 2,500
121	Phenoxyethylhex. bromide	1 : 250	1 : 500
122	*p*–Bromochloroacetylaniline+hex	1 : 2,500	1 : 5,000
126	Chloroacetylaminoazotoluene+hex	1 : 250	1 : 500
134	Chloroacetyl–*p*–anisidine+hex	1 : 2,500	1 : 5,000
138	Chloroacetylphenylhydrazine+hex	1 : 750	1 : 1,000
142	Chloroacetothylamide+hex	1 : 1,000	1 : 2,500
146	Menthyl bromoacetate+hex	1 : 750	1 : 1,000
147	Bromoethylphthalimide+hex	1 : 1,000	1 : 2,500
148	*p*–nitrobenzoic bromoethyl ester+hex	1 : 250	1 : 500
150	Bromoethyl benzoate+hex	1 : 500	1 : 750
158	β–Iodopropionyl–*o*–anisidine+hex	1 : 1,000	1 : 5,000
163	*p*–ethoxyphenyl bromomethyl ketone+hex	1 : 500	1 : 750
164	Chloroacetyl–ψ–cumidine+hex	1 : 2,500	1 : 5,000
168	*p*–Acetamino–ω–bromoacetophenone+hex	1 : 750	1 : 1,000

No.	Preparation	Concentration sufficient to kill T. pallidum	Concentration which no longer kills T. pallidum
171	*m*–Chloroacetylaminomethylbenzamide + hex	1 : 1,000	1 : 2,500
172	*m*–Chloroacetyl–α, α,–phenylbenzylhydrazine + hex	1 : 2,500	1 : 5,000
174	Chloroacetyl–aminoethyl anisate + hex	1 : 500	1 : 750
204	3–(ω Bromoacetyl) quinaldine + hex	1 : 2,500	1 : 5,000
218	Tribromo–*p*–cresyl bromoethyl ether + hex.	1 : 2,500	1 : 5,000
219	Chloroacetyl–*p*–aminoleucomalachite green + hex*	1 : 5,000	1 : 7,500
229	Chloroacetyl–*p* – aminobenzeneazo–p′ – dimethylaniline + hex.*	1 : 500	1 : 750
232	*p*– Chloroacetylaminobenzeneazo – p′ – diethylaniline + hex	1 : 1,000	1 : 2,500
234	α–naphthyl bromoethyl ether + hex	1 : 500	1 : 750
239	*o*–Acetaminophenyl bromoethyl ether + hex.	1 : 1,000	1 : 2,500
242	*p*–chloroacetylaminodiethylaniline + hex	1 : 1,000	1 : 2,500
244	Hex. + chloroacetylaminoethyl *p*–nitrobenzoate	1 : 2,500	1 : 5,000
249	Chloroacetyl–*p*–aminodipropylaniline + hex.*	1 : 500	1 : 750
252	Chloroacetyl–*p* – aminotetraethyl – p′, p″,–diaminotriphenylmethane + hex	1 : 1,000	1 : 2,500
253	Chloroacetyldiethylamine + hex	1 : 1,000	1 : 2,500
255	*p*–Cyanobenzylhex. chloride	1 : 1,000	1 : 2,500
257	Chloroacetyl–*o*–aminophenyl benzoate + hex	1 : 1,000	1 : 2,500
261	Chloroacetyltriphenylmethylamine + hex	1 : 1,000	1 : 2,500
262	Chloroacetylleucoauramine + hex. (* ?)	1 : 1,000	1 : 2,500
263	Chloroacetylaminoethyl *o*–nitrobenzoate + hex	1 : 1,000	1 : 2,500
267	Chloroacetylaminoethyl β–naphthoate + hex	1 : 2,500	1 : 5,000
271	Chloroacetyl – N – phenylaminoethyl – *p* – nitrobenzoate + hex	1 : 1,000	1 : 2,500
272	*m*–Acetamino–*p*–tolyl ω–iodoethyl ketone + hex	1 : 5,000	1 : 7,500
273	Chloroacetylethylaminoethyl *p*–nitrobenzoate + hex	1 : 1,000	1 : 2,500

No.	Preparation	Concentration sufficient to kill T. pallidum	Concentration which no longer kills T. pallidum
278	α, β-Diphenylchloroacetylamino-ethanol +hex	1 : 250	1 : 500
280	Chloroacetyl-*m*-aminoacetophenone +hex	1 : 1,000	1 : 2,500
282	α-Phenyl-α-oxy-β-chloroacetylamino-ethane +hex	1 : 1,000	1 : 2,500
283	*p*-nitrobenzoylaminoisopropyl chloroacetate +hex	1 : 500	1 : 750
288	Iodopropanol +hex	1 : 500	1 : 750
289	2-Chloroacetylamino-3-oxy-3-methylbutane +hex	1 : 2,500	1 : 5,000
291	Chloroacetyl-*o*-methylphenoxyethylamine +hex	1 : 1,000	1 : 2,500
293	Chloroacetyl-β-amino-γ-butanol +hex	1 : 250	1 : 500
298	β-Phenyl-β-oxy-γ-chloroacetylaminopropane +hex	1 : 1,000	1 : 2,500
301	β-Naphthyl bromethyl ether +hex	1 : 1,000	1 : 2,500
303	2-oxy-3, 5-dibromobenzyl bromide (+?) +hex	1 : 1,000	1 : 2,500+
308	Chloroacetyl-*m*-iodoaniline +hex	1 : 750	1 : 1,000
309	Chloroacetyl-5-iodo-*o*-toluidine +hex	1 : 750	1 : 1,000
M1	(4-[p-oxybenzeneazo]-phenylmercuric acetate)	1 : 50,000	1 : 75,000
M4	[o-oxybenzylideneamino] phenylmercuric acetate†	1 : 50,000	1 : 75,000
M7	1-Amino-2-[*p*-naphthaleneazophenylmercuric acetate]-5-sulfonic acid	1 : 25,000	1 : 50,000

Hex. = hexamethylenetetramine.

* = Grind up in a mortar with a little water and add $\frac{N}{10}$ HCl carefully until dissolved.

† = Treat as above, using $\frac{N}{10}$ NaOH instead of HCl.

Table II puts down the strengths of various well-known disinfectants and chemicals for the sake of comparison. Table III gives the resistance of several culture strains of pallidum and other allied species to the action of two different new compounds.

As briefly mentioned, the spironemicidal (or treponemicidal)

TABLE II

Names of substances	Concentration sufficient to kill T. pallidum	Concentration in which T. pallidum survived
Phenol	1 : 2,500	1 : 5,000
Formalin	1 : 750	1 : 1,000
Lysol	1 : 5,000	1 : 7,500
Sublimate	1 : 100,000	1 : 500,000
Salvarsan	1 : 7,500	1 : 10,000
Neosalvarsan	1 : 2,500	1 : 5,000
Atoxyl	1 : 10	1 : 25
Sodium iodide	1 : 10	1 : 25
Potassium iodide	1 : 10	1 : 25
Lugol's solution	1 : 75	1 : 100
Iodox. benz. acid	1 : 500	1 : 1,000
Trypozofrol	1 : 25,000	1 : 50,000
Nectrypozofrol	1 : 250	1 : 1,000
Sodium cholate	1 : 5,000	1 : 7,500
Sodium glycocholate	1 : 2,500	1 : 5,000
Sodium taurocholate	1 : 2,500	1 : 5,000
Sodium oleinicum	1 : 25,000	1 : 50,000
Saponin	1 : 75,000	1 : 100,000
Cholesterin	No action	No action
Cobra lecithid	1 : 1,000	1 : 5,000
Cobra venom	1 : 1,000	1 : 5,000

TABLE III

Names of organisms	Preparation M1			Preparation No. 253	
	1/10,000	1/25,000	1/50,000	1/1,000	1/2,500
T. pallidum, heavy type	..	−	+	−	+
T. pallidum, thin type	−	+	..	−	+
T. calligyrum	..	−	+	−	+
T. mucosum	..	−	+	−	+
T. microdentium	..	−	+	−	+
S. refringens	..	−	+	−	+

power of salvarsan and neosalvarsan is alleged to increase considerably when introduced into the living body. In a series of experiments,[122] it was found that by allowing a sterile extract of freshly removed rabbit's liver or defibrinated blood of the same animal to act upon neosalvarsan for three hours at 37° C. the germicidal power of this drug increased from 1:1000 to 1:2000 in the case of the liver extract, and from 1:1000 to 1:5000 in the case of the blood. The addition of boiled extract had no such activating effect.

Acquisition of Increased Resistance to Drugs.—It will be recalled here that the failure of chemotherapy of trypanosomiasis in man and animals is partly due to the production of so-called drug-fast strains of various trypanosomes after the latter have on several occasions been subjected to the action of certain arsenic compounds. These organisms will be destroyed to a great extent by the first injection of the drugs, but if there remain a few which have resisted the first medication, they will multiply and the animal will once more be infested with the organisms. The offspring is more resistant to the action of the same drug than the preceding generation. A large dose of the medicament is necessary to destroy the organisms and to overcome this increased resistance. But as a matter of fact, the increased resistance of the organism to the drug is relatively much greater than that of the infected hosts, and the limit will soon be reached beyond which the quantity of the drug cannot be further increased without seriously affecting the infected man or animals.

