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FREDERICK G. NOVY AND R. E. KNAPP

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STUDIES ON *SPIRILLUM OBERMEIERI* AND RELATED ORGANISMS.*[1]

FREDERICK G. NOVY, SC.D., M.D.,
Professor of Bacteriology,
AND
R. E. KNAPP, B.S.,
Fellow of the Rockefeller Institute for Medical Research.

(*From the Hygienic Laboratory, University of Michigan, Ann Arbor, Mich.*)

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INTRODUCTION.

EVEN before the discovery by Schaudinn and Hoffmann of the *Spirochaeta pallida*, the accepted cause of syphilis, much interest was attached to the spirochetal infections, several of which had been known for a long time. This is particularly true of the spirochete of relapsing fever, which organism was first seen in 1868 by Obermeier, who, however, published no account of his observations until 1873. Notwithstanding that this organism was quite generally recognized as the cause of the disease at an early date, even in pre-bacteriological times, the fact remains that it is one of the least-known parasites. The reason for this is obvious since the disease is exceedingly rare in Europe outside of Russia and the Balkan peninsula. It is apparently common enough in the regions mentioned and also in Asiatic countries, particularly Turkey, Persia, and India. For more than 25 years it has been a rare exotic in western Europe and on this continent it has been practically unknown. Consequently, nearly all of the studies on this disease and its organism have been carried out in Europe by German, and especially Russian workers, and in India by English physicians, notably Carter.

The net result of these investigations was the firm establishment of the relation of *Sp. Obermeieri* to the disease, in spite of the fact that the organism itself resisted all attempts at cultivation. The transmission of the disease to man and monkeys by inoculation with blood containing spirochetes was easily demonstrated, but all other experimental animals withstood such inoculation without any apparent effect. These facts, together with the geographic distribution of the disease readily account for our rather meager knowledge of the organism as compared with that of most of the known pathogenic bacteria. Nevertheless, in spite of these difficulties, attempts were made, as will be seen, to apply a serum therapy and

even a serum diagnosis for the disease. As to the natural mode of transmission of the disease nothing is definitely known, although the bed-bug is commonly credited with being the carrier of the infection. According to several observers, the spirilla remain alive in these insects for an appreciable length of time. Obviously, after having fed on the blood of infected persons these insects must contain in their stomach the parasites together with the ingested blood. Whether actual multiplication takes place in the stomach and whether these insects really transmit the disease is not established. The mere persistence of the organism in such insects for a month or more is a valuable indication which shows that such transmission is possible.

A second spirochetal infection was discovered, in 1890, by Sacharoff, who found it among geese in the Caucasus. The organism known as *Sp. anserinum* is present at times in enormous numbers. It resembles that of relapsing fever, though it is said to be somewhat shorter, and, like the latter, it has resisted all attempts at cultivation. The disease is very fatal, but it is not accompanied by relapses. Death occurs some days after the disappearance of the spirochetes from the blood. It can be transmitted to geese by injecting blood which contains the organism. Ducks and chickens may also be similarly infected, but they recover more easily than do geese. All other animals seem to be immune. The natural mode of infection is unknown, though analogy would indicate insect conveyance.

A third spirochetal disease, likewise very fatal, is that of chickens. This was discovered in Rio Janeiro in 1903 by Marchoux and Salimbeni. The causative organism, *Sp. gallinarum*, is conveyed by the bite of a tick (*Argas miniatus*) when the insect is kept at a temperature of 30°–35°, but not when kept at 15°–18° (Borrel and Marchoux). Chickens have been immunized against the action of the spirochetes, and it has been further shown that the blood of such immune birds possesses marked preventive properties, and Levaditi has been able to demonstrate even a slight curative action. Moreover, in a mixture of immune serum and spirochetal blood, the organisms become immobile and agglutinate, but do not break up into granules or dissolve. The recent demonstration of diffuse flagella and the occurrence of transverse division may be taken to establish the bacterial nature of the organism.

In 1902, Theiler at Pretoria, Transvaal, noted the presence of spirochetes in cattle, and this observation has since been confirmed by Ziemann in Cameroon, and by Koch in East Africa. The infection is very benign and the organism *Sp. Theileri*, is present in but small numbers and soon disappears. It is transmitted through the bite of a tick (*Rhipicephalus decoloratus*), which may transmit at the same time Texas fever. This fact received an admirable demonstration at the hands of Laveran and Vallée, at Paris. They placed some larvæ of this tick, sent to them from S. Africa, on a cow, which as a result, in the course of two or three weeks, developed a double infection of spirochetes and *Piroplasma bigeminum* (Texas fever).

In the same year Theiler also reported the finding of spirochetes in the blood of sheep in Transvaal. Later, in 1904, Martoglio and Carpano also met with this organism in sheep at Erythrea on the Red Sea. It has been assumed, to some extent, that the cattle and sheep spirochetes are the same, though this is not proven. It is advisable that they be considered as distinct organisms until proven otherwise, and from that standpoint we will designate the spirochete of sheep as *Sp. ovis*, n. sp.

Theiler, likewise in 1902, noted the presence of spirochetes in the blood of a horse and very recently (1906) Martin reported another case in a horse at Timbo, French Guinea. Inoculations of sheep and chickens with this spirochetal blood were negative. It is worthy of note that Martin calls attention, the same as Martoglio and Carpano, to a pale central portion indicating transverse division. We propose to designate this organism as *Sp. equi*, n. sp.

Another spirillosis was observed in a bat by Nicolle and Comte in 1905. Its blood on inoculation into two other bats caused infection of both, but was without effect in white mice and a monkey. According to these observers the spirochete also divides by transverse division. We would suggest for this organism the name *Sp. vespertilionis*, n. sp.

The spirochete of Vincent which he described in 1894 as occurring with the fusiform bacillus in hospital gangrene, and later in ulcerative angina, affords another example of human spirillosis, though it must be said that the exact rôle played by this organism in these affections is by no means established. The cultivation of this spiro-

chete, under anaërobic conditions and in mixed culture, has been reported by Tunnicliff.

Somewhat similar organisms have been found in recent years, by various observers, in different parts of the body, more especially about the genitals. Thus spirochetes have been found in balanitis, balanoposthitis, condyloma, and smegma; also in ulcerative carcinoma and in tumors of mice. Similar spirochetes have been found in tropical ulcers of man (Patton); also in ulcers of dogs in Delhi (James). Balfour has recently described and figured spirilla in blood clots covering ulcers on dogs and monkeys. Similar, if not identical, spirilla have been known, for a long time, to develop in putrefying blood, and examples of such are given in Plates VI and VII of Fraenkel and Pfeiffer's *Atlas*.

In passing, mention may be made that about 12 years ago one of us met with very actively motile, long spirochetes in a submaxillary abscess in a guinea-pig. They were very abundant and apparently in pure culture, for all cultivation experiments failed to give any growth either of spirilla or of ordinary bacteria.

The constant presence of *Spirochaeta dentium* in the mouth is a well-known fact, and Miller has recently called attention to evidence indicating that this organism possesses pathogenic properties.

In this connection reference should be made to the fact that spirochetes are frequently present, at times in enormous numbers, in the intestines of man. They have been particularly observed in cholera stools (Kowalski (*Spirillum hachaizae*), Abel, Rechtsamer, Lustig and De Giaxa, Escherich, Aufrecht); also in cholera nostras (Grassberger); in diarrhea of infants (Escherich) and even in the dejecta of healthy persons (Paltauf, Escherich). Similar forms have also been observed in the ulcers of the large intestine in a hog (Th. Smith); in a cat (*Vibrio felinus*, Escherich) and in the stomach of dogs, cats, and rats (Bizzozero, Salomon). In all these cases, where attempts at cultivation were made, the result was negative. Escherich, however, reported a successful culture on Naegeli's medium.

Of very great importance is the recognition of the nature of tick fever, a disease which is widely prevalent in many parts of equatorial Africa. Spirochetes were first shown to be present in this

disease in November, 1904, by P. H. Ross and A. D. Milne who made their observations in Uganda. The organism was discovered independently, and about the same time, by Dutton and Todd in Eastern Congo. These workers proved that the disease was transmitted by the bite of a tick (*Ornithodoros moubata*), and they also showed that young ticks, hatched in the laboratory, were able to impart the disease, thus proving the hereditary transmission of the organism from the female to the young. Koch, studying the disease in German East Africa, demonstrated the presence of spirochetes in the eggs as well as in the adult ticks. There is no evidence to show that the organisms multiply in the ticks. The presence of tangles, as observed by Koch, in the eggs of ticks, indicates an agglutinated condition, which, as will be shown later, is far from being a sign of active multiplication. That spirochetes may live without apparent multiplication for 40 days *in vitro* will be shown, and it is probable that a somewhat similar condition obtains in the tick and its eggs. About a fourth to a fifth of the eggs were found by Koch to be infected. Of 645 ticks examined at different places along the caravan road he found 71, or 11 per cent, to be infected. In some places the percentage of infected ticks was small, in others it was large, reaching as high as 50 per cent. It will be shown in this paper that the spirochete of tick fever is distinct from that of the European and Indian relapsing fevers, and in view of this fact we propose to name the organism *Sp. Duttoni* in memory of the brilliant Dutton who lost his life while studying this disease.

The work of Schaudinn and Hoffmann (April, 1905) showed the almost constant presence of spirochetes in syphilis, and their subsequent investigations, together with those of numerous other workers in all parts of the world, have made it more than probable that syphilis is a chronic spirillosis. The organism was at first designated as *Spirochaeta pallida*, later as *Spironema pallida*, and still more recently Schaudinn has deemed it necessary to create a new genus of which *Treponema pallidum* becomes the type species.

According to Schaudinn the spirochetes are protozoa and not bacteria. In some, like *Sp. plicatilis* and *Sp. refringens*, he has observed the presence of an undulating membrane but no flagella. In *Trep. pallidum*, however, he demonstrated a flagellum at each

end of the spiral, at times even two, but no undulating membrane. Mention may be made of the fact that Salomon likewise found one whip at each end of the spirillum which occurs in the stomach of cats and dogs. The presence of diffuse flagella on *Sp. gallinarum* and on *Sp. Duttoni* (Zettnow), and of a single whip on the organism studied by us indicates considerable variation among the members of the spirochete group. This matter will be considered more in detail later on.

Bonhoff's observation (1905) of the presence of *Sp. vaccinae* in lymph needs confirmation before any definite conclusion can be drawn. The same may be said of the spirochetes found in yaws by Castellani.

Lastly attention should be called to the presence of true spirochetes in insects. The Sergents have found such in the digestive tube of a larva of *Anopheles maculipennis*. We have found a similar organism in the stomach of two tsetse flies (*Glossina palpalis*). This will be described later under the heading "*Sp. glossinae*, n. sp." The spirochete reported by Petrie in birds does not belong to this group, but in all probability is a hemogregarine such as we found in sparrows.

The flagellated organism which Schaudinn in 1904 designated as *Sp. Ziemanni* is in reality a trypanosome, and that being the case it should no longer be included in this group. Moreover, in a previous paper we have shown that the supposed relationship of this mosquito flagellate to the intracellular parasite of the owl is open to serious question. Further and convincing evidence on this point has since been obtained from a study of mosquito trypanosomes and of bird hematozoa. The papers on these subjects may be expected at an early date.

The spirochete with which we have been working was derived from the first case of relapsing fever described by Dr. Carlisle in this number of the *Journal*. In view of the clinical features of the disease and the probability of its importation by emigrants from the Far East we have assumed that this organism is identical with *Sp. Obermeieri*. As to whether this assumption is correct remains to be demonstrated by comparisons of living and stained preparations

and above all by animal inoculations and serum tests. There is one noteworthy discrepancy arising from the traditional statement that *Sp. Obermeieri* can be inoculated only into man and monkeys. This is certainly not true, either for the organism with which we have been working, or for that of tick fever, since these can be transferred to rats and mice.

The fact of such transmission to rats, recognized for this organism first by Norris and his co-workers, is of fundamental importance, since by passage through successive animals the organism can be kept alive, and is thus made accessible for prolonged study. In this way, by inoculating one or more rats each day, we have maintained the spirillum since the first week of last November, at which time it was received from Dr. Norris.

NATURE OF THE ORGANISM.