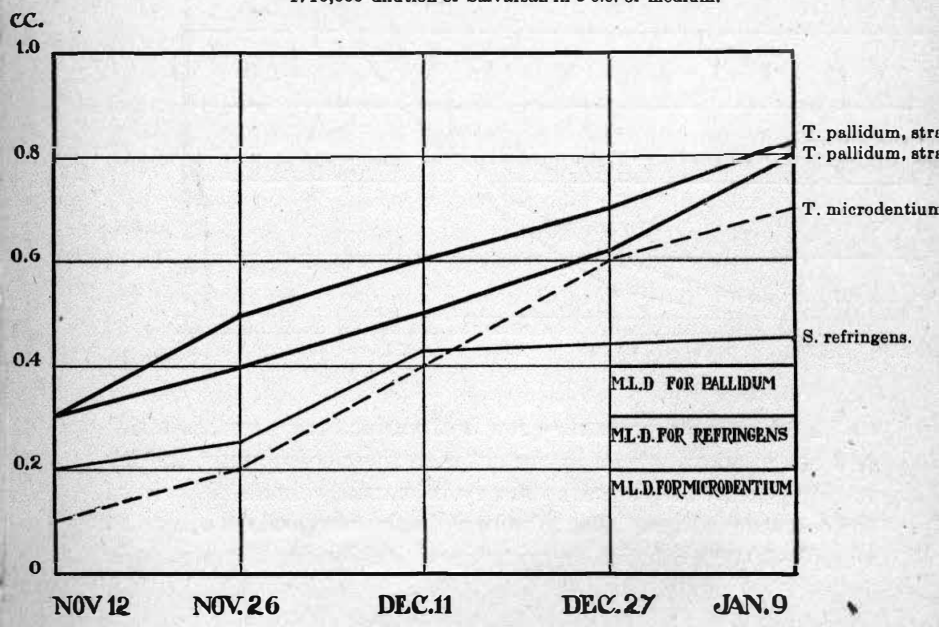
Experiments of this nature have been made with atoxyl, arsacetin, arsenophenylglycin, etc. To employ Ehrlich's terms, the organotropic affinities of these drugs were so close to the parasitotropic, that it was impossible to employ a sufficient quantity to completely sterilize the infected body, since the administration of such a quantity would mean death or the serious impairment of some of the functions. Ehrlich's conception of a specific chemotherapy was based upon the fact that different cell groups are provided with their characteristic receptor apparatus (chemoceptor), to which a given chemical molecule attaches by means of its side chains. Thus, for trypanosomes there are certain receptors which will fit in with a certain atom complex of

atoxyl, arsacetin, etc., while of the infected hosts the organs show much less affinity for them. In developing chemotherapy for syphilis, Ehrlich finally evolved a compound in which the spironematropic atom complexes were far more in excess than the organotropic groups. This compound, as is universally known, is dioxydiamidoarsenobenzol, better known as salvarsan. According to Hata [128] the ratio of the dosis curativa and dosis tolerata of this compound is 1:3 for mice and rats infected with *Spironema recurrentis,* and 1:58 for chickens with *S. gallinarum,* while in the case of experimental chancre in rabbits it is between $^1/_7$–$^1/_{10}$. In these animals Ehrlich's *Therapie sterilisans magna* was achieved, as also in cases of relapsing fevers in man. In human syphilis, however, in spite of the most powerful spironemicidal action, his original aim to sterilize the syphilitic body with a single injection of a large dose was not uniformly attained.

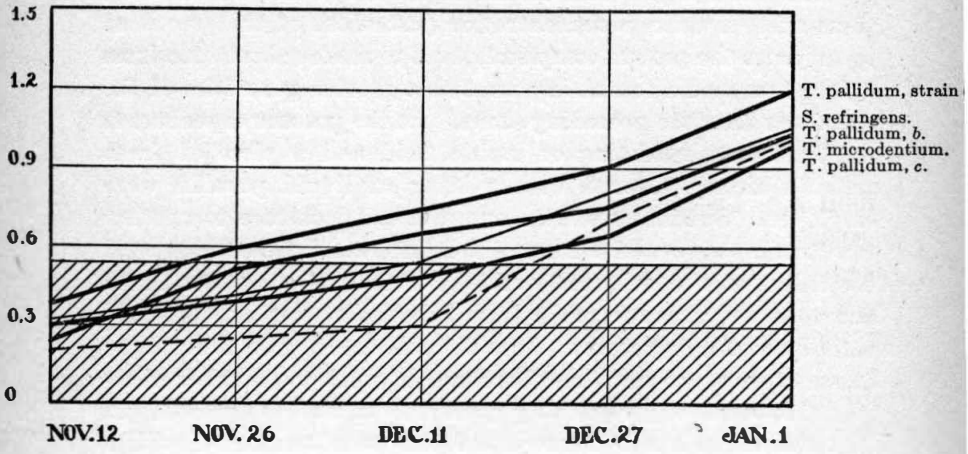
Yet there is no doubt that a prompt administration of salvarsan in sufficient dose during the early stage of infection sterilized the patients, as was evidenced by the increased instances of permanent abortion of the infection and of reinfection after the salvarsan treatment. On the other hand, we are also confronted with repeated recidives in certain patients. We often hear of mercury resistant as well as salvarsan refractory cases. It has been known for some time that *Spironema recurrentis* as well as *Spironema duttoni* produces an arsenic-fast strain in mice or rats when the latter are treated with atoxyl, arsacetin, etc. In this respect these spironemata resemble trypanosomes. Marks [129] once considerably raised the resistance of a bacteria to arsenious acid by allowing it to accustom itself gradually to the action of this chemical in test tube cultures. It therefore seems not at all improbable that Spironema as well as Treponema become more resistant to the parasitotropic effect of arsenic compounds and possibly of mercurial salts, not only *in vivo,* but *in vitro.* Akatsu [282] carried out a number of experiments in my laboratory in which he has apparently succeeded in raising to many times their original degree the resistance of the Treponema group to salvarsan, neosalvarsan, and bichloride of mercury. The experiments were carried out with cultures of these organisms, the

general plan being to cultivate the organisms in media containing these substances in a concentration just short of that required to suppress the growth completely, and to make subcultures from it into new media containing somewhat greater quantities of the chemicals than the preceding series. In the present experiments fluid cultures consisting of ascitic fluid and a piece of fresh rabbit's kidney covered with a layer of liquid paraffin were employed. Subcultures from one medicated culture to another were made at two weeks' intervals, during which time the general condition of the cultures could be estimated. As mentioned above, subcultures are made from tubes still showing numerous actively motile organisms. It is difficult to carry on the culture if one attempts to make a subculture in which too much medicament is present to give a fairly good growth, since no growth will be obtained in a subculture which has been inoculated with a poor culture arrested in its development by an excess of the drugs.

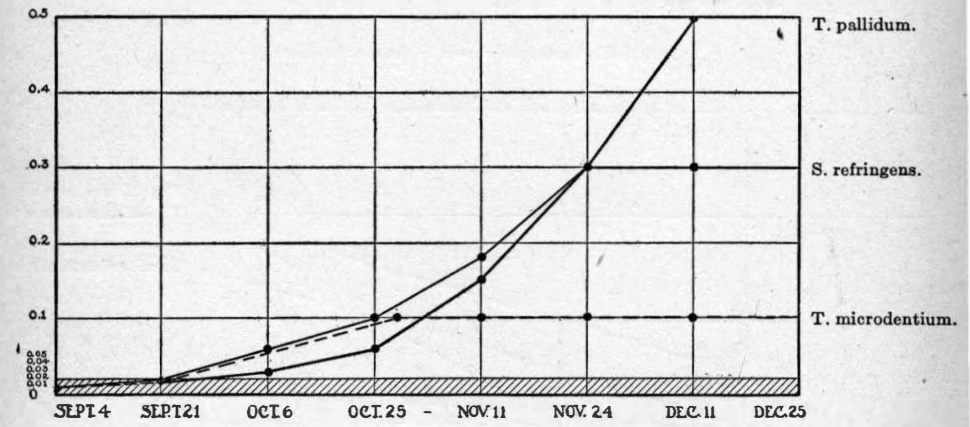
The results of our experiments may be summarized in the following charts:



1: 10,000 dilution of Neosalvarsan in 5 c.c. of medium.



1: 1000 dilution of Bichloride of Mercury in 5 c.c. of medium.



In order to suppress the growth of various treponemata which had not previously been in contact with these compounds, the following doses were found necessary in a total volume of 5 c.c. of the culture medium. The solutions of salvarsan and neosalvarsan were 1: 10,000 dilution in water, and bichloride of mercury in

1:1000 dilution. Salvarsan was neutralized with NaOH, as usual.

	Salvarsan	Neosalvarsan	$HgCl_2$
Refringens	0.4 c.c.	0.6 c.c.	0.02 c.c.
Pallidum (two strains) ..	0.375 c.c.	0.5 c.c.	0.02 c.c.
Microdentium	0.2 c.c.	0.3 c.c.	0.02 c.c.

It will be seen from the charts that the resistance of different species of Treponema and also of different strains of the same species (*T. pallidum*) seems to increase gradually until at the end of ten weeks (namely, five transfers) they were still able to grow very well in a medium which contained several (2–3.5) times the quantity of the arsenic compounds originally sufficient to restrain their growth completely. In case of bichloride of mercury the increased rate of tolerance was still more striking within a certain limit of concentration, but there was no further increase in resistance when the medium contained more than 0.5 c.c. of the 1:1000 dilution of this salt. The tissues which usually remain fleshy pink in color for several days became quickly discolorized into a dirty greyish black when mixed with the above concentration of $HgCl_2$.

The question of the duration of the acquired resistance to the drugs has not yet been studied a sufficiently long time to draw any conclusions, but the resistance has remained unmodified for at least three generations. It may be mentioned that *Spironema recurrentis* was carried through two generations in mice without undergoing any change in its acquired drug fastness.

Transmission of Spironema and Treponema to Man and Animals.—Under natural conditions the transmission of a blood-inhabiting Spironema to man or animals is effected through the bite of an infected blood-sucking insect. The transmitter in each instance is highly, if not strictly, specific, although other blood-sucking insects may also be infected by sucking the blood of an animal which is suffering from an infection with any of the pathogenic blood spironemata. These unnaturally infected ticks, bedbugs, fleas or lice are not good transmitting agents as compared with the natural carrier of the infection. That the spironema

in such non-specific insects can survive for some time can be shown when the disease is produced in a susceptible animal by inoculating it with the crushed material of the infected insects. It is possible, therefore, that an infection can be occasioned by smearing the excreta or crushed body contents of the infected insect over any defect of the epidermic layer of a susceptible subject. For example, in the case of *Spironema recurrentis,* both body-lice and bedbugs may be infected by sucking the blood of a patient suffering from the European relapsing fever, but the lice alone can transmit the disease to the next person they bite. Bedbugs are never known to spread the infection by their bites, although by crushing the infected bugs directly over a minute skin trauma (scratch, etc.), a person may become infected. A brief summary is given below of the natural intermediary hosts of different bearing spironemata, as well as certain experimental data bearing on the rôle of other blood-sucking insects and on the susceptibility of various animals to each Spironema.

Spironema recurrentis, the causative agent of the European relapsing fever, is naturally transmitted by *Pediculus corporis. Pediculus caputi* was found by Gonder to be incapable of transmitting the disease, although its body may contain the organism. The common bedbug (*Acanthia lectuaria*) may likewise harbor the Spironema for as long as 50 days,[130,131, 132] but, according to the experiments of various investigators, does not spread the infection. The rat-louse (*Hematopinus spirulosus*) can carry the infection from rat to rat, while the monkey-louse does the same among monkeys. Breinl and Kinghorn,[133] as well as Neumann,[134] Manteufel,[135] and Sergent and Foley,[136] succeeded in transmitting the infection to rats with ticks (*Ornithodorus moubata*). Schuberg and Kuhn report a successful transmission by *Stomoxys,* the blood-sucking flies.

The infection can be transmitted subcutaneously as well as per os in experimental animals.

Infected organs fed to rats produce the infection in these animals, as shown by Uhlenhuth and Haendel[137] and others.[91] Manteufel considers the uninjured skin permeable to *S. recurrentis,* and Nattan-Larrier produced the infection per vaginam,

per penis, etc., in rats, while Gozony successfully transmitted the disease also by means of subcutaneous, conjunctival, and intestinal application of the Spironema. Schellack,[138] who obtained a positive result in one out of 28 experiments on rats, was able to demonstrate a microscopical defect of the skin at the point of entrance. The organism is experimentally transmissible to monkeys and from monkeys to rats, mice, guinea-pigs, and sometimes rabbits.

S. duttoni, the causative agent of the African tick fever, is normally carried by *Ornithodorus moubata*, as was recognized by Dutton and Todd,[139] and Koch.[139a] The last-named investigator discovered the spironema in the ovaries four to five days after the tick had sucked the infected blood. Carter[140] confirmed this finding, while Neumann[134] found the organisms in freshly laid eggs. Hereditary infection for one or more generations was shown to occur by the study of Dutton and Todd, Wolbach,[141] and others. The tick is infectious an hour after sucking and remains so as long as 90 days (Wittrock[142]). This author always found the Spironema in the infective ticks. *Ornithodorus savignyi* has been suspected of carrying the infection,[143] and Brumpt once succeeded in infecting a monkey by this tick. Robledo holds *Argas americanus* responsible for the spreading of *S. novyi* (the American type of relapsing fever) in Colombia, but this theory calls for further investigation. According to Breinl and Kinghorn,[144] the rat's fœtus may be infected through the placenta when a mother rat is inoculated with *S. duttoni*. The organism is experimentally transmissible to rats, mice, monkeys, guinea-pigs, and rarely to rabbits.

As has been briefly mentioned elsewhere, Leishman, Fantham, Hindle, and others assume a granular or coccoid phase in the life history of this and allied species and maintain that the spironema gradually undergoes granulation when it reaches the tick's body and multiplies in the Malpighian tubules and ovaries. The tick becomes infective after an incubation of 1–2 days at 37° C. Hindle[70] demonstrated the infectivity of the coxal fluid, in which he found numerous granules and some spironemata. This investigator thinks that the Spironema or infective granules in

the coxal fluid enter the body of persons through the wound produced by the bite of the tick. The infected eggs become infective after being incubated, as was demonstrated by Hindle by injecting the crushed material into the susceptible animals; Leishman [145, 146] found numerous spiral rods in the infected tick eggs when the latter were incubated for a few days at 35° C. Schubert and Manteufel showed the infectivity of the ticks to be lost when they are kept at a temperature below 22° C., but Gonder failed to find any such difference. Marchoux and Convy, Gleitmann, Wolbach, Wittrock, Kleine and Eckard, and others, believe that wherever infectivity is present, there are always to be found some typical spironemata, either in the tick or in its eggs.

Spironema berbera (the North African type) is carried by *Pediculus corporis* but not by *Argas,* the flea or bedbug [147, 148] (Sergent, Gillot and Foley). *S. carteri* is also transmitted by body-lice. In this case Mackie [149] found the organisms more numerous in the female lice than in the male; they are distributed in the mouth, stomach, and digestive tract. Mackie believes, however, that *Acanthia lectuaria* sometimes carries the infection.

Among the ticks which transmit spironema in cattle and sheep may be mentioned *Boophilus decolaratus* and *Rhipicephalus evertsi.* The virus is carried by heredity. The causative agent of chicken fever, *S. gallinarum,* is carried by *Argas persicus* under natural conditions, while other species (*Argas reflexus* and *Argas miniatus*) can transmit the disease experimentally (Schellack).[188] *Ornithodorus moubata* is doubtful, as Schellack [188] failed to produce the infection while Fülleborn and Mayer [150] claim a success with this organism. Schellack was able to produce the infection in 3 out of the 15 experiments performed by him on chickens by the percutaneous application of the infected blood. Feeding fowls with infected ticks may cause the infection. The organism is experimentally transmissible to ducks, geese, sparrows, canaries, and sometimes rabbits.

S. icterohæmorrhagiæ, the causative organism of Weil's disease, has been shown by Inada and his associates to be but rarely conveyed by direct contact, but no natural intermediary hosts have been discovered. The organisms are abundantly pres-

ent in the urine during the convalescent stage and they are fully virulent for guinea-pigs. According to the experiments of Inada and his associates, the Spironema as contained in the liver emulsion is capable of penetrating an apparently uninjured skin of the guinea-pig a short time after contact (5 minutes is sufficient to cause infection).* Therefore it is altogether possible to infect a person through direct contact with some of the excreta of a patient. The infection can be induced in guinea-pigs by introducing the infected material into the stomach after it has been previously neutralized with bicarbonate of soda. In regard to the Spironema found by Futaki and others at the site of or in glands adjacent to the rat-bite infection, we must assume that this represents the occurrence in rats of pathogenic Spironema which produces fever and other symptoms when transmitted to human subjects. Further investigation in this direction is most desirable. The organism is most easily experimentally transmissible to guinea-pigs. Monkeys, rats, and mice are less susceptible.

Prior to Futaki's work there appeared a report by Kitagawa and Mukoyama, who also found a spironema in the inflamed tissue of the bitten finger of a woman. By transmitting the tissue into guinea-pigs and white rats, these authors claim to have reproduced symptoms resembling the so-called rat-bite fever. In the smears of the kidney and liver taken from the dead animals, they found two types of spiral organisms, namely, in the guinea-pig tissues the refringens type and in the white rat the minute and short type. In examining the preparations kindly sent to me by the authors, I found their findings to be entirely correct, but the refringens type is more like *T. macrodentium,* and a large number of bacteria, such as fusiform bacilli and big rods, etc., were also present in the same preparations. As to the short type one can only say that its morphology is almost indistinguishable from that of *S. muris* or *S. microgyratum.* These organisms do not agree with the illustrations and description of the spironema reported some time later by Futaki and others. In the case of Kitagawa and

* Of eight guinea-pigs experimented upon only one escaped the infection which developed in from ten to twelve days with typical symptoms.

Mukoyama the local and general symptoms may have been due to a mixed infection by the oral flora of a rat.

Relapses of Infection.—The Spironema of relapsing and tick fevers also causes in man a well characterized type of fever accompanied by two attacks interrupted by a period of apyrexia lasting several days. During the apyrexia the blood is free of the parasites. But it is not at all rare for the recidive to be repeated more than once. While at the highest point of the fever the organisms are most abundant in the blood, they are also present in different organs. Dutton and Todd, Breinl, Leishman and Fantham believe that the spironemata are taken up by phagocytes within which they undergo transformation into the granular phase which in turn gives rise to the new generation of spironema. Balfour considered the intraglobular forms of *Spironema granulosa penetrans* (similar, probably identical, with *S. gallinarum*) as an asexual and the extracellular forms as a sexual phase. Fantham observed some extracellular granules which may start the relapse. Darling would hold the phagocytized spironemata within the endothelial cells of the liver responsible for the source of the recidive, as he found the organism to remain intact for some time during reconvalescence. Gabritschewsky sees in the surviving resistant specimens, which had been shielded from destruction in various organs, the progeny of the organisms producing the second attack. In the case of *S. carteri,* Mackie assumed the possible existence of an ultramicroscopic phase, as the serum taken from a patient at the apyrexic period is said to be infective in spite of the absence of any spiral forms. It may be remarked that to detect a sparse number of any Spironema under the microscope is one of the most difficult tasks, and one is very liable to overlook the organism.

T. pallidum and *T. pertenue* are the only pathogenic varieties among the group. In the case of syphilis our knowledge is quite complete, so far as the mode of transmission is concerned. On the other hand, much still remains to be learned regarding the manner in which yaws is communicated from person to person. Probably, like syphilis, its infection is spread by direct contact with a patient or any object which, after having been in contact

with a patient, harbors the live organisms; although the possibility of transmission through flies, mosquitoes and ticks is not excluded. Castellani and Chalmers [151] quote an instance in which a fly which sucked on a yaws papule infected a monkey whose eyebrow was scarified. Modder [152] assumes transmission of yaws by ticks (Argas and Ixodes) in Ceylon. It is said that vaccination and wet nursing spread the infection. In order to facilitate their entrance into the human body both organisms need only a microscopical defect of the epidermis.

After penetrating the skin or mucous membrane, *T. pallidum* elicits a local reaction characterized by the circumscribed round cell infiltration known as chancre (primary lesion), then several weeks later, at about the time when the chancre recedes, it proceeds to enter the adjacent lymph-glands and general cutaneous and mucous membrane tissue, producing roseola, papules and flat condyloma. At this period the organisms invade almost every tissue, producing the so-called secondary symptoms. Periostitis, meningitis, iritis, laryngitis are very frequently observed. One of the most constant symptoms is the Wassermann reaction in the blood serum.

After a period of several months longer, during which the secondary manifestations abate, another period known as the tertiary stage may supervene accompanied by still deeper tissue destruction caused in the organism than at any previous stages. It affects skin, bones, visceral organs, cardiovascular system and central nervous system. The disease may be progressive or marked with alternate activity and latency. Yet in the latent period repeated abortions may occur. From the time of infection until the central nervous system is affected (general paralysis, tabes), the average period of latency of the disease is from eight to twelve years. During the tertiary stage the lesions are often gummatous and affect the connective tissue, muscles and blood-vessels, while in cases of general paralysis and tabes the parasites diffusely pervade the parenchyma.[153, 154, 155, 156] This form is a syphilitic parenchymatous encephalomyelitis. In acquired syphilis, *T. pallidum* has been demonstrated in every syphilitic condition. It was first demonstrated in the primary and secondary

lesions by its discoverers, Schaudinn and Hoffmann; in liver gumma by Schaudinn; in aortitis by Wright and Richardson,[157] Schmorl,[158] and Reuter;[159] in arteriitis cerebralis by Bender;[160] in heart muscles and pancreatitis by Warthin;[161] in adrenal glands by Hoffman,[162] Jacquet and Sezary;[163] in nephritis by Hoffmann;[166] in the cerebrospinal fluid by Hoffmann,[165] Nichols and Hough,[166] and Suzary and Paillard;[167] in the blood during the secondary stage by Uhlenhuth and Mulzer;[168] in paretics by Graves,[169] in interstitial keratitis by Igelsheim;[170] in cerebral gumma by Dunlap;[171] in the paretic brains by Noguchi and Moore,[172] Marinesco, and Miner,[173] Levaditi, Marie and Bankowsky,[174] Mott, Rosanoff,[175] Tomasczewski and Forster,[176] Wile,[177] and others; in spinal cord by Noguchi,[178] Versé,[179] etc.