In a paper, published early in 1904, Schaudinn propounded the view that the leucocytozoon of the owl (*H. Ziemanni*), after a process of fertilization in the gut of the mosquito and subsequent asexual division, gave rise to an enormous number of trypanosome-like young forms, which he regarded as spirochetes, and from this he reached the conclusion that spirochetes were flagellate stages of an intracellular organism and as such belonged to the protozoa and not to the bacteria. Hence he designated the large intracellular parasite of the owl as *Sp. Ziemanni.* As will be seen, this designation is a mistake, for, in the first place, the flagellates are not spirochetes; and secondly, they have nothing to do with the intracellular parasite, *H. Ziemanni.*

Of particular importance, in this connection, is his statement that he compared his mosquito flagellates with the *Sp. Obermeieri* and *Sp. anserinum.* As a result of such comparison he arrived at the conclusion that these two forms, in the essentials of their morphology, "Kernverhältnisse, Geisselapparat u. s. w.," agreed completely with *Sp. Ziemanni.* In other words, the spirochetes of relapsing fever and of geese were said to have a definite nucleus, blepharoplast, undulating membrane, and flagellum, exactly as in the case of trypanosomes. Since then, however, Schaudinn has practically withdrawn this comparison, for in a recent publication (October 19,

1905) he states that *Sp. Ziemanni* is *very far removed* from the typical spirochetes, such as *Sp. plicatilis* and *Sp. Obermeieri.*

The view as to the protozoal nature of the spirochetes was quite generally accepted, but recent observations have served to throw serious doubt upon its correctness. Thus, R. Koch in his publication on the spirochete of tick fever has shown that this organism presents evidence of transverse division, and that there is absolutely no evidence of a definite nucleus, blepharoplast, or undulating membrane. At the time flagella could not be detected, but since then Zettnow, employing Borrel's method, has succeeded in demonstrating diffuse flagella. These facts go to show that *Sp. Duttoni* is not related to the trypanosomes but belongs to the bacteria.

Prior to Koch, in fact as early as 1889, Fraenkel and Pfeiffer in their *Atlas* called attention to the transverse division of *Sp. Obermeieri,* which condition is shown clearly in their Plate LXVI. Ten years before this Carter pointed out that the spirillum of Bombay divided into two or three parts. Subsequently the transverse division of *Sp. ovis* was emphasized by Martoglio and Carpano; and more recently a like condition was pointed for the *Sp. equi* by Martin.

Of especial importance is the very recent work of Borrel. He has clearly shown that the *Sp. gallinarum* belongs to the genus Spirillum since it divides transversely, is provided with diffuse or peritrichous flagella, and has no undulating membrane. The demonstration of these facts definitely relegates this organism to the group of bacteria. Up to the present, then, two spirochetes, *Sp. Duttoni* and *Sp. gallinarum* have been shown to be non-protozoal in nature. It is more than likely that most if not all of the spirochetes will be returned to their former place among the bacteria.

Our own work, as will be seen, leads to exactly the same conclusion as regards *Sp. Obermeieri.* We have endeavored to approach this question from several standpoints, and all the results obtained point very definitely to the bacterial nature of this spirochete. The evidence on this point will be considered under nine heads.

1. *Structural characteristics.*—The examination of the living preparation failed to reveal the presence of an undulating membrane or of a flagellum, structures which are readily recognizable in even the smallest cultural forms of trypanosomes. The contents of the

cells appeared perfectly homogeneous. The nucleus and blepharoplast, if present, should be easily demonstrable by staining reactions, but such was not the case. The contents, whether stained lightly or deeply by Romanowsky's method, invariably gave a solid stain, exactly as in the case of ordinary spirilla or bacilli. At no time was there the slightest evidence of nucleus, blepharoplast, undulating membrane, or flagellum, all of which, as indicated above, were reported by Schaudinn. Our observations agree with those of Norris and his co-workers, and are in perfect accord with those of Koch on *Sp. Duttoni* and of Borrel on *Sp. gallinarum.* They show conclusively that these spirochetes do not possess the structure of a trypanosome.

2. *Presence of a flagellum.*—Although a flagellum of the protozoal type, easily recognizable either by direct examination of the living preparation or of specimens stained by the Romanowsky method, could not be demonstrated, nevertheless by means of Loeffler's whip stain it was possible to show the presence of a whip having all the characteristics of those seen on bacteria.

From the photographs shown on Plate 10 it will be seen that *Sp. Obermeieri* possesses but a single whip at one end of the short spiral. This is as long as the spiral itself, and the wavy turns of the whip correspond with those of the spirochete. The wavy character of the flagellum should be particularly noted, for, in this respect, it resembles exactly the flagella of bacteria. The flagella of protozoa, such as trypanosomes, are coarse, thick, and do not show any indication of regular wavy bends as is the case with this organism.

The end opposite the flagellum terminates in a short, faintly stained appendage which presumably serves as the starting-point in the development of a new flagellum when division takes place. An examination of the photographs will show that the flagella are present on very short spirals. It would seem as if the longer forms, prior to transverse division, would have a flagellum at each end, but our search for such examples has thus far been unsuccessful. This may be largely due to the method of preparation, as a result of which the longer spirals would probably break up into their smaller components.

At all events it is perfectly certain that the smaller spirals, which probably represent the actual unit or cell, possess but one flagellum

which is terminal. In this respect *Sp. Obermeieri* differs from *Sp. Duttoni* and *Sp. gallinarum* which have been shown to possess diffuse flagella, and from *Sp. pallida* which has a flagellum at each end. The presence of a single flagellum will recall the cholera spirillum, an analogy which, as will be seen later, is borne out to a striking extent in the properties of the immune blood.

The successful staining of the flagellum we owe to Mr. C. T. Burnett, assistant in this laboratory. The method of securing preparations satisfactory for staining purposes, though similar to that employed by Borrel, was devised quite independently. Previous attempts showed the futility of trying to demonstrate the flagella on the spirochetes as they are present in the blood. The excess of organic material was an effectual bar to success, and some method had to be devised whereby clean, washed organisms could be obtained.

It had been noted previously that when rich spirochetal blood was placed in a test tube and allowed to stand for about 24 hours, at room temperature, white patches or islands appeared on the surface of the sedimented corpuscles. These patches were found to be masses of spirochetes which, being lighter, floated above the deposit of red cells. By means of a finely drawn-out pipette these patches were removed with as little serum and corpuscles as possible and transferred to a centrifuge tube. Citrated salt solution was then added to the material and the whole was centrifugated. The clear liquid was then removed, more salt-citrate solution added, and the centrifugation was repeated. Two or three such washings were sufficient to remove the excess of organic matter. The preparation was then mordanted with tannate of iron and stained with a saturated solution of anilin water fuchsin. Best results were obtained by repeated, alternate treatment with mordant and dye. The deposit which formed was cleared up in part by immersion for a few seconds in dilute nitric acid.

3. *Transverse division.*—At the very outset of our work it was noted that the longer spirochetes of the blood when stained showed in the middle a pale transverse band. This pale band corresponds to the faint tips which are seen at each end. This appearance is very suggestive of a cell wall, and as such it indicates that the organism multiplies by transverse division. It is inconsistent with a pro-

tozoal organism which should show longitudinal division. We have looked almost daily for evidence of the latter form of division, in living and in stained preparations, and have never met with the slightest indication of such a change. Moreover, in living preparations we have repeatedly seen a long spirillum separate into two halves. Such observations may be taken as positive evidence of a transverse division, unless it be assumed that in such cases two individuals had become temporarily joined or agglutinated, only to separate later on. End to end agglutination does occur as will be shown later. The union of two cells by means of flagella is well shown in Figs. 4 and 5, Plate 9.

In support of the view that division takes place transversely is the fact that in the earliest stages of infection, before agglutination can be said to occur, the long forms predominate. If longitudinal division was the mode of reproduction one would expect to find at this stage short and thick forms instead of the long ones actually observed.

Our observations on transverse division accord fully with those of Norris and his co-workers. They are also in agreement with those of Borrel on *Sp. gallinarum;* of Koch and Zettnow on the *Sp. Duttoni;* of Martoglio and Carpano on *Sp. ovis;* of Martin on *Sp. equi;* and of Fraenkel and Pfeiffer on *Sp. Obermeieri.*

4. *Rapid multiplication.*—This fact should be taken into consideration in dealing with the nature of the organism in question. At the present time an injection of 0.1 c.c. of spirochetal blood into a rat or mouse is followed by the appearance of the spirochetes in the peripheral blood in about 15 hours. The number then rapidly increases and reaches its maximum in about 36 to 48 hours, after which they decrease and finally disappear. Such rapid multiplication agrees perfectly with a bacterial nature, and is decidedly at variance with all known protozoal infections. In the latter, a period of incubation of several days' duration is the rule and the subsequent multiplication is relatively slow. This is certainly true for the various trypanosomes and plasmodium infections, and something analogous might reasonably be expected if the organism in question was a protozoon.

The fact alluded to above finds a remarkable parallel in the causa-

tive agent of yellow fever, which likewise disappears from the blood in about two days. It goes to show that this unknown organism may be related to the spirochetes, and, as such, may belong, not to the protozoa, as is quite generally accepted, but to the bacteria.

5. *Plasmolytic changes.*—In order to throw further light upon the nature of *Sp. Obermeieri* several series of comparative experiments were made by subjecting the spirochetes, *Tr. Lewisi*, *Tr. Brucei*, the cholera vibrio, *Sp. rubrum*, and the virus of rabies to the action of distilled water. For this purpose the thinnest possible collodium sacs were prepared by the method worked out in this laboratory by Gorsline. These sacs were about 15 mm. in diameter and 60 to 80 mm. long. They were so thin that they collapsed when empty. They were attached to cut test tubes and sterilized at 105° for 15 minutes. The material to be tested was then placed in the sterile sac and suspended in running distilled water. A very good control of the efficiency of the sacs was obtained by placing in one some defibrinated rabbit or rat blood. With a sac of proper thickness such blood will be completely laked in from one to two hours, and a microscopical examination will reveal only the stroma or "shadows" of the corpuscles. Obviously, every sac was subjected to a pressure test to make sure that it was perfectly tight.

When rat blood containing *Tr. Brucei* is placed in such sacs, there is noticed at first a marked hyperactivity of the organism. The motion then becomes sluggish and at the same time the form alters. The posterior end rounds up, and, as a result, nearly all of the trypanosomes assume the kite- or tad-pole-form while the flagella show a sluggish or only occasional motion. Eventually, only round forms can be seen together with tangles of free flagella. In some experiments where the plasmolytic change was very rapid, as shown by the prompt laking of the red blood cells, the trypanosomes were all dead within an hour. This result was usually obtained within two hours, and only exceptionally, when the membrane was not in perfect condition as shown by the persistence of the blood corpuscles, was the *Tr. Brucei* found alive beyond this limit. With one exception mice and rats inoculated with the liquid, dialyzed for two hours, did not become infected.

Similar experiments with *Tr. Lewisi* seemed to indicate a greater

resistance of this organism. The first effect was likewise seen in a pronounced hyperactivity. Within six hours, however, they became swollen and distorted in form and were nearly all dead.

Spirochetal blood, in parallel experiments, under identical conditions, showed an entirely different behavior. The organism became very active and remained so for six to eight hours. They then become somewhat sluggish, and such motion may be observed for 20 hours, although complete hemolysis occurs within a few hours. When the spirochetes come to rest they retain their form perfectly, and at no time is the slightest morphological variation observable.

Furthermore, the spirochetes when they thus come to rest are by no means dead. They may be readily revivified, after which they become as active as in the beginning of the experiment. This fact was first observed under the following conditions: A drop of the liquid from a sac was placed under a cover-glass and apparently showed only dead spirochetes. About two hours later the specimen was again examined. In the mean time the liquid had all but evaporated from under the cover-glass. Only a few small patches or ponds were left, and in these we were surprised to find very active spirochetes. Evidently in the evaporation of the liquid the traces of salines, either in the liquid or from the glass, had become concentrated to a point which enabled the vitality of the organism to assert itself. This observation suggested the possibility of restoring the vitality by the direct addition of a salt solution. Accordingly, in one experiment, where the spirochetes, after dialyzing for 20 hours, shows no motion, a drop of the liquid was placed on a slide and to this a drop of a salt-citrate solution (0.5 per cent each) was added. In less than two hours this specimen showed extremely active spirochetes.

These observations show that *Sp. Obermeieri*, unlike *Tr. Brucei* and *Tr. Lewisi*, retains its motion for a longer period, when dialyzed in distilled water; that while the trypanosomes are actually killed under these conditions, the spirochetes merely come to a rest, on account of the decrease in osmotic tension, and can be revivified many hours later by the addition of salines.

The above behavior of the spirochetes is similar to that of the cholera vibrio and of *Spirillum rubrum*. For experiments on this

point we are indebted to Mr. R. G. W. Owen, Assistant in the laboratory. The cholera vibrio in a very thin collodium sac in running, distilled water still shows some motion at the end of six hours, occasionally even at the end of 12 hours. The non-motile organism, when transplanted to broth, gives positive growths at the end of 18 hours but not at 24 hours. Suspensions of *Sp. rubrum*, under like conditions, show some motile forms for 12 to 18 and even at times at the end of 24 hours. The non-motile forms invariably give cultures at the end of 24 but not at the end of 30 hours.

It will be seen from the above that the *Sp. Obermeieri*, under the conditions of the experiment, retains its motility as long as, and even longer than, the cholera vibrio and for about the same length of time as the *Sp. rubrum*. In other words, it possesses about the same resistance as these well-known examples of spirilla.