It should be mentioned that the first demonstration of *T. pallidum* in sections of tissue from acquired syphilis was accomplished by Bertarelli and Volpino [180] by means of their silver impregnation; a method which has since been superseded by a similar procedure amended by Levaditi.

In congenital syphilis the number of organisms present in the different organs and in different fœtuses varies greatly. In some it may be extremely tedious to demonstrate the organisms, in others the whole fœtus may be thickly interwoven with the intertwining nets of treponemata. The favorite site of invasion is the liver and skin, although stomach, intestines, adrenals, kidney, spleen, heart muscles, pancreas, bone-marrow, lymph-glands, thymus, testes, ovaries, and brain have been shown to contain the parasites, even in large numbers in certain instances.[181] The placenta and navel cord are also affected. For the first demonstration of the organisms in congenital lues, we are indebted to Levaditi,[182] who introduced his well-known silver impregnation method for this study. According to personal experiences in connection with syphilitic infants who lived several days after birth, the number of pallida present was always very small and it sometimes required many hours' search to find a single specimen. A striking difference between syphilis and yaws is the absence in yaws of visceral affections and of the nervous involvement. Much yet remains to be investigated with regard

to the relationship between syphilis and yaws, the causative agents of which bear so great a morphological, and to a certain extent, a biological resemblance toward each other.

The transmissibility of syphilis to animals was long the subject of study by earlier investigators, but the first conclusive experiments in this connection were furnished by Metschnikoff and Roux,[183] who succeeded before the discovery of *T. pallidum* by Schaudinn in producing the primary and secondary lesions in chimpanzees. It was also shown that these lesions were transferable to further series of animals. Immediately after the discovery of *T. pallidum* in human syphilitic tissues, Metschnikoff and Roux [183] found the same organism in experimental syphilis, thus closing up the first link of the chain of evidence which was to prove the specificity of the organism for syphilis. They also infected macacus monkeys with the virus derived from chimpanzees. Soon afterward Schultze [184] and Bertarelli [185] produced syphilitic keratitis in rabbits, while Parodi [186] selected the testes (intratesticular) to transmit the human strain to the rabbit. This work has been extended and elaborated by later investigators, particularly by Neisser,[187] Hoffmann, Locke and Mulzer,[188] Uhlenhuth and Mulzer,[189] Grouven,[190, 191, 192] Nichols,[193] Tomasczewski,[194, 195, 196, 197] and others. In monkeys the best site for inoculation is the eye-brow, while in rabbits intratesticular, scrotal, intraocular and intracardial inoculations were recommended. For the purpose of keeping up the pallidum strain the intratesticular mode is preferable, especially when it is desired to obtain a pure material for cultivation (Uhlenhuth, Noguchi); but in case of utilizing the lesions in order to determine the effect of a therapeutic agent, Hata [128] recommends the scrotal chancre method (introduced by Tomasczewski) wherein he is supported by the experience of Brown and Pearce.[198] With the purpose of causing a generalized syphilis in the rabbit—which animal is usually refractory to the systemic pallidum infection—Grouven reports the intracardial introduction of a large quantity of the pallidum in half-grown rabbits. My own numerous attempts to produce generalized syphilis by this method completely failed, probably owing to the difference in the strains employed. It may be mentioned, how-

ever, that with certain pallidum strains symptoms similar to human secondaries or tertiaries could be produced by means of intravenous or intratesticular inoculation. I have a few times observed iritis, keratitis, and squamatous or ulcerative skin lesions, in the last of which the pallidum could be demonstrated. Nichols and Hough [166] were the first, however, to isolate a strain from a case of nervous recidive which constantly invaded the cornea, even before the local symptoms (testis) commenced to appear. This strain has most persistently caused keratitis and choroido-retinitis in rabbits. This phenomenon led Nichols to assume that the strain possessed a highly invasive character.[199] The patient from whom this strain was obtained died several months later of a rapidly progressive form of meningo-encephalitis, and the duration of the infection (from the time of the chancre to death) was very short. Nichols, therefore, considered that this case was explained by the character of the strain. Reasoner [200] also obtained a strain from a rapidly fatal case which was characterized by the early production of choroiditis in rabbits. In some rabbits the choroiditis was the only symptom in spite of its being introduced into the testis or vein. While studying ten different strains of Treponema pallidum I was once struck with the constancy with which the various types were associated with certain distinct characters of the lesions produced in rabbits. For example, I could discern the differences among different strains in the width, length, and number of curves to a given space, etc. I divided these strains into a thick, a thin, and a medium type. The differences were great enough to enable me to identify different strains as belonging to any one of the three types.[201] In a series of passages covering a period of about one year and a half, it was found that the thin type produced a soft, diffusely swelling orchitis within 10 to 14 days and did not form any definite nodules even after six weeks. On the other hand, the thick type produced a hard, circumscribed nodule of varying size within about six weeks. Its development was unusually slow and the nodule remained for several more weeks. The character of the syphiloma produced by the medium type was a large, moderately firm orchitis, which started to be palpable at the end

of about four weeks. As will be mentioned later, these three type strains were cultivated in an artificial medium and were found to retain their morphological characteristics unchanged. Again, my experience with the two paretic strains of Treponema pallidum transmitted from human brains to rabbits' testicles (using 36 rabbits for six specimens of brains) showed me that they were of lower virulence than the ordinary chancre strains in my possession, as they required 97 and 102 days, respectively, before the lesions could be definitely demonstrated.[119] With the usual skin strains four weeks' incubation is the average. Wile [177] recently reported a successful transmission of the pallidum from the living paretics to rabbits' testicles (using one rabbit for six specimens of brains) in which the lesions appeared within 14 days, and he concluded that the paretic strains were more virulent than the ordinary strains. It may be recalled that the persistent endeavors to produce syphilitic orchitis in rabbits by means of the paretic brains was not limited to a few investigators. Tomasczewski and Forster,[202] who in 1913 performed the Neisser-Pollack puncture on 62 cases at the University Institute in Berlin and found numerous examples of the motile pallida in 29 cases of the removed material, inoculated a large number of rabbits. Their results were uniformly negative. Marie, Levaditi and Bankowsky, Marinesco, Mott, and others also failed to obtain a single positive result. Another interesting feature characteristic of the strain obtained by Wile [203] is the readiness with which it at once adapted itself to an artificial culture medium generally known to be unsuitable for the purpose of obtaining an initial growth with any strain which is transmitted to the rabbits' testicle. As I have pointed out on several previous occasions, a solid medium consisting of fresh tissue, ascitic fluid and agar is not suitable for such a purpose, and this fact has been confirmed by numerous investigators (Zinsser and Hopkins, Uhlenhuth, etc.).

It will be incomplete if we pass on without reviewing the interesting observations of Graves,[169] who succeeded in infecting a certain number of rabbits by injecting the blood of paretic patients. Graves obtained the blood in small glass ampules (sterile) which were immediately sealed. The different speci-

mens were put in an incubator at about 37° C., and after a number of days the contents of these ampules were inoculated into the testicles of rabbits. Although the majority of the inoculations were negative he found a strain developing in one of the animals. Morphologically, the organisms were the typical pallidum and produced local as well as generalized reactions (ulcerative lesions near the nostrils, anus, prepuce, vagina, etc.) wherein the organisms were demonstrated. The incubation period of average duration is about 3–4 weeks. This strain was characterized by the early appearance of keratitis in rabbits. The observation of Graves furnishes us with a problem, *viz.*, the fact that the sample of paretic blood sealed in a tube and left many weeks and months at an indifferent temperature was still capable of infecting a rabbit with such extreme severity. Yet Graves never succeeded in cultivating any strain of such examples; neither could he demonstrate the presence of any definite pallidum. Therefore, as Graves seems to think, Treponema pallidum must possess a stage of its life-cycle which is still little understood by us. Can there be a resistant form which remains dormant for years until favorable conditions are secured? Clinical evidence has suggested this idea to certain syphilologists (Pollitzer). Personal experiences with cultivated strains of Treponema pallidum do not justify my assuming the existence of a resistant form, except for the fact that the pallidum under cultural conditions is one of the most viable organisms. In suitable media it survives over one year when kept at 37° C., and it is not impossible that under naturally favorable conditions it may remain dormant for many years.

Several other animals besides monkeys and rabbits are susceptible to the disease. In dogs and sheep (Bertarelli, Hoffmann, Brüning), in guinea-pigs and goats (Bertarelli), and in cats (Levaditi and Yamanouchi) specific keratitis has been produced. Testicles of guinea-pigs (Truffi, Tomasczewski, W. H. Hoffmann, Uhlenhuth and Mulzer) and goats (Uhlenhuth and Mulzer) are also susceptible to infection by Treponema pallidum. Schereschewsky reported a scrotal chancre experimentally produced in a pig.

Treponema pertenue has been successfully transmitted into

monkeys by Neisser, Baermann and Halberstädter [204] with skin papules, and by Castellani [205] with a punctate of the spleen of a patient. Nichols [206] transmitted it from man to Macacus rhesus and then from the latter to the rabbit. In the rabbit's testicle it produces a hard induration much like a syphilitic chancre. The parenchymatous orchitis finally extends over to the tunica and scrotum, in which an extensive ulcerative indurated lesion results. When inoculated to the eyebrows of Macacus rhesus the yaws organism produces highly destructive ulcerative papules which may remain unhealed for many months. In these lesions Treponema pertenue can easily be demonstrated. Halberstädter [207] observed a generalized eruption in an orang-utan four months after inoculation. In lower monkeys the lesion remains localized and heals in from three to thirteen weeks; sometimes it may result in a serpiginous recidive which tends to become diffuse.