Similar experiments to the above were made with "virus fixe" by Dr. J. G. Cummings, in charge of the Pasteur Institute here. They are of interest inasmuch as they show that the rabic virus is destroyed quite as readily as *Tr. Brucei*—a fact which indicates that the microbe of this disease in all probability belongs to the protozoa. In several experiments the dilute filtered suspension of the virus, after dialyzing for one hour, failed to infect. This result was invariably obtained when the dialysis had proceeded for three hours or more. Controls inoculated with the same suspension, kept under as nearly identical conditions as possible, but not dialyzed, invariably developed rabies. Very thin sacs and very dilute suspensions are necessary here, as in the above experiments, otherwise the virus may not be destroyed completely in from 6 to 12 hours. It is of interest to note that in some of the animals inoculated with the dialyzed liquid a typical excited stage developed, which, however, was of short duration and complete recovery followed. As to whether such recovered animals are immune to a minimum fatal dose remains to be established.

Results somewhat like those with collodium sacs may be obtained by the addition of distilled water to the infected blood. Thus, if one drop of blood containing *Tr. Brucei* is added to 1 c.c. of distilled water the trypanosomes at the end of half an hour will be found to be all rounded up and dead. A similar test with spirochetal blood

will show some actively motile individuals together with many unaltered motionless forms.

The foregoing experiments go to show that dialysis in running distilled water, with the aid of thin collodium sacs, brings out marked differences between the known protozoa and known bacteria. Just how far this difference in behavior will hold true for other representatives of these groups remains to be established by further experiment. It is quite possible that this reaction will be found to be a fairly general means of distinguishing between protozoa and bacteria. In this particular case it certainly proves that *Sp. Obermeieri* differs markedly from trypanosomes, so much so that there can be no question but that it is wholly distinct from the latter. Moreover, the close similarity in the behavior of the spirochete under these conditions to the typical spiral bacteria strengthens the evidence, if it does not actually prove, that this organism belongs among the bacteria and not among the flagellated protozoa.

6. *Action of heat.*—Comparative determinations of the thermal death-point of the spirochete and of *Tr. Lewisi* were made to ascertain if there was any notable difference in this regard between the two types. The defibrinated blood of infected rats was used direct, that is, without dilution. This was taken up in drawn-out, thin-walled tubes about 3 mm. in diameter. The sealed tubes were then immersed in a water-bath, the temperature of which was kept constant at 45°, 50°, and 60°.

In the case of *Tr. Lewisi* an exposure of five minutes at 60° was sufficient to cause the complete destruction, and even the disappearance, of the trypanosomes. After an exposure of five minutes at 50° the trypanosomes were all dead and scarcely recognizable, the bodies being shrunken, rounded up, and granular. In 10 minutes not even dead trypanosomes could be detected. At 45° the trypanosomes were hyperactive at the end of five minutes; at the end of 15 minutes only a few sluggish trypanosomes were present together with many distorted dead forms. At the end of 30 minutes the trypanosomes had entirely disappeared.

The spirochetal blood, drawn during the decline stage, was tested at the same time, side by side with the trypanosome blood. In five minutes at 60° only agglutinated masses of non-motile spirochetes

were present. The same result was obtained in five minutes a 50°. At 45° they were very active at the end of 15 minutes; at 30 minutes there were many sluggish spirochetes present with some small agglutination groups.

According to Heydenreich the *Sp. Obermeieri*, kept at 42.5° to 46°, remains alive for one and three-fourths to three and one-half hours. The difference between his results and ours is easily explainable. In the first place he employed human blood with relatively few spirochetes, whereas we used rat blood which contained about 10 spirals per field of the 2 mm. objective. Of more importance than the mere number of spirochetes is a second factor which is more difficult to control. In the infected animal germicidal substances appear in the blood and their amount increases with the increase in the number of organisms. As will be shown, the disappearance of the spirochetes from the blood of the rat and the absence of a relapse, as in the case of man, is due to the formation of a large amount of such anti-bodies. In our experiments we used a "decline" blood, that is to say, the rats were bled about 48 hours after inoculation, at which time the spirochetes usually reached their maximum number and were even on the decrease. Consequently in such blood the germicidal agent is already present in appreciable amount, and is therefore not without action upon the vitality of the organism. It follows from these considerations that the spirochetes employed in these tests were already in an enfeebled or weakened state. Moreover, it is self-evident that a germicidal agent will act more promptly the higher the temperature. The fairly rapid death of the organism at 45° is largely if not wholly due to the combined action of heat and the specific bactericidal body. It is reasonable to believe that if "onset" blood, in which the germicidal agent is present in minimal amounts, is employed the spirochetes will be found to live considerably longer than 30 minutes at 45°. However, no experiments were made to test this point.

The experiments, such as they are, demonstrate two things: first, that *Tr. Lewisi* at 45° is killed, and disappears in less than 30 minutes; second, that the spirochetes may be alive for more than 30 minutes at that temperature, and that they do not undergo, when dead, any alteration in form. The latter is a particularly important

fact and holds true for all of our experiments, with the exception of Pfeiffer's phenomenon. Even after an exposure of 30 minutes at 60°, the spirochetes, though they agglutinate, show a perfect spiral form, whereas *Tr. Lewisi* disappears almost completely after it has died. The action of the heat, it will be seen, is very much like that observed in the plasmolytic experiments. There, also, the trypanosomes dissolved or disappeared almost completely as soon as they became dead, whereas the spirochetes, though immobilized and eventually killed, retained their form without the slightest alteration.

The absence of any change in form of the dead spirochetes, corresponds to the behavior of bacteria under like conditions and this observation, together with the greater resistance to heat, may be taken as an additional proof of the bacterial nature of the *Sp. Obermeieri.*

7. *The persistence of form.*—In the preceding paragraph special emphasis is placed upon the fact that the spirochetes do not undergo any alteration in form as the result of the action of heat. This fact holds true for all the other conditions which we have studied. Thus, in the dialysis experiments, though the spirochetes became immobilized and eventually dead, yet at no time was there the slightest change in form. The dead cell does not dissolve, or fade away or even become indistinct, but on the contrary it is sharply defined as a rigid spiral. No matter what the condition is which causes the death of the spirochete the latter retains its form perfectly and apparently so for an indefinite length of time. Thus, perfect dead spirals may be seen in blood which has been kept *in vitro* for more than 40 days. In the hundreds of attempts which have been made at cultivation, the dead spirochetes, normal in form, could always be found regardless of the temperature or the medium employed.

Under the influence of the immune serum the spirochetes may be agglutinated and killed in a few minutes, but in such cases also, the typical form persists and not the slightest variation or departure from this can be detected. The only exception to this statement is presented by Pfeiffer's phenomenon, in which, as will be shown later, granulation may result, within or without the body, exactly as in the case of the cholera vibrio.

It will be shown that under the influence of large doses of immune

blood the spirochetes may be killed *in vivo*, and a cure may be sometimes effected within half an hour. In the many tests which we have made along this line we have scarcely ever seen any other than perfectly preserved dead spirochetes. Occasionally, drawn-out or filamentous forms are met with in smear preparations, probably due to traction, since these are not observed in drop examination.

The persistence of the spiral form under the most varied injurious conditions is a fact of much importance, since it is incompatible with a flagellate or protozoal nature. Under adverse conditions protozoa tend to round up, to a greater or less extent, and the absence of any indication of this sort effectually removes this spirochete from the flagellates, and for that matter from the entire group of protozoa.

8. *Active immunity.*—The production of active immunity in several protozoal diseases is well established, though at the same time it must be conceded that immune sera are of little value as curative or preventive agents in such affections. The production of even a relatively active germicidal serum with respect to protozoa is a difficult task which requires much time, usually many months. In the case of the trypanosomes, with the exception of *Tr. Lewisi*, a really active serum is practically unknown.

On the other hand, the bacteria, as a rule, readily react with the production of powerful anti-bodies. Thus, to take one of the earliest and best studied organisms, one which forms a fitting parallel to our spirochete, the cholera vibrio on intraperitoneal injection promptly gives rise to a germicidal agent. The amount of this substance present in the blood of the immunized animal can be rapidly increased by successive injections. In a few weeks it is possible to secure a serum which will act as a preventive or curative agent in extremely small doses.

In the case of *Sp. Obermeieri*, as will be shown later, active immunity can also be produced with an ease which is quite comparable to the example just given. Repeated injections of spirochetal blood cause a rapid increase of the immune and germicidal bodies and to such an extent that the blood of the actively immunized rat has a powerful curative action. Thus, we have been able to obtain a blood which in a dose of 0.002 c.c. protects absolutely a 100 g·

rat against a simultaneous injection of 0.1 c.c. of spirochetal blood. In other words one part of the immune blood protects 50,000 parts of body-weight. The same blood in a dose of 0.5 c.c. causes the spirochetes to disappear, sometimes within half an hour, from the peripheral blood of an infected rat.

The readiness with which active immunity can be established and the ease with which the spirochetal infection can be prevented and cured is to our mind an additional and clinching proof of the bacterial nature of *Sp. Obermeieri.*

9. *Absence of aërotropism.*—During the past three years we have had occasion to study a very large number of cultures of trypanosomes representing many different strains and a considerable number of species. All these eultures present an interesting behavior with reference to air. Thus, if a small bubble of air remains in the liquid under the cover-glass it will be found that the cultural trypanosomes will mass themselves around its border. They form a compact layer of three to five or more cells deep, and each cell points with the flagellum toward the air-bubble. This radial massing about an air bubble appears to be a very general reaction for trypanosomes and as such it may be utilized as a means of separating trypanosomes from accompanying bacteria. Bacteria under similar conditions are in no wise responsive to the presence of air. In some instances it is true that hyperactivity may be observed, but the bacteria never assume the characteristic arrangement as described above for trypanosomes. Furthermore, in our daily examinations of spirochetal blood we have always looked for some evidence of a trypanosome-like behavior with reference to air. This we have never observed. On the contrary, the spirochetes show the same indifference to the air globules as do ordinary bacteria.

This fact is mentioned as an additional proof of the nature of the spirochete.

The several facts which have been brought out under the preceding headings point clearly to the non-protozoal nature of *Sp. Obermeieri.* The only escape from this conclusion is the assumption that there are protozoa wholly unlike those known at the present time. Such an assumption would be unwarranted, to say the least,

especially in view of the marked agreement which the spirochete shows with the well-recognized bacteria. We must conclude, therefore, that the *Sp. Obermeieri* belongs to the bacteria and more especially to the Spirillaceæ. As pointed out, the same conclusion has been reached by Borrel as regards *Sp. gallinarum*, and the facts brought out by him with reference to this organism hold true for *Sp. Duttoni*. That is to say, the latter presents no evidence of nucleus, micro-nucleus, undulating membrane, etc., but does show transverse division and is provided with diffuse flagella. Three typical spirochetes are therefore demonstrated to belong to the group of bacteria. The other spirochetes which show transverse division, as *Sp. ovis*, *Sp. equi*, *Sp. glossinae*, etc., will undoubtedly eventually find their place in the group of spiral bacteria. The wavy flagella on *Treponema pallidum* also indicate a relationship to this group. On the other hand, the position of *Sp. plicatilis* and *Sp. refringens*, which, according to Schaudinn, possess an undulating membrane but no flagella, is less certain. The possibility of capsule formation among the spiral bacteria should not be lost sight of.

As further work is done with these organisms, it may be found necessary to create new genera based upon the number and arrangement of the whips. We now know of spirilla with a bundle of whips at one end, also such as have but one whip, also such as have one at each pole and lastly such as have diffuse flagella. For the present the creation of new genera does not seem advisable and for that reason we will retain the old, established term "spirillum," especially since the type species of the genus Spirochaeta, *Sp. plicatilis* of Ehrenberg, is said to have an undulating membrane but no flagella.

Hitherto it has been assumed that insect transmission indicates a protozoal organism, and in so far as the spirochetes are concerned, the chief evidence which can now be adduced in support of their animal nature is the fact of such transmission in the case of *Sp. Duttoni* and *Sp. gallinarum*. The persistence of the spirochetes in such insects for months, and the infection of their eggs constitute the remaining argument in support of this view. There is as yet absolutely no evidence that the spirochetes actually multiply in these insects, much less any indication of the existence of a life-cycle in any way comparable to that of the malarial organism. The

occurrence of relapses, as will be shown, is readily explainable without the assumption of such a cycle. On the other hand, the facts brought out in this study point so conclusively to the bacterial nature of the organisms that there can be little or no doubt of the correctness of the conclusion arrived at. With the recognition of this proof it follows that insect transmission is no longer a criterion of the nature of an organism.

MORPHOLOGY.

The spiral forms as met with in the blood vary considerably in length, usually from 7 to 19 μ and even more. The short forms vary from 7 to 9 μ in length and hence are as long as, or a trifle longer than, the diameter of the red blood cell. These probably represent the actual size of the young or single cell. The disintegration within phagocytes shows a tendency of the spiral to break up into comma or S forms, and these may represent the actual unit. Further evidence on this point will be brought out under the head of tick fever. The contents of the cell usually stain solid by the Romanowsky method with the exception of the ends, which take a very pale tint and fade away to a point. At times a tendency to granular staining will be observed, but this is probably due to the dye employed. Heavily stained portions or nodes can also be observed, but these cannot be taken as evidence of structure. Examples of the above will be seen in the photographs which accompany this paper.