Filterability of Spironema and Treponema.—According to the experiments of Novy and Knapp,[18] Spironema recurrentis and S. duttoni pass in one form or another through the pores of Berkefeld filters, the walls of which were either previously shaved off to a thickness of 1.4 to 2.5 mm. or left intact (4.2 mm.) as the filtrates obtained by this procedure were able to produce in susceptible animals a slight infection accompanied by sparse spironemata appearing in the blood. The scarcity of the organism is ascribed to the presence of immune substances in the filtrate which was simultaneously introduced. Breinl and Kinghorn [132] obtained similar results with unmodified filters. In neither instance did the infective filtrates contain the Spironema in its spiral form. Their experiments tended to suggest a filterable phase in the life cycle of this organism. Todd and Wolbach [208] report the successful filtration of the organism through the Berkefeld filters N and V by pressures of fifty to ninety pounds to the square inch. Under these conditions the organism traversed the tortuous pores of the filters and was seen to have retained its usual spiral form when it appeared in the filtrate. Todd succeeded in finding the organisms in the filtrates of one experiment into which the control bacteria did not pass. Wolbach found the Spironema in the act of passing through the pores,

by preparing a thin section of the filter which had been employed for the filtration. He is of the opinion that the infectivity of a filtrate is due to the presence of the regular organism and not to that of filterable granules, as is assumed by others. C. Fraenkel failed to obtain an infective filtrate with any filters whatever.

Spironema icterohæmorrhagiæ was found by Inada and his associates [79] to pass through the Berkefeld filters, grades V and N. Out of 28 experiments the filtrates were found to be infective for guinea-pigs 15 times. It is not stated whether the filtrate contained the spironema in a regular form. Huebener and Reiter [209] also report the filterability of the virus of Weil's disease prevalent in Germany. Since they claim Spirochæta nodosa, found by them, to be the etiological agent, the same organism must be considered filterable. It passes through the Berkefeld filters V and N. As mentioned elsewhere, Spironema nodosum (Spirochæta nodosa) is probably the same organism as Spironema icterohæmorrhagiæ (Spirochæta icterohæmorrhagiæ) discovered a year earlier by Inada.

Treponema pallidum and Treponema pertenue are unable to pass through any bacteria-proof filters when filtered by the usual processes (application of a vacuum or a positive [compressed air] pressure). Metschnikoff, Klingmüller and Baermann,[210] Casagrandi and de Luca,[211] and many others established this fact in the case of syphilis, and Castellani in the case of yaws. On the other hand, the pallidum can grow through the pores of the Berkefeld filters,[57] grades V and N, and appear in the filtrate when provided with favorable cultural conditions for several days. On the fourth day the young forms commence to appear in the fluid which collects in the empty tube which is fitted up to receive the drops that fall by spontaneous diffusion without suction or pressure. This phenomenon, which was first noticed and utilized by myself when obtaining a pure culture from mixed cultures, has since been confirmed by Nakano [212] and others.

There are yet other spiral organisms which are of great interest from the standpoint of filterability. Thus, Wolbach and Binger described Spirochæta elusa [213] and Spirochæta biflexa,[214] which they obtained in a filtrate of stagnant water taken from

the shores of a fresh water pond in the vicinity of Boston. The first was cultivated but the second was not. With the culture of S. elusa, which measures about 0.5μ wide and 20μ long with an average of six to eight curves, they were able to demonstrate the organism in the filtrate within about fifteen minutes. The filtration was made by suction with the Berkefeld filters, V and N. The organism is provided with one terminal flagellum at each end and is extremely motile. Spirochæta biflexa was a much more delicate organism. Another filterable organism, morphologically considered a "spirochæta" in the loose sense of the term, was obtained by Wolbach and his associates from human fæces. In explaining the filterability of these rather coarse spiral organisms, which are larger than many bacteria, Wolbach considers their plasticity to be one of the important factors.

Cultivation.—Only a comparatively limited number of "spirochætes" have been cultivated on artificial media. Of the free-living varieties Spirochæta plicatilis was cultivated by Zuelzer [22] in a flask containing ¾ liter of stagnant lake water and ¼ liter of water to which a certain amount of hydrogen sulphide had been added. According to this procedure the flask is hermetically (anærobic) sealed after the inoculation and hydrogen sulphide occasionally introduced. The rôle of H_2S is to produce sulphur by oxidation ($H_2S + O = H_2O + S$). By this means the organism can be kept in culture for an indefinite period. Wolbach's Spirochæta elusa was cultivated on a hay infusion (ærobically) where it propagates indefinitely. This organism is not allied to Ehrenberg's organism, but appears more like a Spirillum. No culture has been obtained of the molluscan cristispira. Of the Spironema group several varieties have been cultivated. Attempts at the cultivation of Spironema novyi by Norris, Pappenheimer and Flournoy [215] were partially successful in that these investigators were able to notice a definite multiplication of the organism in human or rat citrate blood at room temperature within 24 hours, a second generation cultivated in a similar medium bringing about the same increase the next day. A third generation was not obtained. Occasional multiplication of the Spironema in a defibrinated blood had previously been shown by

earlier investigators (Lachmann, Albrecht, Gerhardt). Cultivation of the blood Spironema in the strict sense of the term was achieved by the writer [216, 217] for the first time by employing a culture medium containing a piece of fresh tissue (rabbit's kidney, etc.) and ascitic fluid (10 to 12 cm. deep). This medium provides a condition that I proposed to designate as an *aërotropic anaërobiosis;* that is, a strictly anaërobic state is produced around the base of the fresh tissue, while the top of the ascitic fluid column has access to a certain quantity of oxygen. The whole medium may be covered with a layer of sterile paraffin oil in order to prevent evaporation of the fluid, and this regulates at the same time the amount of oxygen admitted. When the medium is inoculated with a minute quantity of the Spironema-containing blood and then incubated at 37° C., the organism multiplies steadily until every field will show numerous motile specimens which may occur singly, in pairs, or in chains of three or more individuals. The height of multiplication is reached within four or six days, and a sudden degeneration of the organisms sets in on the seventh to the ninth day. By making subcultures on the fourth or fifth day the culture can be carried on indefinitely. It was found that the success or failure greatly depends upon the suitability of the ascitic fluid samples. A sample which forms a loose fibrin web with the fresh tissue (rabbit's kidney) within 24 hours at 37° C. gives the best empirical results. The addition of glucose or pepton seems to hinder the growth of the Spironema, and sterilization by filtration or fractional heating also impairs the nutrient value of the medium. Invasion of the culture by any other bacteria quickly destroys the culture; in other words, no mixed culture has been obtained. So far I have been able to cultivate S. recurrentis, S. novyi, S. duttoni, S. kochi and S. gallinarum by the same method. Plotz [218] successfully applied this method in order to obtain a culture directly from patients in Bulgaria suffering from the European relapsing fever.* According to Hata,[219] instead of the fresh tissue and ascitic fluid, a medium consisting of a piece of blood coagulum and of the serum of the horse may be satisfac-

* Personal communication.

torily used for cultivating S. recurrentis. At the temperature of 26° C. the culture in this medium remains actively motile for a month (Hata). None of the spironemata causing septicæmia in man or birds have been cultivated on a solid medium, and nothing is known about such colonies. In a fluid medium they produce a diffuse opalescence but no definite change of the medium is noticed. No odor or gas or change in the reaction has been detected in the culture. Their virulence remains unattenuated for many generations of artificial cultivation.

From the oral cavity S. vincenti and several spiral organisms have been obtained in pure cultures by various investigators. Tunnicliff [220] considers S. vincenti and Bacillus fusiformis to be identical, but in different phases of development and under varying conditions. All these organisms are strictly anaërobic and can be cultivated by the usual anaërobic methods on solid media (glucose agar with or without animal proteids). They form definite colonies comparable to any other bacteria, and some are putrefactive or acid-producing organisms. I am inclined to regard them as allied to Spirillum rather than to Spironema.

Spironema icterohæmorrhagiæ has been cultivated by Inada [79] and Ito in the same medium as was originally employed by the writer for the cultivation of S. recurrentis, S. duttoni, S. gallinarum, etc. Ito [221] later succeeded in cultivating the organism on agar or gelatine containing human or guinea-pig defibrinated blood in the ratio of equal parts, or one to two, of blood and agar or gelatine. The organism is said to grow readily on these media at a temperature of 26°–37° C. A good growth takes place even at room temperature. Characteristic cultural features, such as gas or odor production, colonies, turbidity, etc., have not been recorded. In the fluid as well as in the solid media no visible growth was obtained. The culture is said to remain virulent for many generations and lives over one month in a solid medium when kept at about 15°–26° C.

In proportion to its clinical importance Treponema pallidum has ever since its discovery in 1905 been the subject of correspondingly more numerous investigations. Volpino and Fontana [222] (1906) observed a temporary increase of the organisms

after a piece of syphilitic tissue had been put in human serum or defibrinated blood and then incubated at 37° C., but no culture was obtained. Lebailly [223] claims to have kept alive the pallida in the syphilitic fetal tissue for 15 days when put in human serum at 37° C. Levaditi and McIntosh [224] inoculated the inactivated human serum with the expressed serum of a syphilitic lesion of a monkey containing a few pallida and, after sealing it in a collodion sac, introduced it into the peritoneal cavity of a monkey. It was taken out after a month and was found to contain numerous motile pallida along with certain contaminating bacteria. The contents of the sac cultivated *in vivo* could be successfully transferred from one sac to another for many passages with the same result. The impure pallida cultivated by this method were avirulent for monkeys. Mühlens and Loehe [225] failed to confirm the above findings. In 1909 Shereschewsky [226, 227] claimed to have succeeded in starting an impure culture of Treponema pallidum by implanting a semi-solidified, clear horse serum with a piece of chancre or condyloma inserted several inches below the surface. The serum commenced to liquefy around the tissue and within several days more liquefaction took place. On examining the fluid or solid medium about the tissue, he found very numerous actively motile spiral organisms resembling the pallidum. Some of them were coarser and less regularly curved and looked like S. refringens. An enormous number of cocci or bacilli were also present. The culture gave off an intensely offensive odor. Subcultures were carried on indefinitely. The impure culture was avirulent for experimental animals. About the same time Mühlens [228] obtained a pure culture of an organism from a syphilitic lymphadenitis of man by first using Schereschewsky's medium and then transferring the culture to another kind of solid medium consisting of horse serum and agar. In the latter he succeeded in purifying the treponema from the contaminating bacteria. Notwithstanding the fact that the organism was derived from a material in which the pallidum would be the only small treponema, and in spite of its great resemblance to the pallidum, Mühlens's culture has certain characteristics which, as will be seen later, render the organism distinguishable from

the pallidum cultures which were obtained by others (Noguchi,[229, 230] Sowade,[231] Tomasczewski,[232] Baeslack,[233] Zinsser, Hopkins and Gilbero [234]). Thus the organism isolated by Mühlens was avirulent, produced a strong odor and could grow from the beginning in a horse serum agar without the addition of any fresh tissue. W. H. Hoffmann [235] (1910–1911), a co-worker of Mühlens, obtained several strains which were identical with that of Mühlens, except for the fact that he was able to produce in the rabbit's testicle a somewhat acute or subacute inflammation by injecting a large quantity of solid culture.[236] His description of the experiments leaves the syphilitic nature of the lesion indefinitely established. The extract of the organism acted as an antigen in the Wassermann reaction, as was also shown by Schereschewsky [237] in the case of his impure culture; but Mühlens as well as Schereschewsky obtained similar results when extracts of other bacteria were used. Recently Zinsser and Hopkins have confirmed the non-specific nature of so-called antigens in this type of complement fixation. Bruckner and Galasvesco [238] (1910) and Sowade [231] (1911) reported the successful inoculation of rabbits by means of their impure cultures (Schereschewsky medium) given intratesticularly and intracardially. Sowade claims to have produced generalized syphilis by the intracardial injection, into half-grown rabbits.

During 1910–1911 I was engaged in cultivating T. pallidum.[57, 120] Unlike the previous investigators, I had chosen the testicular syphiloma of rabbits as the material for cultivation, for the reason that in this material we have a constant and unlimited supply of a practically pure pallidum and as many strains simultaneously as one desires to try. Besides, the rabbit strains being already acclimatized to the animal, this would more readily take on when a culture derived from this source is to be tested for its virulence. After unsuccessful attempts to cultivate the pallidum in all the various media previously reported suitable for cultivation of the pallidum, and a large number of culture media, and conditions having also failed, the following two methods were found to yield a positive growth of the organism on an artificial medium. As has been mentioned elsewhere, neither method is a

perfect one, and only a limited percentage of attempts is ever successful. The inconstant results are due partly to the different resistance offered by various strains to the artificial cultivation, and partly to certain still unknown factors which enter into the composition of the media. At all events, the greatest difficulty in cultivating the pallidum is to obtain the first growth. As the number of generations increases, the organism acquires an easier growth, and after a period of years of life in the culture the organism becomes quite saprophytic and may grow even without the addition of fresh tissues. The strict requirements demanded by anaërobiosis and by the reactions and compositions of the media become more and more lax until the culture may adapt itself to a great many cultural conditions. The two methods above mentioned are (1) a fluid medium consisting of a suitable sample of ascitic fluid or sheep serum water (His) with the addition of a piece of freshly removed kidney or testicle from a normal rabbit; and (2) a solid medium consisting of a mixture of ascitic fluid and agar with the addition of a piece of fresh tissue as above described. The use of the fresh tissue seems to offer two-fold advantages. First, as an oxygen absorbent as originally recommended by Th. Smith,[239] and secondly, as a source of nutrient substances needed for the pallidum. The first method (fluid medium) is applied exclusively for the cultivation of the testicular pallidum from rabbits, and the second (solid medium) is only used to cultivate the impure material derived directly from human syphilitic tissues. The first method requires an anaërobic apparatus, as a complete removal of oxygen from the atmosphere in which the cultivation is to be carried out is essential, while for the solid medium a layer of sterile liquid paraffin poured on the top of the culture medium suffices to prevent evaporation and possibly to minimize the diffusion of oxygen into the medium. The requisite anaërobiosis is produced by the fresh tissue which lies at the bottom of the tube. I shall not enter into any technical details, but suffice it to say that nearly a dozen strains were obtained within the last few years by the use of these two methods. The strains obtained by means of the fluid medium remained

for many generations unadaptable to the solid medium to which they finally grew. On the other hand, the strains grown on a solid medium could readily be made to grow when suitable conditions were provided.* Impure pallidum cultures in a fluid medium can be purified by allowing the pallidum to grow through the pores of a Berkefeld filter. Before the associating bacteria passes, the pallidum will appear in the filtrate (by gravitation), probably on the fourth or fifth day. Some of the strains of T. pallidum obtained by these methods were virulent to rabbits and monkeys when tested within a few months. The lesions produced were typical in every respect, although once the organism had entered the animal body it resisted recultivation just as much as before the first cultivation. In this respect they differ from the strains of W. H. Hoffmann,[236] who was able to cultivate the organisms back from the lesions into the horse serum agar without the addition of any tissue. His strains produced a strong offensive odor when recultivated. The strains cultivated in my laboratory did not, and still do not, give any offensive odor such as is described by Mühlens and W. H. Hoffmann. As has been stated, no growth could be obtained without the aid of fresh tissue during the first year after these strains were isolated. Nor could they be induced to grow on a plain horse serum agar of Mühlens or semi-coagulated horse serum of Schereschewsky. Since attention had been called to the differences which existed between the cultures of Mühlens and Hoffmann and my own cultures, later investigators gave special attention to detecting any possible production of a peculiarly offensive odor. Sowade, Tomasczewski, Baeslak, Nakano, Zinsser, Hopkins and Gilbert failed to find any such characteristic odor in their strains. Erich Hoffmann [240] considers that the cultures of Mühlens and W. H. Hoffmann either contained the pallidum and a second odor-producing organism or were not Treponema pallidum at all, since there exist certain pallidum-like, easily cultivated, saprophytic treponemata which in pure cultures produce a strongly offensive odor (T. microdentium, T. mucosum, *vide infra*).

That the cultivated strains of Treponema pallidum gradually become tolerant to various media and conditions had been strik-

* Fluid medium method.

ingly demonstrated by the recent investigations of Zinsser, Hopkins and Gilbert.[234] Thus the investigators found that a pallidum strain which they had isolated by the original fluid culture method, described by me, gave a good growth after the tenth generation in fluid media containing different kinds of autoclaved tissues of rabbits and a mixture of slightly acid meat infusion broth with heated sheep serum. This strain likewise grows well in symbiosis with staphylococci, streptococci, Micrococcus candicans and bacillus fæcalis alkaligenes added to the sheep serum agar mixture without tissue. Addition of dead staphylococci had the same effect as symbiosis. The gelatinized horse serum or sheep serum with or without the tissue proved to be a good medium for the growth of this strain. They have obtained the same results with two other strains which were isolated in my laboratory several years ago. The fact that during the first few years after isolation all my pallidum strains failed to grow on various media similar to those now successfully used by Zinsser, Hopkins and Gilbert seems to indicate that rigid parasitic properties of the organism have gradually deteriorated, due to the artificial cultural conditions, some undergoing the changes more abruptly than others. It is not at all improbable that in time the saprophytized strains of Treponema pallidum will adapt themselves to still simpler ordinary culture media. It is to be desired that efforts be directed toward improving the condition under which the organisms can be kept as nearly natural as those in living tissues, since the results obtained with completely denatured material might be different from those derived with the less modified material.

In summing up the pallidum cultivation one may say that the methods hitherto proposed are still imperfect and that much patience is still demanded in order to isolate a strain. Some strains remained persistently unamenable to cultivation in my hands. The strains isolated by Mühlens may have been Treponema pallidum; but there was no way of proving this, as his culture, which was avirulent, possessed certain properties inconsistent with those of the pallidum as subsequently defined by other investigators. The first instance, therefore, of a successful culti-

vation of Treponema pallidum was that which was carried out at the Rockefeller Institute in 1910–1911, in which were brought out not only the demonstration of the pathogenicity of the organism isolated but also the studies of other biological characteristics of the culture. Treponema pertenue [241] was also successfully cultivated in 1911 by the same method as that given for the cultivation of the pallidum. The material used for this work was in the form of a testicular lesion of experimental yaws in the rabbit and was supplied by Captain Nichols. The organism possessed the same cultural characteristics as the pallidum, but it was probably slightly thicker and less regularly curved. Preliminary attempts to produce lesions in rabbits ended negatively, and the strain was lost before further comparative studies could be undertaken.

Several spiral organisms were isolated from unclean lesions around the genital regions. Spironema refringens [242] and Treponema calligyrum [104] (from a condyloma) were cultivated by me in the pure state by methods similar to those used for the pallidum and pertenue. Levaditi and Stanesco [243] obtained impure cultures of S. gracilis and S. balanitidis by means of gelatinized horse serum. A spiral organism, *S. phagedenis,* was also obtained by me in a pure culture from a phagedenic ulcer on the genitalia of a woman, but its systematic affinity is quite uncertain.[95] T. calligyrum is slightly coarser than the pallidum but is apt to be mistaken for the latter in cultures. It grows easily in tissue-free media.

From dental deposits of normal oral cavity Treponema macrodentium and T. microdentium [102] were cultivated in the pure state by means of the same methods, and Treponema mucosum [103] from the scraping of the pyorrhœal gum. The microdentium and the mucosum appear very similar, but can be distinguished by the production of a thin, but tenacious, mucin in the culture of the latter. When the culture gets old, both of these give a strong, somewhat offensive odor. This faculty to decompose the proteids (thus causing a slight turbidity in the fluid media) makes this culture readily distinguishable from the pallidum cultures, because the latter do not produce such an odor. Morphologically they bear a great resemblance to the pallidum,

although their curves are set somewhat more closely than in the pallidum. The macrodentium is more difficult to cultivate and, according to my experience, requires fresh tissue in the culture media. Morphologically it is coarser than the pallidum and its serpentine movements and irregular, stretchable, and wider curves are characteristic enough to distinguish this species from other varieties. It does not produce an odor.

Mühlens and Hartmann [244] succeeded in 1906 in obtaining a pure culture of S. dentium in the horse serum agar medium of Mühlens. In their culture they recognized a minute form of Koch's S. dentium type and another which approached the dimension of Hoffmann-Prowazek's S. media type. They suggested the possibility of these representing different stages of development or even a sexual differentiation. It appears as though their so-called pure culture may have contained more than one species. No admixture of tiny and coarse forms has been observed in the culture of the macrodentium or microdentium. The dentium culture of Mühlens produced a strong offensive odor.