The long forms may result from either of two wholly distinct causes —multiplication or agglutination. The evidence on this point we consider to be quite conclusive. When the blood is examined at the earliest stage of infection (15 to 18 hours after inoculation) thin, long, and very active spirals can be seen together with the short forms. The presence of these very long cells at the very beginning of infection indicates that they give rise by transverse division to the shorter forms. Their length (16 to 19 μ) corresponds to that of two short forms. An examination of the stained preparation confirms this view in so far as it shows the presence of a pale central zone, corresponding to the pale tips or appendages which can be readily seen on the free ends (see Plate 8).

In the later stages of infection, even longer forms than those men-

tioned appear as a result of the formation of agglutinating bodies. Moreover, when a very active immune blood is injected into an infected rat these very long forms promptly appear in the blood together with agglutination groups of various sizes. The long forms of this type vary in length from about 18 to 100 μ and even more. They result from the end to end agglutination of two or more cells. That such is the case we have been able to determine by the aid of Loeffler's flagellar stain. In a good preparation stained by this method it is easy to find many pairs of cells held together by the flagellum. Two photographs showing this manner of attachment are given in Plate 9. The recognition of this linking by the staining method at once explained a peculiarity frequently observed in fresh blood. It is not an unusual occurrence to find two spirals, separated by an appreciable distance, about the length of the cell, which maintain a perfect alignment and move together, backward and forward. The invisible bond holding together such cells is the flagellum.

The actual width of the spirillum probably does not exceed 0.25 μ. The apparent width depends largely upon the amount of dye which has been deposited in and upon the cell. Hence in feebly stained preparations they may appear as mere lines. The heaviest deposit of dye, as obtained with mordant and anilin water fuchsin, in Loeffler's method, does not increase the width beyond 0.3 μ (see Plate 10). In fresh onset blood the spirilla appear to be thinner than in the latest stages and because of their tenuity and rapid motion they are very difficult to see. In the later stages, and particularly upon the addition of immune blood, the spirochetes become slower and apparently much thicker. They can then be readily seen even by untrained persons.

At times there are found, especially in the later stages of infection, relatively long, thick spirals which are about twice as thick as the ordinary ones. They are usually of the long form and may be considered either as involution forms, or, what is not improbable, as two cells which have become woven together much as in the case of the formation of giant whips. Similar observations have been made by Koch as regards the spirochete of tick fever.

The number of turns in the short spirochetes (7 to 9 μ) which, as indicated above are to be considered as the individual cell, is usually

but two or three. At times the number may be increased to four or even to six which, however, is probably a condition preliminary to division. In the latter kind the turns are but 1.5 μ apart (from crest to crest) while in the former they are 2 to 2.7 μ apart. The width of the turns, that is the extreme width of the spiral, is from 0.6 to 1.5 μ, the average being about 1.0 μ. These measurements, it may be added, are best made on photographs of the object which have a magnification of 3,000 diameters; a micron then corresponds to 3 mm. on the photograph.

The short form of *Sp. Obermeieri*, as shown heretofore, is provided with a long flagellum at one end, while the other has a faintly stained appendage, corresponding to the pale tips seen in the ordinary Romanowsky preparation. Tinctorially, this behaves like the whip, and may possibly give rise to the latter. The width of the flagellum is probably 0.1 to 0.2 μ, and the length is from 5 to 7 μ. It is usually as long as, or even a trifle longer than, the cell to which it is attached and has from three to five wavy bends or turns. At no time have we seen any indication of the presence of lateral flagella.

The above demonstration of the presence of a single flagellum is at variance with the statement of Karlinski who believed he saw, in living preparations, a pair of whips at each end. No other observer has been able to get any indication of the presence of flagella.

The motility of *Sp. Obermeieri* is exceedingly pronounced, and, as will be shown, it may persist for days and even weeks. Under suitable conditions, such as a proper thickness of the fluid under the cover-glass, the absence of a fibrin network, and of immune bodies, the spirilla will be found to travel with extreme rapidity; so much so that it is quite impossible to follow the organism as it darts in a straight line through the field. This may be regarded as the normal motion of the uninjured or unhindered organism and is particularly in evidence in preparations made during the early stages of infection.

Usually, however, on account of the adverse conditions mentioned, the motion is limited in extent. The cell then travels back and forth over a distance of not more than two or three times its length. It may even adhere, presumably by its whip, to the glass surface or to

the fibrin network, and when this occurs the rapid screw motion continues until the cell is released from its attachment.

The flagellum not only causes the spirillum to move from place to place, but it also effects at the same time a rotation of the cell along its long axis and thus imparts the characteristic screw motion to the organism. Rotation along the long axis is a common occurrence among the rod-shaped bacteria and also among the cultural forms of trypanosomes, and, indeed, must be looked upon as a necessary condition to propulsion, especially where a single lashing whip constitutes the organ of locomotion. Such rotation is easily overlooked in the case of a bacillus, whereas with a spiral organism, on account of its very structure, it becomes a decidedly noticeable feature. Hence, it is manifestly wrong to speak of a real wave motion passing over the spirillum from one end to the other. It is only an apparent motion for the form of the cell is that of a rigid spiral.

The swaying lateral motion, from side to side, is seen only in the long forms which consist of two or more cells. This is also a secondary condition to the real motion, and although it imparts to the long spirochete the so-called flexible character, the latter feature is hardly of sufficient importance to justify its employment as the basis of a generic difference among the spiral organisms. The long form of the *Sp. rubrum* or of the cholera vibrio, as is well known, will show similar lateral swayings.

Under unfavorable conditions the active motion, from place to place, ceases and the organism comes to a rest except for the apparent wave motion which passes alternately backward and forward over the spiral. This motion becomes slower and slower and eventually can be readily followed under the microscope. A brief quiescent stage may intervene before the reverse motion begins. This interval of rest becomes longer and longer, and finally all motion ceases. The cessation of motion does not necessarily imply that death has taken place. Thus, in the dialysis experiments described above, the motionless spirals could be revivified under certain conditions. Moreover, we have seen infection result, with the usual period of incubation, by the injection of blood in which all the spirals had lost their motion.

A most important cause affecting the motion and viability of the

spirilla is the presence in the blood of specific germicidal substances. This fact can easily be demonstrated, either by injecting an immune blood into an infected rat, or by drawing the blood during the decline stage and keeping it in a test tube. In either case, the organism soon comes to rest and is apparently dead. As this subject will be discussed farther on it may be dismissed for the present.

The spiral motion is seen at its best in the agglutination rosettes which form *in vivo* or *in vitro* under the influence of immune blood. Hundreds of spirals arrange themselves in perfect radiating rosettes and maintain for hours an extremely active rotation along the long axis. The first effect of the anti-bodies referred to is to induce a marked hyperactivity on the part of the cell. A similar effect is observed when the blood is submitted to dialysis in collodium sacs, as mentioned above.

VIABILITY.

A characteristic feature of infection with *Sp. Obermeieri* is the rapid and even sudden disappearance of the organisms from the circulating blood. In man, monkeys, and mice, after a period of six to seven days, the spirilla reappear and a day or two later they again disappear. Several such relapses may follow each other before final recovery takes place. In the case of a rat there is no relapse, and hence the organisms once gone do not reappear. In view of these facts several interesting questions arise: first, what causes the disappearance of the organisms; second, what becomes of them; and third, how they manage to reappear. The answers involve the question of immunity, and as this subject will be considered by itself, it is undesirable to take up their consideration at this time. Certain facts, however, in connection with the subject of viability have a direct bearing upon the queries propounded.

1. *Extravascular viability.*—When spirillar blood is drawn from a number of rats it will be found that in some cases the organism dies out in about one to two days, while in others they may live for several weeks. We have found living spirilla in blood kept *in vitro* for 40 days, which is by a trifle the longest survival hitherto noted, that of 37 days by Moczutkowsky.

The best procedure for obtaining blood from small animals

is to draw it directly from the heart into a sterile pipette. The latter is made from a small test tube the lower end of which is drawn out into a capillary which is then bent at a suitable angle. A narrow glass rod, passing through the cotton plug of the tube, serves to defibrinate the blood. The pipette is sterilized by dry heat. The heart of the etherized animal is exposed, and the cut, flamed tip of the pipette is then inserted. The filling of the pipette is aided by suction, and, when all the blood has been drained, the tip is sealed in a flame after which the blood is defibrinated and transferred to sterile tubes, planted on media, or taken up in a syringe for purpose of inoculation.

The spirillar blood, drawn by the procedure indicated, was transferred in quantities of 1 to 4 c.c. to test tubes, which were then closed with rubber caps and set aside at the ordinary room temperature. From time to time the contents of the tubes were examined microscopically and portions were injected into rats.

In these experiments it was soon found necessary to distinguish between the blood drawn during the early stage of the infection and that drawn during the later stages. The former, for convenience' sake, will be designated as "onset" and the latter as "decline" blood, whereas that drawn after the spirilla had disappeared from the circulation will be known as "recovered" blood.

In decline blood of rats, kept under the conditions mentioned, all the spirilla have been found to be dead in from 36 to 40 hours. This result may even be obtained with blood but 24 hours old. In this time, the corpuscles settle to the bottom, leaving a perfectly clear serum above. Lying just above the corpuscles will be seen several white patches or islands, reference to which has already been made. When these are removed by means of a fine pipette and examined, dense agglutinated masses of thousands of dead spirals will be found. In one experiment two rats inoculated with 0.5 c.c. of such blood failed to develop an infection thus confirming the microscopical observation as to the death of the spirilla. These rats, when reinoculated three days later, each with 0.1 c.c. of fresh spirillar blood, did not become infected, whereas a control which received 0.5 c.c. of the dead spirillar blood plus 0.1 c.c. of fresh spirillar blood became infected. This experiment it will be seen

indicates: first, the presence of a germicidal substance; and second, the presence of an immunizing body. The amount of the latter, however, was not sufficient to protect against a simultaneous injection, rat 2/253, but when given three days before it did manifest a preventive action.

While in the decline blood as shown above the spirilla may die out in from one to two days, an entirely different result is obtained with onset blood. The patches or islands above the corpuscles, if they form at all, are very small and quite numerous. More often they are not formed, but instead the surface of the corpuscles will show a light film which on examination will reveal considerable numbers of very actively motile spirilla, some of which may form small agglutination groups or rosettes. These are insignificant in size compared with the large masses, referred to above, which sometimes fill several fields of the 2 mm. objective. In such onset blood the spirilla will live for a variable length of time. We have found actively motile forms in specimens kept for 30 to 35 to 40 days, and without doubt, with special care, in the selection of the onset blood, they may be found to be viable for a much longer period. It may be added incidentally that under these conditions no evidence of multiplication of the spirilla was obtained.

The presence of living spirilla in kept blood does not necessarily mean that infection of rats can be induced with such material. Thus, a rat which was inoculated with blood 40 days old, in which a few living spirilla were present, failed to become infected. Several days later, it was reinoculated with fresh spirillar blood, but did not develop an infection. From this it will be seen that a small amount of immune body may protect against a very small number of enfeebled spirilla. It should be added that we have infected a rat with blood 37 days old.

It is evident from the foregoing that a germicidal substance, which is not present in blood at the time of the onset, forms as a result of the multiplication of the spirilla. It eventually accumulates in sufficient amount to inhibit their further increase and finally brings about their destruction within the body exactly as it does in the test tube. At times, such action can actually be seen to occur within the body. In the later stages of infection dead spirilla and

even small tangles may be observed; especially is this true when the infection is cut short or modified by an injection of immune blood.

2. *Intravascular viability.*—In the case of animals which suffer relapses it is evident that the destruction of the spirilla is not complete. A small number survive, in some out of the way place in the body, and when the germicidal agent decreases in amount, either by elimination or by oxidation, they reappear again for a short time. Inasmuch as the rat shows no relapse, it seemed particularly well adapted for determining the question as to whether spirilla could live or persist for any length of time in the blood of the recovered animal. It is well known that many protozoa, such as trypanosomes, piroplasmata, and plasmodia, may remain in the blood long after recovery from the disease.

In order to solve this question, three rats were repeatedly examined to determine the exact time of the disappearance of the spirilla from the blood. This was found to be 66 hours in two and 78 hours in the third rat. The rats, 2, 3, 4/232, were then bled 12, 24, and 36 hours respectively after the organisms had disappeared. Two c.c. of the blood of the rat killed after the 12-hour interval was injected into another rat, 3/242. Likewise 1.5 c.c. of the blood of the rat which was bled after 24 hours was injected into a fresh rat, 5/242, and the same dose of the blood drawn at the end of 36 hours was injected into a third rat, 6/242. The rat which received the blood drawn 24 hours after the disappearance developed a mild infection, whereas the other two, inoculated with the 12-hour and 36-hour blood failed to show spirilla. Moreover, these two when reinoculated nine days later with spirillar blood resisted infection.

The variation noted in the above experiment is due either to individual peculiarity of the animal, or, what is even more probable, to the overlooking of a few spirilla. In the latter case the spirilla, instead of having disappeared at the end of 66 hours as was supposed, may have persisted in small numbers for about 80 hours. The detection of a stray spirochete is obviously largely a matter of chance, and hence the supposed interval of 24 hours may have been actually considerably less. This was substantiated by the following check experiment. A rat, 3/77, showed spirilla for about 82 to 84 hours. Its blood, drawn twelve hours later, when injected into a

rat in a dose of 0.5 c.c. not only failed to infect but actually protected rats against a simultaneous injection of spirillar blood.

An objection may be raised to the conclusion that the spirilla are killed off in the recovered blood in 12 hours or less. This may be based upon the immunity result just mentioned and upon a similar result obtained with blood which had been kept *in vitro* for 40 days. In the latter instance, though a few living sluggish spirilla were present, the blood failed to infect and actually conferred immunity. It is therefore supposable that in the negative experiments above the failure to infect was due, not to the absence of spirilla, but to the presence of immune bodies. That such bodies were present was demonstrated by the subsequent failure to infect with spirillar blood. This view, however, has no basis in fact because a germicidal agent is present in such quantities as to destroy rapidly any organisms which might be present. The experiments with decline and recovered blood *in vitro* prove this fact. The recourse to Metchnikoff's theory that the germicidal substance is formed outside of the animal body from damaged leucocytes, is offset by the fact, as will be shown under Pfeiffer's phenomenon, that in actively immunized rats the bactericidal action can be demonstrated within the body.

In his recent paper on African recurrens, Dr. Koch attempts to explain the fact that a large per cent of the ticks containing spirochetes are found in huts in which no cases of the disease exist. One view, which he offers, is that persons who have recovered from tick fever are not free from spirochetes, but on the contrary may harbor a few of the parasites for a greater or less length of time, possibly for years. This assumption is based on the known facts regarding trypanosomatic and piroplasmatic diseases. The close similarity of the *Sp. Duttoni* to *Sp. Obermeieri* leads us to believe that this view is not well founded. In relapsing fever, unlike in the protozoal diseases mentioned, the blood *in vivo* possesses a powerful germicidal and immunizing action and it is inconceivable that the spirilla should exist for any length of time in the presence of such agents. The alternative hypothesis which he gives, namely, that the ticks may derive their spirochetes from infected rats, is more plausible. It is very desirable that an examination be made of a large number of rats in the tick-fever regions to determine whether

these rodents are the carriers of the disease. On the other hand, the viability of the spirochetes in ticks is not fully determined, and it is quite possible, as seen from our experiments *in vitro*, that the organisms may survive for many months in ticks which have fed upon a case of relapsing fever. It would be of interest to know just how long the ticks may retain their infective properties. As a matter of fact, ticks have been sent from Africa to Europe, and, when placed on animals, have caused infection.

PATHOGENESIS.

It is generally stated in literature that *Sp. Obermeieri* can be transferred by inoculation only to man and monkeys. This, apparently, is incorrect as regards mice and rats, which are susceptible to our organism as well as to that of tick fever. The error, if any, of the the early observers may in part be attributed to a failure to examine such animals on the first and second days after inoculation. It is possible, however, that the European organism does not infect these animals in which case it would represent a distinct species. It is therefore very desirable that the spirilla met with in Russian and Indian relapsing fevers be carefully tested as to their behavior in rats and mice. But, whatever may be the cause of this discrepancy, the fact remains that the organism which we provisionally regard as *Sp. Obermeieri* can be transmitted to man, monkeys, mice, and rats.

Rabbits and guinea-pigs appear to be refractory. Thus, two young rabbits, weighing about 800 g. each, were injected intraperitoneally* with 1 c.c. of rich spirillar blood. They were examined daily for 10 days with wholly negative result. Norris, Pappenheimer, and Flournoy have been more successful and have found spirilla in the blood of rabbits on the second day, but none thereafter.

Three young guinea-pigs were inoculated in like manner, and their blood was also examined daily for a period of ten days. Two of these were wholly negative; in the third only two spirilla were found on the third day, but none before or after. It is quite likely that

*To avoid repetition it may be stated that all injections were made intraperitoneally except in the case of monkeys, where subcutaneous inoculation was resorted to.

the stray organisms thus found represent mere survivals of those injected, and that no actual multiplication takes place. According to Sawtschenko spirilla may be found in guinea-pigs for 24 to 36 hours after injection into the peritoneal cavity or into the subcutaneous tissue.

Monkeys.—Inasmuch as Norris, Pappenheimer, and Flournoy have tested very fully the behavior of these animals to the spirillum, it was not deemed necessary to use them except for the purpose of demonstrating the preventive and curative action of immune blood. These experiments will be described in their proper place.

White mice.—The white mice are very susceptible to spirillar infection. The organisms appear in the blood within 24 hours after injection and persist up to about the end of the third day (80 hours). They then disappear for several days, after which a relapse occurs. This is followed, usually, by a third, and even a fourth relapse. The interval between the successive relapses, counting from the appearance of the spirilla in one, to their appearance in the next attack, is about seven days. They may, however, reappear as early as the fourth and as late as the tenth day. So regularly do they return that one can quite accurately predict when this will occur.

The following details serve to show the progress of the infection in mice. Only the days on which spirilla were found are given, the others being negative.

Mouse 6/198, received 0.5 c.c. of spirillar blood on January 13. It was examined daily for 40 days. Spirilla appeared on January 14, 15.

Again on January 21 (7-day relapse).
Also " " 28 (7 " ").
" " February 4, 5 (7 " ").

They were missed on February 11 and although examinations were made until February 22 the spirilla did not return.

Mouse 2/213, received 0.1 c.c. of spirillar blood on January 21. The organisms appeared on January 22. The mouse was then given 0.25 c.c. of an immune blood (result of five injections); the spirilla decreased in number, but did not disappear until after two hours. This was regarded as a cure experiment and no further examinations were made until exactly four weeks later, when it was calculated the parasites should appear, if the cure was not permanent. The examinations made on February 19 and 20 showed a very few spirilla. This represents then a fourth relapse with seven-day intervals (see Monkey No. 1). Examinations made for the next 10 days were negative.

Mouse 6/229 received 0.25 c.c. of spirillar blood on January 29 and its blood was examined every day until February 27. The organism was found on January 30, 31, and February 1;

Again on February 9 (10-day relapse).
And " " 15 (6 " ").

Mouse 7/229 received on January 29 the same injection as the preceding and was subsequently examined for a like number of days. The spirilla were found on January 30, 31.

Again on February 7 (8-day relapse).
Also " " 14 (7 " ").
And " " 21 (7 " ").

Mouse 2/244 received 0.1 c.c. of spirillar blood on February 7. The organisms appeared on the eighth, when it was given an injection of 2 c.c. of immune blood (result of 13 injections). The spirilla disappeared within one hour. No examinations were then made until February 21, after which they were made daily until March 12. Spirilla present February 8, first attack.

Also on February 23.
And " " 27.

The presumption is that a first relapse occurred about February 15, in which case the second and third relapses came on in eight and four days respectively.

Mouse 4/244 served as a control for the preceding. It received the same injection of spirillar blood but none of the immune blood. Daily examinations were made until February 27. Spirilla present on February 8 and 9. Again on February 16 (8-day relapse).

It is evident from the above that relapses occur in treated as well as in untreated mice. The number of relapses varies with different individuals. Particular attention should be called to the fact that the number of spirilla which appear in the blood during a relapse is much less than in the first attack, which clearly indicates the existence of a partial immunity. At first appearance, as many as 50 per field* may appear in the blood, especially is this true when a large dose (0.5 c.c.) is injected. With smaller doses (0.1 to 0.25 c.c.), the number rarely exceeds 10 per field. In a relapse, the number of spirilla is usually quite small; thus, in some instances, but one or two spirilla could be found in a specimen. This was particularly true in the mice which received immune blood. In the untreated mice, the number of spirilla found during a relapse was usually appreciable, from 0.1 to 1 per field.

Recovery in mice seems to be the rule as with other animals. In only one instance have we found what might be interpreted as a fatal result. This mouse, 7/198, received an injection of 0.5 c.c. of spirillar

* The expression "per field" refers to the number of spirilla in a living preparation examined with a 2 mm. objective and No. 1 eyepiece.

blood. On the following, or first day, the organisms were 1 per field; on the second day they were 10 per field; on the third day 30 per field, and the following morning it was found dead. The fatal result may of course, have been due to some other cause than the infection, but the steady increase of spirilla for three days was a very unusual occurrence.

White rats.—The white rat, either pure or piebald, was chiefly employed in this investigation. Up to the present time about 500 of these have beeen used. No difference was observed in the susceptibility of the two varieties.

As a result of the consecutive passage of the spirilla through this long series an increase in virulence, if this expression can be used, was noted. At first the period of incubation was about 40 hours. The spirilla then became numerous on the second day after the inoculation and even persisted into the third day, after which they usually disappeared. Only very rarely have they been found after 80 hours.

It was soon found that the period of incubation was decreasing and instead of being 40 hours, as at first, it has of late been reduced to 15 to 18 hours. The consequence has been that the rats now show as many as 10 per field, 24 hours after inoculation, and in a few instances it has happened that they disappeared within 48 hours. Usually, however, the spirilla now disappear in about 60 hours after inoculation. Exceptionally, a rat is met with in which the period of infection lasts three or four days. In such which show a delayed infection, the spirilla come on in very small numbers about the third or fourth day and persist for only one day.

Young rats are more susceptible than the old since the spirilla are more numerous and persist longer. They often show as many as 100 per field and in small numbers may be found as late as four and one-half days after inoculation. This behavior of young rats recalls that of children having relapsing fever.

The injection of living spirilla may be said invariably to produce an infection. There are, however, apparently exceptions, for we have met with several rats where living organisms were known to be injected and yet those animals did not become infected. It might be assumed that these few exceptions indicate a natural resistance, and, without doubt, considerable variation due to this factor exists. However,

a more plausible explanation is based upon the fact that immune bodies appear in the blood and reach their maximum about the end of the decline stage. That being the case, it is evident that an injection of only a few living spirilla, and these probably having a lessened vitality, together with a relatively large dose of the immune body, may result in non-infection. An example of this kind has been mentioned in the experiment with blood which had been kept for 40 days. In this case, although a few organisms were present no infection, followed. Several such examples with blood kept in tubes could be given. Similarly, a large injection (0.5 to 1.0 c.c.) of blood drawn at the close of the decline stage may fail to infect. This is particularly true when such blood is extremely rich, 75 to 100 per field, and is kept *in vitro* for a few hours before injection. This explanation, however, does not always hold true. Thus, in one experiment three rats were inoculated, each with 0.25 c.c. of fresh spirillar blood. Two of these developed the usual infection while the third failed.

A number of rats were examined daily for a long period, but at no time could a relapse be observed. In this respect, then, the infection in rats differs from that in man, monkeys, and white mice. It indicates that the cells of the rat readily respond to the presence of the spirilla and give rise to an abundance of immune bodies which persist in the rat and, as a result, all the organisms are destroyed. It will be shown in the part on immunity that rats, which have recovered, retain their resistance for months.

The prompt response of the body by the production of anti-bodies accounts for the remarkable fact that, notwithstanding the severity of infection, death never occurs. In not a single instance among the large number of rats experimented upon has a fatal result been observed.

The only noticeable pathological change in the rat is an enormously enlarged spleen. This was especially the case in the early part of the work when the incubation period was twice as long as at present. Of late, owing to the more rapid onset of the disease, this alteration of the spleen is not as great as formerly, though at times it is still very pronounced.

As to the distribution of the spirilla it may be said that they are present, not only in the circulating blood, but also in all the organs of

the body. This fact has beeen repeatedly demonstrated on rats from which every drop of blood, so to speak, had been drawn from the heart. The detection of the spirilla in the exsanguinated organs is not easily effected by staining methods. The procedure which we have followed was to add a drop of salt-citrate solution to the moist smear which was then examined for living spirilla. In this way they were detected, without any special difficulty, in the spleen, liver, and bone-marrow.

Wild rats (*Mus decumanus*).—Several of these, caught in the laboratory, were inoculated with spirillar blood. In one experiment a young rat, 3/20, was given 0.25 c.c. of the infected bloood. On the following day it showed spirilla, three per field; on the second day 30 per field; on the third day, three per field, after which they disappeared. In another rat, 6/92, somewhat larger than the preceding, the spirilla did not appear until 72 hours after inoculation. The blood then showed but one per field and on the following day none could be found. The infection in the wild rat is therefore essentially the same as with white rats, although possibly greater individual variation may exist.

ATTEMPTS AT CULTIVATION.

From the very beginning of our efforts to secure artificial cultures of the spirillum it was realized that the problem was an extremely difficult one, perhaps more so than that of the cultivation of trypanosomes. A long series of failures was expected and in this we were not disappointed, for, up to the present time, we have recorded nearly 500 wholly negative results. It is unnecessary to consider these experiments in detail, though a general outline of the ground covered may possibly be of interest.

Several sets of tubes were inoculated each time and these were placed at different temperatures such as 20°, 25°, 30°, 35°, 39°. The tubes kept under aërobic conditions were sealed with rubber caps to prevent evaporation.