Immunity and Immunization.—The very name, relapsing fever, suggests the possibility of the development of some sort of protective power in the infected hosts against a third attack. In fact, the second attack is often milder than the first and a third relapse is rare. Persons who have had the fever are usually immune to subsequent infection for a period of several years. The same is true of the African tick fever, although repeated recurrences are more frequent in this instance. Susceptible animals such as monkeys, mice, and white rats, enjoy a period of immunity extending over about three months after recovering from the second attack. In rats no relapse has been observed. In the fowl and geese spironematosis similar immunity follows recovery. The studies of various investigators, especially Gabritschewsky, Pfeiffer, Novy and Knapp, Manteufel, Marchoux and Salimbeni, Levaditi and Manouélian, Prowazek, Neufeld and others, have contributed in explaining the mechanism upon which the immunity depends. Gabritschewsky [245] demonstrated the presence of a specific antibody against S. recurrentis in the blood of convalescent patients by mixing it with the spironema-contain-

ing blood *in vitro*. The destruction of the organism occurred within a short time when the mixture was kept at a temperature of 37° C. This author considered that the development of a germicidal substance in a patient's blood was the cause of the crisis and subsequent immunity. He also recognized the appearance of a similar specific immune substance in the geese recovering from the attack of S. anserina. The convalescent recurrentis blood had no effect upon the organisms of goose fever, and the anserina blood did not affect the organisms of the relapsing fever. The phenomena observed were agglutination, immobilization and dissolution of the organisms when mixed with their correspondingly specific bloods. Gabritschewsky produced an immune serum by injecting the horse with the spironema-containing blood. It was tested by Löwenthal in 83 cases and in 39 cases (47 per cent.) no relapses occurred, while in 140 untreated cases 65 had three attacks (46.5 per cent.). Novy and Knapp [18] confirmed and greatly extended the experimental parts of Gabritschewsky's work and pointed out that the protection afforded by active as well as passive immunity is not wholly dependent upon the germicidal property but also upon the immune bodies, since a comparatively weak germicidal blood may protect the animal against the infection in small quantities. Besides, Novy and Knapp hold the rôle of the phagocytes (mononuclear, but not polynuclear) to be very important, as they ingest the dead as well as the enfeebled spironemata under the infiuence of immune bodies. Levaditi and Manouélian [246] suggest the existence of an opsonin in this phenomenon. Manteufel [247] believes the lysis of the spironemata in the immune serum to be due to the co-operation of complement and a specific amboceptor. The rapidity and intensity with which the spironemata are destroyed within the peritoneal cavity of actively or passively immunized rats is variable. In the peritoneal cavity of a hyperimmunized animal, the organisms become granular in from 2 to 5 minutes, while in rats recently recovered from the attacks the organisms are ingested in 15 minutes. In passively immunized rats the spironemata are first agglomerated and temporarily immobilized, and this is followed by the appearance of some leucocytes on the scene; but the effects of the immune

substances gradually wear off within about an hour. The leucocytes disappear in 30 minutes (Novy and Knapp). The germicidal and bacteriolytic actions are parallel. The duration of passive immunity in rats is less than 40 days while that of the active immunity lasts nearly four months. Novy and Knapp succeeded in preparing in rats by means of hyperimmunization a powerful immune serum which contained in each cubic centimetre 500 immunity units; that is, 0.002 c.c. of the serum was able to protect the rat against 0.1 c.c. of the infective blood showing 10–50 spironemata per field (2 mm. objective). In ordinary recovered rats there were only about 2 immunity units per cubic centimetre. The use of the immune blood from a hyperimmunized rat prevented the infection in the rat and cured it on its onset, but a greater amount is found necessary in order to obtain similar results in monkeys and mice as these animals are subject to a relapse after the treatment. Novy and Knapp suggested the inoculation of the spironema during the apyretic period in order to increase the amount of immune principles in the victim's system and thereby ward off a relapse. They found an interesting phenomenon, *i.e.*, the injection of too much immune blood proved to be less effective than a moderate quantity. This was explained by assuming the production of a specific precipitin which acted as an anticomplement. In regard to the use of hyperimmunized blood serum in human relapsing fever, they calculated that about 375 c.c. of a serum such as mentioned in the experimental part would be necessary, and that the future of a serotherapy much depended upon the success attained in cultivating the organism in an artificial medium in large quantities. As a matter of fact we have been able to collect large quantities of comparatively pure organisms from each of the cultures of S. recurrentis, S. duttoni, S. novyi, S. gallinarum, etc., for various purposes (immunization, vaccinotherapy, etc.). In the serum of those who had just recovered from the relapsing fever a complement-fixation principle was demonstrated by Kolle and Schatiloff [248] and Korschum and Leibfried.[249] The reaction was said to be positive after the second attack.

In Weil's disease Inada and his co-workers found the presence

of a specific spironemalysin in the serum of convalescent man or guinea-pigs. The immune bodies develop after the second week of the disease and may be still present in individuals who had the attack more than four years previously. The Pfeiffer phenomenon is easily demonstrated by using the organ (liver or kidney) emulsions rich in the spironemata, or a culture and the immune serum in the peritoneal cavity of the guinea-pig. These investigators immunized goats and horses with the cultures of the causative agent (S. icterohæmorrhagiæ) for a period of more than a year and succeeded in producing a serum which prevents the infection against the lethal dose in guinea-pig in the amount of about 0.001 c.c. The clinical experience of this serotherapy which has now extended over many hundreds of cases proves to be highly encouraging.*

The question of immunity in syphilis is rather imperfectly understood. In human subjects it was once assumed that after the first infection complete immunity occurs, as evidenced by the extreme rarity of a reinfection. Later investigations seem to consider this assumption as incorrect, inasmuch as it was based upon the fact that the syphilitic individuals do not a second time contract a chancre or show a general skin eruption in spite of exposure to such an infection. This fact does not, however, necessarily denote immunity in the usual sense of the word. This state of refraction to the second infection is said to be due to the pre-existence of the same virus in the same individual who no longer reacts to the second inoculation with the original intensity or vigor, and the condition is designated by Neisser as "Anergie." At the same time Hutchinson showed the possibility in rare instances of an auto-inoculation, while Finger and Landsteiner [250, 251, 252] believe that a superinfection may take place in certain syphilitics. The effect of a superinfection may be a purely local manifestation or it may be subsequently followed by generalization; or it may again cause a general mobilization of the virus without a local manifestation. The character of the lesions produced by superinfection agrees with that of the lesions peculiar to different stages of the disease. If it occurs during the secondary stage the superinfected lesion will be a papule or other exuda-

* Personal communication soon to appear in print.

tive varieties, and if during the tertiary stage the result will be a gummatous product. This alteration of various tissues of a syphilitic individual in their reactivity to the syphilitic virus is designated as " Umstimmung " by Neisser, who regards this condition as a morbid state of the tissues brought about by the presence of Treponema pallidum. There once prevailed a vague impression that when cutaneous tissues are extensively involved there is less likelihood of the visceral organs being invaded by the syphilitic virus and *vice versa*,[253, 254] but there is not experimental proof to support this contention. Since the introduction of salvarsan and its derivatives in the treatment of syphilis, the instances of reinfection with typical or sometimes atypical chancres are not so rare, thus indicating that after a cure has been effected the human body reacts in the usual, or nearly usual,[255] manner. This also points to the absence in such cases of any lasting immunity after the first infection has been eradicated. A thorough investigation is required in order to ascertain whether or not a certain degree of immunity develops in some of the cured cases, thereby affording protection. In some ways the question of immunity in syphilis is comparable to that in protozoan diseases, in which, though latent, no typical infection can be reinduced until the first attack is completely cured, and where no congenital immunity has yet been demonstrated.

Let me now review the situation of the immunity question in experimental syphilis. Metschnikoff and Roux, Neisser and Bruck, and others found that monkeys which have once been infected with Treponema pallidum may prove refractory to subsequent inoculation. Metschnikoff [256] thought he succeeded in protecting a monkey against the infection by inoculating it with an attenuated living virus which was no longer able itself to produce typical reactions. That the vaccination against syphilis was not equivalent to that against variola in its fundamental principle was later demonstrated by Neisser and others, who were able to show that the monkeys which had been " vaccinated " with an attenuated virus and which were rendered "immune" to the subsequent inoculation with a fully virulent material were harboring the infection in various localities escaping the usual clinical

detections. Thus the emulsions, prepared from the bone marrow, spleen, etc., of the " vaccinated " animals were able to infect new susceptible animals. This phenomenon is similar to the state of anergy observed in syphilitic human subjects. Fontana,[257] Uhlenhuth and Weidanz,[258] Bertarelli,[259] Truffi [260, 261] and others pointed out that a rabbit which carries syphilitic keratitis in one eye is not refractory or immune to the infection in the other eye. A rabbit, one of whose testicles is infected with T. pallidum, offers no greater resistance in the other, which may be infected with the virus at any stage of orchitis preceding that on the opposite side. Tomasczewski [262] thought that a skin infection produced in rabbits in which scrotal lesions had been persisting for about two months was much milder than in normal animals. According to personal observations a rabbit in which a syphilitic orchitis, or keratitis, or scrotal chancre has been cured either spontaneously or through the administration of salvarsan, enjoys no perceptible immunity to syphilis. Truffi repeatedly inoculated rabbits with a fetal liver emulsion containing an abundance of T. pallidum, but found no immunity to develop. Uhlenhuth and Mulzer [189] immunized rabbits with the testicular pallidum emulsion without obtaining any decisive result, although in some cases they thought it exerted a beneficial influence upon the syphilitic process. In my personal experience it has been found that the susceptibility of the rabbit to syphilis is decidedly diminished in some animals by immunizing them with T. pallidum for several months. With a strain which gave 100 per cent. takes in normal rabbits' testicles only about 60 per cent. positive results were obtained in the immunized animals. This tends to show that the lower percentage of positive takes in the immunized rabbits may be due to the destructive influence of the treatment upon the invading pallida. But it was also found that in the immunized rabbits in which the inoculation succeeded the symptoms were not any milder. In fact, not only were the local reactions just as marked as in the control animals, but there was a tendency to the formation of generalized lesions. In two of the rabbits scrotal lesions developed after the intravenous inoculation of a virulent strain. It appears that an incomplete immunization exerts an adverse influence on the de-

fensive factors of the rabbit. This phenomenon finds verification in the work of Grouven and Sowade [263, 264] who recommended for the animal a few preliminary intravenous inoculations of the pallidum in order to insure a generalized infection through a subsequent intracardial introduction of the organisms in huge quantity. I also endeavored to ascertain whether a local administration of devitalized pallida (killed at 60° C.) on many successive occasions will not bring about a state of local immunity to Treponema pallidum, but my results were rather unsatisfactory, for the reason that the testicular parenchyma which was repeatedly inoculated with the pallidum emulsion underwent gradual atrophy and the resulting hard fibrous structure was no longer a suitable test-object for this fastidious parasite. Nevertheless I was able to produce small nodular lesions in two out of several rabbits so treated. Moreover, reinfection of the same tissues (cornea, testis, skin) after a spontaneous or chemotherapeutic healing has been found possible as long as the suitable structures of the tissues are preserved.