A considerable variety of media, chiefly containing blood constituents, was tested. Thus defibrinated rabbit or rat blood or serum was added in different proportions to agar; in other instances the blood or serum was first dialyzed to effect a partial decrease of the saline constituents; and at other times the addition of diverse salts to the

blood or serum was resorted to. In other trials the percentage of the proteid constituents of the blood was decreased and even hemoglobin agar, wholly free from serum proteids, was made use of. Again, the blood or serum was supplemented with auto-sterile brain, liver mush, yolk of eggs, etc.

Anaërobic cultures were attempted by the pyrogallate method with varying partial pressure. Modifications of the glucose-litmus-gelatin process of cultivating anaërobes in the open air, as used in this laboratory for many years, was tried, together with cultivation under neutral oil, with the usual result. The direct inoculation into the yolk of fresh eggs was likewise negative.

Defibrinated spirillar blood, either with or without citrate or glucose solutions, was transferred to tubes and kept under diverse conditions. With these an interesting *pseudo-multiplication* was observed. On standing the corpulscles settle to the bottom, while the specifically lighter spirilla remain in the serum and eventually settle forming a light-colored deposit above the corpuscles. When decline blood is used the spirilla agglutinate and form large patches or islands which lie or float on the surface of the corpuscles. Consequently, when material is removed from this surface layer, it will be found to be enormously rich in very actively motile spirilla, and the first impression is that a successful cultivation has been obtained. A careful examination of the sedimented corpuscles, as well as of the upper layers of the serum, will show an entire absence of the organism. *The apparent multiplication resolves itself into a mere concentration by process of sedimentation.*

Before proceeding to the discussion of another series of experiments, reference should be made to some of the very early investigations which at times are cited as demonstrating extravascular multiplication. The first of these is that of Albrecht (1880), who claimed to have observed an increase in spirilla in blood drawn from the finger before the onset and kept under a cover-glass for some days. Similar observations were made by Lachmann (1880) and by Gerhardt (1881), but these have not been confirmed by subsequent workers. Inasmuch as this work was done in pre-bacteriological times, it is easy to see that contaminations were necessarily present, and hence no value can be attached to such experiments. Reference perhaps should be made

to the statement of F. Cohn (1879) who referred to a letter of Dr. Koch in which the latter claimed to have cultivated the spirillum in the same way as the anthrax bacillus. In view of the fact that no further mention is made by Dr. Koch it may be assumed that he encountered a mere pseudo-multiplication.

The observations of Norris, Pappenheimer, and Flournoy with regard to multiplication of the spirilla in citrated, undefibrinated blood are of fundamental importance, if they can be corroborated. Borrel and Burnet have recently (March 17, 1906) reported like results with *Sp. gallinarum*. In defibrinated blood, with or without addition of citrate solution, we have obtained no evidence of an increase and for that reason it seemed very desirable to repeat the observations of Norris and his co-workers under as nearly the same conditions as possible. We did not, however, employ human blood.

In these experiments the pipette employed for drawing the heart blood was provided with an S-shaped capillary in order to hold back the citrate solution. After sterilization by dry heat, about 3 c.c. of a solution of sodium citrate was introduced into the pipette. Different strength, from 1.5 to 5 per cent, citrate solutions were used. The blood was drawn directly from the heart, through the bent capillary end of the pipette, into the citrate solution, and the two fluids in equal parts were at once mixed by means of the rod, in order to prevent coagulation. Portions of 1 to 2 c.c. of this uncoagulated, citrated blood were then transferred to test tubes which were placed at different temperatures, 25°, 30°, 35°, and 38°. Portions were also transferred to citrated, uncoagulated, normal rat blood.

In these tubes the corpuscles settle in a few hours, leaving above a whitish, cloudy plasma. The latter, on gentle agitation, shows currents of fine particles *strikingly suggestive* of a culture. Moreover, on further standing, a conspicuous whitish deposit, resembling the sediment in fluid cultures of bacteria, formed. The microscopical examination, however, soon dispelled the illusion, for the cloudiness of the plasma and the deposit was found to be due to enormous numbers of well-preserved platelets. The spirilla were in good condition and about twice as numerous as in the original, mixed citrated blood. This increase was apparent and not real, for on thoroughly mixing up the plasma and corpuscles the number was found to be the same as

in the beginning of the experiment. This apparent increase, then, is merely due to the fact that the corpuscles, being heavier, readily settle, leaving the spirilla and platelets in the plasma which makes up about one-half of the total volume.

Cultures were attempted by this method with about 10 rats. The results were no more encouraging than by the other procedures. In a few instances only were scanty, sluggish spirilla found at the end of 48 hours.

In view of the fact that during infection germicidal bodies develop and, in the decline blood, *in vivo* as well as in *in vitro*, actually cause the death or disappearance of the organisms, it is clear that such blood should not be used for culture work. One obvious essential to success, then, is the use of the earliest onset blood (drawn 15 to 20 hours after inoculation), in which the number of spirilla is less than one per field. Such blood, it may be added, was employed in the preceding experiments.

It is worthy of note that in all these cultural experiments the original spiral form of the organisms was found to persist, wholly unchanged, for weeks after they had died. As in the case of bacteria, the dead spirilla retain their form almost indefinitely, and, in this respect, besides many others as shown, they differ from the protozoa.

As to the viability of the spirilla under the conditions tested it should be said that the longest survival is to be noted in plain defibrinated blood, this being in one instance 40 days. On the various agar media, the spirilla may be found alive for two to three days but not longer.

IMMUNITY.

One of the most interesting and, at the same time, most important subjects developed in this study is that of immunity to *Sp. Obermeieri*. The results obtained have been satisfactory in the highest degree, for they not only explain the course of the disease, that is, the crisis and the relapse, but they definitely establish a sound basis for the prevention and cure of relapsing fever and of the related tick fever. Before taking up the discussion of our own work, it may be desirable briefly to indicate the results heretofore obtained by other workers.

In connection with his early studies on immunity, Metchnikoff sought for a germicidal substance in the blood of relapsing fever. He allowed critical or recovered blood, one from which the spirilla had disappeared, to remain in contact with fresh spirillar blood for some hours, and, not observing any injurious action, he concluded a bactericidal agent was absent. The error in this experiment lies in not having employed a highly immune blood, for with such, as will be shown, the destruction of spirilla can be demonstrated within a few hours and even within a few minutes, *in vivo* as well as *in vitro*. In part the error was due, as Gabritschewsky pointed out, to the fact that the test was made at room temperature and not at that of the body. Specific bactericidal bodies may be expected, as in the case of the ordinary enzymes, to act best at, or near, the temperature of the body.

Pfeiffer, in connection with his memorable studies on cholera immunity, postulated the existence in relapsing fever of specific anti-bodies. A short time afterward (1896) Gabritschewsky showed that when equal parts of spirillar blood or serum and normal serum of man or monkeys are brought together, the spirilla will live longer than when recovered blood or serum is used. This difference in behavior was clearly due to the presence of a specific bactericidal body in the recovered blood. In a mixture of spirillar blood and recovered serum, kept at 37°, the organisms died out within less than one hour, whereas when similar mixtures were kept at room temperature a much longer time was necessary. Gabritschewsky accordingly arrived at the conclusion that the cause of the crisis and of the immunity was a germicidal agent.

This view, however, was not accepted by Metchnikoff, chiefly on the ground that Gabritschewsky's experiments were merely reactions *in vitro* and that there was no evidence to show that a similar reaction occurred within the body. He explained the reaction *in vitro* as due to products which escape from leucocytes after these are damaged outside of the body, and not as due to substances existing in the blood at the time it was drawn. Cantacuzène and Bardach have arrived at conclusions supporting that of Metchnikoff. It will be shown in the following that a germicidal substance is formed *within the body* and that its action can be demonstrated *in vivo* either in the circulating blood or in the peritoneal cavity (Pfeiffer's reaction).

Gabritschewsky, as well as previous workers, noted that a monkey which had recovered from the spirillar infection was immune to subsequent inoculation. The latter test was made 21 days after the first inoculation. Active immunity has been found to persist in a monkey for six weeks (Iwanoff), but how much longer this condition will exist is not known. Our experiments on rats show that such immunity may last for at least three months.

In the course of time, with the disappearance of the active immunity, reinfection is possible in man (one to one and one-half years) as well as in monkeys. Such a second attack is invariably mild, is of short duration, and is not followed by relapses.

Passive immunity to *Sp. Obermeieri* was obtained by Iwanoff who injected two monkeys with 20 and 50 c.c. of apyretic blood. In the course of a day or two these were reinoculated with spirillar blood, but no infection followed.

Gabritschewsky was the first to apply serotherapy in relapsing fever, but his results were by no means convincing. In the only experiment made, he inoculated two monkeys with spirillar blood, and on the evening of the third day, 12 hours after the appearance of the spirilla in the blood, he injected into one of them 5 c.c. of the serum from a recovered monkey. The monkey received, 12 hours later, a second injection of 5 c.c. of the serum. Crisis occurred during the following night, and hence the

disease lasted about 48 hours, whereas in the control monkey the crisis occurred after 72 hours. With reference to the monkey which supplied the serum it should be stated that it was first inoculated with several drops of spirillar blood and then 18, 23, and 25 days later it received further subcutaneous injections. Finally, on the 27th day it was given an intravenous injection of 12 to 15 drops of defibrinated spirillar blood. It is obvious from this that the blood which was drawn on the 30th day did not possess a very high immunizing value.

A result similar to that just given was obtained by Bardach, who injected 6 c.c. of serum of a recovered monkey (drawn four hours after crisis) into another one in which the spirilla were just appearing. The spirilla persisted into the evening of the same day, but were gone on the following day. The dose of the recovered blood, however, was insufficient to prevent a relapse on the seventh day.

By inoculating human spirillar blood into three horses Gabritschewsky obtained an anti-spirillar serum which Löventhal tested on a considerable number of patients. While the latter did not succeed in curing an attack, his results show that a weak serum may be given successfully to prevent a relapse. Of 83 patients thus treated, 39 (=47 per cent) recovered without a relapse, whereas of 140 untreated cases, 65 (=46.5 per cent) recovered after three attacks; practically the same per cent, it will be seen, as of the treated persons who recovered after the first attack.

The results of immunity experiments made with the other pathogenic spirochetes are on similar lines to those indicated above. Thus, Gabritschewsky in 1898 showed that a germicidal substance was formed in the blood of geese inoculated with *Sp. anserinum*, and that its action was specific for this organism; that is to say, the blood of immune geese which killed *Sp. anserinum* in a few minutes at 37° was without effect on *Sp. Obermeieri*, and vice versa; the recovered blood of relapsing fever of man, while it destroyed promptly *Sp. Obermeieri*, was without action on *Sp. anserinum*. The latter lived longest *in vitro* in onset blood. Geese which recover acquire an active immunity corresponding to that of the rat in our experiments. They never suffer relapse and are not reinfected by subsequent inoculation even as late as 113 days. Infection terminates not by crisis, as in relapsing fever, but by lysis, and death usually occurs several days after the disappearance of the spirilla.

He also noted that in serum drawn toward the close of the infection, large agglutination groups formed and that the individual cells rapidly became immobilized and finally dissolved. This he interpreted as a Pfeiffer reaction *in vitro*, the only difference being that Pfeiffer in his cholera work employed hyperimmunized animals. Consequently in the decline stage in geese, germicidal, agglutinating, and bacteriolytic properties were demonstrated. On the other hand, in the blood of recovered geese, while the germicidal action persisted, the bacteriolytic property disappeared, as indicated by the fact that when spirilla were added to such blood they promptly died but were not dissolved.

As in his previous work, Gabritschewsky held that the lysis and recovery of geese was due to bactericidal substances, thus clearly overlooking the presence of specific immune bodies. By injecting intravenously, in four doses, 30 to 40 c.c. of spirillar serum from geese into a horse he was able to obtain a serum which possessed a distinct preventive, but no curative, action. Thus 2 c.c. of this serum injected into geese 24 hours before inoculation with spirillar blood protected, but 1 c.c. failed to do so. The duration of such passive immunity in geese exceeded 20 days, but was less than 30 to 40 days, whereas the active immunity was found to persist for 45 and

even 113 days. Likewise 3 to 6 c.c. of the serum given 24 hours after the inoculation with spirillar blood, and before the organisms appeared in the circulation, afforded protection. On the other hand, 10, 30, and 40 c.c. of the serum had no appreciable effect after the spirilla once appeared in the blood. This result is in agreement with the experience of Löventhal, who was unable to shorten the paroxysms in man with the aid of serum although the latter clearly prevented the occurrence of relapse. It will be seen from our results that the failure to cure with either serum was due to insufficient immunization of the horses.

Chicken which have recovered from infection with *Sp. gallinarum* likewise do not suffer relapses and are not reinfected on subsequent reinoculation. The duration of this active immunity has not been established. Marchoux and Salimbeni induced passive immunity in chicken by injecting blood which contained dead or weakened spirilla. They also showed that the serum of recovered chicken possessed a marked preventive action, either when injected before, or simultaneously with, the spirillar blood. They failed, however, to obtain any curative action; but later Levaditi was able to show that while large doses of immune bood caused death, because of thrombosis due to the agglutination of spirilla, smaller doses brought on a crisis in less time than would ordinarily take place.

In the case of tick fever, Koch has shown that monkeys which recovered from a severe attack were perfectly protected against subsequent inoculation, whereas recovery from a mild or aborted attack did not afford such protection.

THE GERMICIDAL AGENT.

The rat is an admirable reagent for demonstrating the presence of a germicidal body for the reason that its freedom from relapses is probably due to the formation of such substances in larger amount than is the case in man or monkeys. Four methods have been employed to show the presence of a powerful germicidal substance. These, it may be added, have been made use of repeatedly with results so perfectly concordant as to leave no room for doubt that the destruction of the spirilla *in vivo* is accomplished largely, if not wholly, through its action.

The first method consists in comparing the viability of the spirilla in onset and decline blood. As shown heretofore there is an enormous difference in the behavior of the two kinds of blood. Thus in the decline blood which has been kept *in vitro* for 24 to 48 hours no living spirilla may be found, whereas in onset blood they have been observed in living, motile condition for 40 days. The animal experiment confirms the microscopical findings. Thus rats have been successfully infected with onset blood kept for 37 days, whereas decline blood in a day or two loses its infectiveness.

The second procedure is essentially the same except that recovered blood is used; that is to say, blood drawn 12 to 24 hours after the disappearance of the spirilla is used. When such blood is mixed with equal parts of decline blood the spirilla have been observed to die out within an hour at room temperature.

Thus in one test the blood of Rat 3/177 was drawn about 12 hours after the disappearance of the spirilla. Two drops of this were added to a like amount of very rich spirillar blood, at room temperature. In 20 minutes most of the spirilla were dead; a few sluggish cells were found at the end of 25 and 40 minutes. But not a single living organism could be found at the end of 50 and 60 minutes.

The third method consists in the use of blood of an animal which has been hyperimmunized by repeated injection of spirillar blood. One series of experiments of this kind will be sufficient for purpose of demonstration.

Rat 2/127, after receiving 26 injections of spirillar blood in doses of $\frac{1}{4}$ to $\frac{3}{4}$ c.c., was bled and its blood used in the following experiment. It was added to the blood of another rat, which contained about 25 spirilla per field, in varying proportions, 1:1; 1:10; 1:20, 1:50; 1:100.

In the case of the 1:1 mixture, the agglutination took place almost instantly and within two minutes all the spirilla were immobilized.

In the dilution of 1:100, small agglutinations were immediately produced; in 10 minutes the spirilla were very sluggish; in 20 minutes all but a very few were apparently dead. In 35 minutes not a single motile cell could be found.

The dilution of 1:20 behaved in exactly the same manner as the preceding.

The dilution of 1:50, when examined in less than two minutes, showed numerous small agglutination rosettes of 10 to 50 very active spirilla. One or two of these groups were present in nearly every field of the 2 mm. objective. At the end of 30 minutes the agglutination rosettes had broken down to a mass of granules (Pfeiffer's phenomenon), and only a few single, motile spirilla could be found. At the end of one hour the masses of granules had increased, but a very few motile spirilla persisted. The same condition existed at the end of two hours.

In the dilution of 1:100 identically the same results were obtained as in the preceding trial. Some of the agglutinated masses of spirals filled two to three fields of the 2 mm. objective, and, though at first they were made up of actively motile forms, within 30 minutes they had broken down into an unrecognizable mass of granules. Some free actively motile spirilla were present at the end of two hours, perhaps in larger number than in the preceding case.

The fact that in this and the foregoing trials a few organisms survived the intense reaction is explainable on the supposition that

the agglutinating and germicidal substances were insufficient to affect every one of the large number of spirilla present. Exactly analogous results have been observed *in vivo* when the dose of the immune blood employed was not sufficient to destroy, at once, all of the organisms present (see Rat 4/40, below). Moreover, it should be noted that this behavior in a way parallels the lysis observed in geese and the relapses with *Sp. Obermeieri.*

The above experiments clearly demonstrate the presence of agglutinating and germicidal substances in the blood of an hyperimmunized rat when tested *in vitro.*

The fourth method demonstrates that the agglutinating and germicidal bodies exert their action *in vivo.* An indication to this effect is easily observable when the blood of a rat in the decline stage is examined from hour to hour. It will be found that the motion of many of the spirilla becomes quite sluggish; end to end agglutination with the consequent formation of spirals 50 and 70 μ long is quite common, and, at times, even small tangles or agglutination groups may be seen. These, however, are never as perfect and as pronounced as when a large dose of a curative serum is given, as can be seen from the following:

A rat, 4/40, when it had about 40 spirilla per field, was given an injection of 0.25 c.c. of immune blood from Rat 2/127, the same as used above. A drop of blood drawn 30 minutes later *and examined at once* showed numerous tangles of 10 to 20 spirilla; also long agglutination spirilla, some of which measured 100 μ in length. The rat, re-examined 60 minutes later, showed perfect, radiating, agglutination rosettes composed of very active spirilla. Again re-examined at the end of two hours, the free spirilla were very scarce, not over three per field, and most of these were quite motionless. At the three-hour examination the spirilla were showing signs of recovery from the effects of the injection. They resumed their normal activity, but agglutination groups and spirals, 75 to 100 μ long, still persisted.

In a second experiment 0.5 c.c. of the immune blood, 2/127, was injected into a rat, 6/40, which showed about 20 spirilla per field. In this, as in the preceding, there was a prompt decrease in the number of free spirilla (down to one per field), whereas agglu-

tination groups and long spirals formed. It is hardly necessary to emphasize that, in both these instances, the agglutinating and germicidal actions took place *in vivo*, for the above results were noted on immediate examination of the blood. The motility of the organism was perceptibly decreased, but after three hours recovery seemed to take place.

The above facts which might be multipled by many similar experiments conclusively prove that a germicidal agent is present in the blood of rats which are in the decline and in the recovered stage and especially in those which are hyperimmunized. Moreover, such blood exerts its specific action *in vivo* as well as *in vitro*. Needless to say that control experiments with normal rat blood show no such action.

Additional examples of germicidal action will be given under agglutination, cure, etc., and especially under Pfeiffer's phenomenon, which will be considered next.

BACTERIOLYSIS AND PFEIFFER'S PHENOMENON.

It has been shown above that under the influence of hyperimmune serum the spirilla, *in vitro* and *in vivo*, agglutinate to form enormous masses and that these *in vitro* soon become an unrecognizable mass of granules. As this reaction corresponds to the well-known Pfeiffer's phenomenon, it was deemed desirable to demonstrate that it takes place in the peritoneal cavity as well as in the test tube. For this purpose a series of experiments were made with: (1) *recovered*, (2) *hyperimmunized*, and (3) *passively immunized* rats. As will be seen these experiments throw a flood of light upon the manner, as well as the rate of destruction, of spirilla within the living body. They effectually do away with the objections which have been heretofore formulated against the humoral destruction of the organisms.

1. *Reaction in recovered rats.*—Rat 5/88, from which the spirilla had disappeared 30 to 36 hours before, was given an injection of 0.5 c.c. of spirillar blood (20 per field). The abdomen was then thoroughly kneaded for about two minutes. At the intervals noted a drop or two of the blood was removed by means of a capillary from the peritoneal cavity and examined direct.

EXPERIMENT A.

Three minutes. Single, hyperactive, distorted or bent spirilla were present in small numbers; apparently instant, positive chemotactic reaction, for the phagocytes, though few in number, are each surrounded by a compact fringe of actively motile spirilla.

Seven minutes. Only three free spirilla found. Phagocytes as before, but the spirilla in the fringe are perceptibly shorter, as if ingestion was slowly taking place.

Ten minutes. No free spirilla found. Phagocytes still fringed with short projecting spirals. Several large masses of granules, probably result of disintegration of spirilla.

Fifteen minutes. Only one sluggish free cell found. Phagocytes have disappeared and reaction is seemingly at an end.

In this experiment the phagocytes apparently ingested still active though enfeebled spirilla.

EXPERIMENT B.

The above rat was given one hour later another injection of 0.5 c.c. of the same spirillar blood. It showed at the end of —

Two minutes. Agglutination groups of 10 to 20 very active cells. One rosette filled half the field of the 2 mm. objective. Only one phagocyte in preparation and this was surrounded by hundreds of spirilla.

Eight minutes. Several, single, dead spirilla; also large agglutination rosettes of very active spirilla. Only two phagocytes found and these were fringed with spirals as above.

Fifteen minutes. One dead and one sluggish free spirillum. Phagocytes as scarce as before. No indication of Pfeiffer's granules.

Twenty-three minutes. Three single and fairly active spirilla and 10 dead; no phagocytes. Reaction almost at end.

It would seem from the foregoing that the first injection had exhausted the phagocytes, which either retired or were killed by the enormous ingestion of spirilla, and hence but very few were available for the second injection. In the absence of phagocytes which exert a marked positive chemotaxis, the spirilla agglutinate about masses of débris, platelets, etc. At the close of the experiment the rat was killed and examined. It showed intensely injected, mesenteric blood vessels.

2. *Reaction in hyperimmunized rats.*—Four rats which previously had received 26 injections of spirillar blood were used for this experiment. Each rat was injected with 0.5 c.c. of spirillar blood (40 per field), after which the examinations were made as above.

EXPERIMENT RAT 3/132.

Two minutes. Showed enormous numbers of phagocytes *but no spirilla, alive or dead.* Masses of granules resulting from the destruction of the spirilla were present.

Five minutes. The same condition as before.

EXPERIMENT B, RAT 4/132.

Two minutes. Enormous numbers of phagocytes; *not the slightest sign of living or dead spirilla.*

EXPERIMENT C, RAT 6/132.

Two minutes. Showed a very few, single, dead spirilla; also a few agglutination groups in one of which a single sluggish spirillum was noted. Enormous numbers of phagocytes some of which show fringes of dead spirilla. The latter retain the spiral form but present a pale appearance.

Preparations, made from this material stained by MacNeal's method, showed the marked phagocytosis as indicated in Plate 11. In some phagocytes the plasma was found to be literally one mass of chromatin granules resulting from the digestion or breaking down of the spirilla. In some cells, comma or S-shaped remnants were still to be made out. This condition can be readily recognized in one or two of the photographs. It is important to note that the *phagocytes are not polynuclear* cells such as Metchnikoff described in the spleen. The study of the origin of these mononuclear phagocytes will be reserved for another time.

EXPERIMENT D, RAT 4/142.

Three minutes later. Showed phagocytes in enormous numbers as before, but no sign of dead or living spirilla.

The above experiments show that the destruction of spirilla in the peritoneal cavity of hyperimmunized rats is almost instantaneous. In only one out of four experiments could the spirilla be detected two minutes after injection. The phagocytes are all mononuclear cells, and, under the conditions of the experiment, it must be assumed that they ingest spirilla which were previously killed by the germicidal constituent of the body fluids. It is further noteworthy that the intracellular digestion of the spirilla takes place with enormous rapidity. The almost instantaneous destruction and digestion of the countless spirilla introduced can be well compared to the rapidity of a chemical reaction. These observations are of interest as bearing upon the rate of the metabolic processes in the animal body.

3. *Passively immunized rat, 1/89.*—This was given an injection of 0.5 c.c. of blood from the hyperimmunized Rat 6/132 used in the above experiment. It was reinoculated 24 hours later with 0.5 c.c. of spirillar blood (50 per field). The fluid was drawn from the peritoneal cavity and examined as before. It showed at the end of—

Two minutes. An intense positive chemotaxis. The phagocytes were very numerous and every one was fringed with enormous numbers of very active spirilla. Intense hyperactivity is a feature. Some free agglutination rosettes, also dead, single spirilla were present.

Six minutes. Exceedingly active, single spirilla are fairly common. Some dead

spirilla also met with; also loose tangles and perfect rosettes. Phagocytes fringed as before.

Twelve minutes. Condition about the same as above.

Twenty minutes. Condition unchanged.

Thirty minutes. The most notable feature is the great and sudden decrease in the number of phagocytes. The excessive hyperactivity continues. A few single spirilla are present, also many long forms 50 to 100 μ; also rosettes of 20 to 100 very active spirilla.

Forty minutes. Phagocytes are very rare and are fringed with active spirilla as in the beginning. Rosettes are very numerous and are composed of very active cells.

Fifty minutes. Each field has two to three rosettes consisting of several hundred very active spirilla. A few fringed phagocytes persist as before. The free, single, actively motile cells are apparently more numerous; no sign of dead cells.

Apparently the spirilla are recovering from the effects of the agglutinating and germicidal agents.

Sixty minutes. Rosettes are not as numerous, are smaller and less compact than before. Few phagocytes, fringed with active spirilla, still present. *Actual increase* in number of free, single, actively motile forms.

In the above experiment, the spirilla seemingly recovered from the effects of the anti-bodies in about one hour. The change was so marked that a general blood infection was confidently expected on the following day. This, however, did not occur, and at no time during the subsequent daily examinations were spirilla found in the blood of this rat. The passive immunity was therefore sufficient to protect against the enormous dose of spirilla, although, at the end of the first hour the indications pointed to a probable infection. It would seem as if the immediate resources of the passively immunized rat were insufficient in the first struggle, but that reinforced later by additional newly made anti-bodies they eventually destroyed the organisms before general infection occurred.