Our knowledge pertaining to the immunity phenomena *in vitro* is of more recent date, for the test-tube experiments with T. pallidum were made possible since the discovery of the organism and were particularly facilitated by the successful cultivation of the parasites on artificial media. Attempts to demonstrate the presence of a specific agglutinin for T. pallidum in the sera of human and experimental syphilis were made by Hoffmann and Prowazek,[265] Herxheimer and Löser,[266] Hoffmann[267] Brönnum and Ellerman,[268] Babes and Pinea,[269] Metschnikoff and Roux, Landsteiner and Mucha,[270] Zobolotny and Maslakowetz,[271] and others, with the pallida derived from the syphilitic tissues. Their experiments were indecisive, owing to the difficulty found in obtaining a pure material free from various tissue constituents. Uhlenhuth and Mulzer[189] found no agglutinins in the sera of the rabbit, goat and monkey after repeated intravenous injections of the rabbit's testicular emulsion rich in the pallidum. In 1910–1911, soon after obtaining pure cultures of T. pallidum, we started the immunization of rabbits with different strains of the organism. In the sera obtained from the immunized rabbits we were

able to demonstrate the presence of the specific agglutinins and complement binding principles for the cultivated pallidum strains. We were unable to produce with the sera any unmistakable agglutination of the pallidum derived directly from the syphilitic orchitis of the rabbit, but considered this to be due to the simultaneous presence of tissue debris and other cellular elements which may have interfered with the agglutination phenomenon. These sera were not strictly specific, but contained a small quantity of agglutinins for other treponemata obtained in pure cultures. There were also a sufficient number of specific complement-binding bodies, but there was at the same time a more or less definite group reaction for other treponemata. The work was continued later (1915–1916) by Akatsu at my laboratory with similar results. He was able to obtain a serum which could agglutinate the pallida in a dilution of 1 : 50,000.

In order to know whether syphilitic human sera have any definite agglutinating and complement-binding properties, a number of sera obtained from the various stages of syphilis were examined with pure cultures as well as with the tissue pallidum derived from rabbits' testicles. All experiments were unsatisfactory owing to the difficulty experienced in reading the reaction in the case of agglutination and also owing to the high anti-complementary powers of the antigens and the feebleness of the reaction in the case of the complement fixation test, except in the case of the pure culture antigens which fixed complement with the immune rabbits' as well as with some of the syphilitic human sera (chiefly late and tertiary cases). According to our experiments there is a certain degree of group reaction or the other treponemata (T. calligyrum, T. microdentium, T. mucosum, and S. refringens).

Kolmer [272] first described the agglutination of a pure culture of Treponema pallidum by the sera of rabbits injected with a living and heat-killed culture furnished by our laboratory. His results show that normal rabbit sera do not agglutinate the culture pallidum in dilutions as low as 1 : 20, while the sera of immunized animals produced agglutination in dilutions as high as 1 : 1280. No definite agglutination was observed with human syphilitic sera in

a dilution of 1:20 or higher. Nakano [273] also reported the presence of agglutinins in the sera of rabbits injected intravenously with a pure culture in dilutions from 1:10 to 1:70. Kissmeyer [274] immunized rabbits with a pure culture of T. pallidum and was able to obtain agglutinins in dilutions as high as 1:200,000 to 1:500,000 of the immune sera, while the sera from individuals with primary, secondary, tertiary and congenital syphilis contained agglutinins for the pallidum in dilutions of 1:100 and higher in a percentage of 40 to 60 out of 59 cases. Normal human sera may agglutinate the pallidum on dilutions as high as 1:50. Zinsser and Hopkins [275] state that normal rabbit serum may agglutinate the pallidum in dilutions lower than 1:10, but the sera of their immunized rabbits (intravenous injections of the pallidum cultures) agglutinated it in dilutions as high as 1:2000. They added that the normal as well as certain syphilitic human sera may agglutinate the culture pallidum in emulsions. Zinsser, Hopkins and McBurney [276] failed to observe any agglutination when the pallida from human lesions were mixed with the immune sera (rabbits and sheep) produced with the culture pallida. Zinsser and Hopkins demonstrated the treponemicidal bodies for T. pallidum (cultivated) in the immune serum produced by them.[277]

In the sera of animals experimentally infected with syphilis the presence of specific complement-binding antibodies for T. pallidum has not been satisfactory proved. It is true that we were able to demonstrate the positive complement fixation in the sera of animals immunized with cultivated treponemata, but this does not hold good when dealing with the syphilitic animal sera and the virulent pallidum strains found in tissues. On the other hand, these syphilitic sera do bind complement when mixed with pure cultures, not only of T. pallidum, but also of various bacteria, such as colon bacilli (Zinsser and Hopkins). Undoubtedly the phenomenon is non-specific but pathognomonic, as is the Wassermann reaction which is caused by certain lipoidal substances. These cultures must serve as the containers of the similar lipoids. Indeed, Craig and Nichols [278] long ago showed that the alcoholic extracts of the pure pallidum and pertenue cultures

produced almost equally strong complement fixation when mixed with the human syphilitic sera giving a positive Wassermann reaction with pure lipoidal antigens derived from other tissues. In a word, a syphilitic animal may give a positive complement fixation with various lipoids without at the same time containing any specific antibody for T. pallidum. In human syphilitic sera the same is also true, except in the sera of certain late and tertiary cases in which there may be a positive reaction due to the specific antigens and antibodies in the strict sense of Bordet-Gengou's phenomenon.[279]

The nature of the Wassermann reaction in the sera of human experimental syphilitic subjects is still unexplained, but one fact has been established *viz.*, that it is due to a peculiar change of the sera not specific for syphilis; it occurs in yaws, leprosy, trypanosomiasis, malaria (febrile period), and sometimes in malignant tumors. The fact that so many lipoidal substances as well as certain salts (sodium taurocholate, sodium cholate, etc.,) derived from different sources can bring about a positive fixation precludes any strict specific antigen-antibody reaction. According to personal observations, the lipotropic complement-fixation reaction is not present in immune rabbit sera which have been obtained by injecting the pallida repeatedly, and which contain a large number of specific complement fixation bodies from the pallidum strains employed for their production.

Closely related to immunity is the question of allergy in syphilis. From the chronic nature of the disease many investigators considered the possibility of its occurence at one stage or another. Jadassohn, Meierowsky, Ciuffo, Fontana, Neisser, Bruck and others made numerous observations which rendered the presence of allergy still more probable. These investigators were handicapped by not having a pure culture of T. pallidum. Soon after the isolation of the pallidum strains Professor Welch suggested that I undertake a study of this subject in human syphilis with the pure material. In the meanwhile it was ascertained experimentally that the prolonged treatment of rabbits with intravenous injections of the pure pallidum culture as well as with the organisms obtained direct from the rabbit's orchitis

lead to the production of a state of hypersensitiveness of the skin to the inoculation of the extract of a pure, heat-killed pallidum culture.[280] The reaction was found to be apparently specific for T. pallidum. There was no injurious effect following the injection into the rabbits of the heat-killed pallidum emulsion. The emulsion, since known as luetin, was employed as a means of diagnosing human syphilitic cases, with the result that the luetin reaction was found to be most frequently present in the latent tertiary and congenital syphilis cases where one would naturally expect most constantly to find the allergetic or hypersensitive state of the skin. As an auxiliary or supplementary factor in producing a positive luetin reaction I have already pointed out that the pathological state of the skin of chronic syphilitic patients designated by Neisser as "Umstimmung" a rôle in nearly 10 per cent. of tertiary cases in which the skin reacted intensively to the inoculation of the control emulsion without the pallida. No efforts were made to explain this peculiarity of hypersensitiveness of the skin of certain syphilitics. But a recent work of Camp [281] points out that the administration for many days of potassium iodide to a non-syphilitic individual produces in the skin a hypersensitiveness to any trauma, including the inoculation of the leutin. Probably this finding may furnish the solution of the problem of Neisser's " Umstimmung," or at least of one of the contributing factors. The clinical evidences thus far accumulated seem to show, however, that in a large number of cases the luetin reaction was positive in spite the fact that no iodide had been given during the period when the test was applied. Recently Akatsu [282] at my laboratory carried out several series of experiments regarding the influence of potassium iodide upon the reactivity of the skin of rabbits to the intradermal inoculation of the luetin, control fluid and plain bouillon. The iodide was administered intravenously for a period of from 7 to 9 days, given in increasing doses of 0.5 to 2 c.c. of a 10 per cent. aqueous solution. At the end of seven days or later the skin was tested for the luetin, control and plain bouillon. It was found that the skin of normal rabbits did not react to the injections after the iodide treatment. There was no change in its reaction to the trauma. The skin of

the rabbits which had been previously rendered hypersensitive to the luetin by means of prolonged immunization with pure pallidum cultures mostly remained the same, that is, it reacted to the luetin with the same intensity as it did before the administration of potassium iodide. Only in a few instances was the reaction somewhat intensified. There was no definite reaction to the control emulsion of plain bouillon. In some rabbits in which the testicular orchitis after several months had shrunk to a small fibrous nodule the luetin reaction was mildly positive, but the intensity of the reaction was but little influenced after the injection of potassium iodide, except in a few rabbits where the second tests came out more distinctly. The above findings show that the potassium iodide has no noticeable influence upon the reactivity of the skin of normal as well as of syphilitic rabbits. It would be interesting to study whether in other spironematoses (relapsing fever, tick fever, rat-bite disease, infectious jaundice) there appears any skin allergy comparable to that described for other bacterial infections (typhoid, gonorrhœa, etc.). In cases of yaws the skin reacts to the intradermal inoculations of the luetin and of the framboesin with equal intensity and cannot be differentiated by this method (Baermann and Heinemann) [283]

The last and probably the most important field is chemotherapy. The inauguration of modern chemotherapy by Ehrlich is as interesting as it is romantic. It can be traced back to Schaudinn's suggestive but unsupported theory that the spirochætes represent a stage of the life-cycle of trypanosomes, or at least were closely related to the latter. The introduction of organic compounds of arsenic into the treatment of trypanosomiasis was promising much when Schaudinn discovered T. pallidum which he regarded as a protozoa allied to the trypanosomes. Ehrlich took up experimental chemotherapy in connection not only with the latter, but also with the newly discovered spirilloses, as he called them, including syphilis and the fowl fever caused by S. gallinarum. The achievements of Ehrlich and his collaborator Hata, in discovering salvarsan for the treatment of these two diseases, mark a new era in modern chemotherapy. To review this phase of the spirochæte problem would be out of the scope of

my present paper. Suffice it to say that to the great pioneers, Schaudinn and Ehrlich, Metschnikoff and Neisser, we owe an inestimable debt, not merely for their own researches, but also for rekindling in us the sublime stimuli which have already inspired so many investigators to discover new facts, and which will continue to urge us still more to take up this task and to extend our knowledge regarding the classification, morphology, biology, pathogenesis, and experimental as well as clinical aspects of the micro-organisms known as spirochætes.

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