The significant feature of this experiment is the inability of the phagocytes to destroy the living spirilla. The rather sudden disappearance of most of the phagocytes in about 30 minutes corresponds to the observations made above with recovered rats. On comparison it will be seen that the reaction in the recovered rat is much more intense than in one passively immunized in the manner indicated.

From the three series of experiments outlined above it will be seen that Pfeiffer's reaction is almost instantaneous when a hyperimmunized rat is employed. The germicidal and bacteriolytic actions go hand in hand and the phagocytic ingestion is probably

consequent upon the death of the spirilla. The reaction as observed in recovered rats is less rapid, but nevertheless clean cut. The germicidal and bacteriolytic actions are present as before but in less degree, and there is an apparent phagocytic destruction of living though weakened spirilla. The reaction in the passively immunized rat was the least marked of the three, though the same defensive properties were brought into play.

PRESENCE OF AN IMMUNE BODY.

It has been shown conclusively, in the preceding part, that germicidal and agglutinating substances are present in the blood as the result of spirillar infection. It becomes of interest to establish the similar presence of an immune body. The latter is not necessarily identical with the germicidal substance; indeed, there is good reason to believe that they are wholly distinct. Thus, it can be shown that blood which has little, even minimal, germicidal action is yet capable of preventing infection. As pointed out heretofore, Gabritschewsky considered immunity to be due to the presence of a specific germicidal agent. One of his own experiments clearly shows that this is not the only factor. Thus, the bactericidal coefficient of the serum of an immune monkey was but a trifle above that of a normal serum, and yet, notwithstanding this evidence, he concluded that protection was obtained even when this coefficient was comparatively low.

The evidence as to the presence of an immune body in the blood, during and after infection, will be presented under the following heads:

1. *Inoculation with old kept blood.*—In defibrinated blood, which is kept in capped sterile tubes for a varying length of time, the spirilla die out, soon or late. It was repeatedly observed that when such blood was injected into a rat, even if it contained a few living, but sluggish spirilla, no infection resulted. The inoculation of such rats a few days later with spirillar blood invariably gave negative results. Such tests clearly show that the germicidal constituent must be very small in amount, if any, and consequently, the protection must be due to some other agent. In other words, an immune

body is present in the kept blood and persists long after the spirilla have died out. The following examples are selected from many to illustrate this point.

Rat 5/10 received on February 22 an injection of 0.5 c.c. of spirillar blood, 6/183, kept at room temperature for 46 days. Daily examinations of the rat were negative. It was reinoculated on February 26 with 0.1 c.c. of fresh spirillar blood. Result equally negative; control positive.

Rat 6/10 inoculated on the same day as preceding with spirillar blood, 4/191, kept 40 days under like conditions. Daily examinations were negative. Reinoculated on February 26, with 0.1 c.c. of fresh spirillar blood, was found to be immune.

Rat 7/10 inoculated at the same time as the preceding with spirillar blood, 1/198, kept 38 days as above, failed to develop infection and when reinoculated on February 26 with 0.1 c.c. of fresh spirillar blood gave like result, showing that immunity had been conferred by the previous inoculation.

Experiments like the preceding show conclusively that immunity can be obtained by means of a blood which does not contain a sufficient amount of the germicidal substance to destroy the spirilla until after the lapse of a month or more. In other words, they show that the germicidal and immune bodies are distinct.

2. *Inoculation with blood one to two days old.*—The presence of a germicidal substance in decline blood has been demonstrated above, and, as pointed out, in such blood when drawn near the close of the infection, the spirilla may die out within 24 to 48 hours. No living organism can be found at the end of that time in the blood, and injection of such into a rat will fail to produce infection. The rats thus treated on subsequent injection with fresh spirillar blood will not become infected. This fact, like the preceding, points to the presence of an immune body in the drawn blood. However, the non-identity of the latter with the germicidal agent is not as clear as in the first set of experiments.

Rat 1/10 was inoculated on February 21 with 0.25 c.c. of spirillar blood, 1/7, which had stood for 24 hours in the room. No infection resulted and the rat was found to be immune to subsequent inoculation with fresh spirillar blood.

Two other experiments of the same kind are referred to under the head of "Extravascular Viability." It will be noticed from the control mentioned (2/253) that 0.5 c.c. of this dead spirillar blood immunizes when given three days before, but not when given simultaneously with 0.1 c.c. of the spirillar blood.

3. *Inoculation with recovered blood.*—The disappearance of the spirilla from the blood is largely, if not wholly, due to the action of the specific germicidal substances. And, in view of the preceding experiments, it is evident that such recovered blood must be capable of conferring immunity, though naturally enough it leaves the question open as to whether such immunity is due to the germicidal substance or to an immune body. One experiment of this kind (see "Intravascular Viability") shows clearly the immunizing properties of the recovered blood. Such blood, however, is rich in the germicidal body as seen from the fact that when mixed with an equal part of spirillar blood it will destroy all the organisms in less than one hour.

Another very instructive experiment is presented in the following: A rat, 3/77, was bled 12 hours after the disappearance of the spirilla from its circulation. The given dose of this blood was mixed with 0.1 c.c. of fresh spirillar blood and injected at once into a rat. The several tests are given below in a tabular form.

Rat, No.	Simultaneous Injection of		Result
	Recovered Blood	Spirillar Blood	
3/88	1.5 c.c.	0.1 c.c.	No infection
4/88	0.75	0.1	" "
5/87	0.5	0.0	No infection and when reinoculated 4 days later with 0.1 c.c. spirillar blood it became infected. See below
9/90	0.5	0.1	No infection
10/90	0.5	0.1	" "
3/92	0.1	0.1	Very slight infection 3 days later
4/92	0.2	0.1	" " " 4 " "

It will be seen from the above that while 0.5 c.c. of the recovered blood protects against a simultaneous injection of spirillar blood it does not show this action when given four days previous to the latter.

The injection of an emulsion of half the spleen of Rat 3/77 failed to infect Rat 6/87. This rat, when reinoculated with 0.1 c.c. of fresh spirillar blood at the same time as Rat 5/87, developed a very slight infection on only the fourth day. The long period of incubation and the mild infection is in striking contrast with Rat 5/87, in which the infection was prompt, the spirilla appearing on the

first day and persisting for three days after inoculation. It would seem from this single trial as if the spleen was richer in the immune body than the blood. In another experiment, somewhat similar to this, a rat was bled shortly after the disappearance of the spirilla from its blood. Its blood in a dose of 1 c.c. produced infection in a rat, whereas a thick emulsion of the spleen failed to infect and actually imparted immunity.

4. *Inoculation with filtered blood.*—The details illustrative of this point will be discussed later under a separate head. In this connection, however, it will be sufficient to make the general statement that filtered blood when it does not actually cause infection may confer partial or complete immunity. That is to say, rats which received 5 to 10 c.c. of a Berkefeld filtrate of diluted spirillar blood, without becoming infected, when inoculated several days later with fresh spirillar blood, developed either a very slight and late infection, or none at all. This evidence is essentially the same as that presented under 1. In both these instances, blood was employed before the decline stage had set in, and consequently it was relatively weak in germicidal bodies. In the one set of experiments the organisms were allowed to die off slowly in about five or six weeks, whereas in these trials they were removed by the filter.

5. *Inoculation with dialyzed blood.*—It has been shown heretofore that, when spirillar blood is dialyzed in running distilled water, the spirilla retain their vitality for a much longer period than do the trypanosomes. In one experiment, after dialyzing for 20 hours, 2.5 c.c. of the laked blood, containing apparently dead spirilla, were injected into Rat 2/251. The subsequent daily examinations being negative the rat was reinoculated four days later with 0.1 c.c. of spirillar blood with like negative result. Controls positive. In the dialyzed blood, the spirilla were not actually dead, for it was possible to resuscitate them under a cover-glass, and yet they failed to cause infection as did also the subsequent inoculation with fresh active spirilla. This result, together with the preceding, is clearly due to the presence in the blood of immune bodies which appear early, even before the decline stage.

Inoculation with heated spirillar blood can also be resorted to in order to demonstrate the presence of an immune body; a proced-

ure which Marchoux and Salimbeni have followed with *Sp. gallinarum.*

6. *Inoculation with hyperimmunized blood.*—This affords final and positive proof, if any be needed, of the presence of an immune body. As will be shown, the blood of a rat which has been actively immunized possesses a very marked preventive and curative action, sufficiently so to justify the belief that when the artificial culture of the spirilla is once realized, the sero-therapy of relapsing fever, as well as of tick fever, will be an accomplished fact.

ACTIVE IMMUNITY.

The fact that man is immune to subsequent infection, after recovery from relapsing fever and from tick fever, is abundantly demonstrated by clinical observations. Moreover, the experimental confirmation of this fact has been supplied by many investigators, and reference to some of these observations has already been given.

The duration of this active immunity has not been fully established, but in some experiments on monkeys it has been found to persist for six weeks (Iwanoff). There is reason to believe that it will last much longer. Norris, Pappenheimer, and Flournoy have shown that monkeys inoculated with their spirillum likewise became immune to subsequent inoculation.

In our own work on rats and mice we have demonstrated by numerous trials that these animals, when once recovered, become wholly immune. This condition can be easily demonstrated in the case of rats. As shown heretofore, on inoculation with spirillar blood the organisms appear in the blood in from 15 to 40 hours, depending upon the number of successive passages, they then persist for 24 to 48 hours, rarely for a longer time, after which they disappear for good. No relapse has as yet been observed in a rat. That the rat from whose blood the spirilla have disappeared is immune is shown by the fact that on subsequent inoculation no infection results.

The duration of the active immunity, thus established, is of interest. The exact length of time which elapses before the rat becomes again susceptible has not been determined, but that the immunity conferred is solid and lasting, of that there can be no doubt. We have repeatedly endeavored to inoculate rats one, two, and

three weeks after a previous infection with wholly negative results. The following experiments will clearly show that the immunity is not of short duration.

Two rats, 7 / 114 and 8 / 114, which were inoculated with spirillar blood on November 27, were reinoculated *55 days later*, on January 21, with 0.25 c.c. of spirillar blood (10 per field). No infection resulted.

Rat 2 / 116 received its first inoculation on November 28. The spirilla persisted until December 1 and were gone on the following day. Reinoculation was made on January 21 (*54 days later*) with negative result. Controls positive.

Rat 1 / 131 was inoculated with 5 c.c. of a Berkefeld filtrate on December 7. The number of organisms in the filtrate was evidently very small, for they did not appear in the blood until December 10 and were gone on the following day. This rat was reinoculated on February 11 (*66 days later*) and failed to become infected, whereas the control was positive.

Rats 3, 4, 5 / 138 were inoculated on December 9 with 5, 5, and 7 c.c. respectively of a Berkefeld filtrate. All three became infected. They were reinoculated on March 27 (*108 days after the first inoculation*), but did not become infected. Control positive as usual.

In white mice, as in rats, a condition of active immunity is established but with this difference, that a solid, lasting immunity is not acquired until after one or more relapses have taken place. That a condition of active immunity exists in a mouse at the close of the first attack can be readily demonstrated. Thus, if a mouse from whose blood the spirilla have disappeared, a few hours before, is given an injection of 0.25 c.c. of richest spirilla blood (50 to 100 per field), it will not become infected but a relapse may follow in the usual time.

The theory of relapses.—The condition at the close of the first attack in a mouse, monkey, or man may be designated as a relative or temporary immunity which is due, in the first place, to the destruction of the spirilla by the specific germicidal agent, and secondly, to the presence of a specific immune body. The amount of the germicidal substance is sufficient to kill or weaken most of the spirilla *which are then taken up* by the phagocytes. Some of the spirilla, however, escape this fate because they are sheltered in some part of the body, possibly in extravascular places. Eventually, as the result of a partial elimination or oxidation, or both, of the anti-bodies mentioned, the spirilla are enabled to multiply and hence reappear in the blood. In the resulting relapse, which occurs usually after an interval of about seven days, they are never as numerous as at first and, as a rule, in each suc-

cessive relapse they appear in less numbers. Each such relapse has the same effect as an injection of spirillar blood, and hence after several such attacks a condition of solid immunity is established, due chiefly to the presence of a sufficient amount of immune bodies or to an increased production of such substances.

It is our belief, based upon experimental evidence, that the germicidal and immune bodies are two distinct substances and that the relative amounts of these two substances at the close of the attack determine whether or not a relapse will occur. It is wholly unnecessary to assume the existence of spores, as many of the older writers have done in order to explain a relapse. Neither is it necessary to assume that the organism passes through a cycle analogous to that of the malarial parasite. The fact that the spirillium is not a protozoon effectually eliminates this theory of a cycle. The survival of a few normal spirilla in some protected spot in the body is quite sufficient for this purpose. Moreover, that such a survival is possible is indicated by the observation of Cantacuzène, who, after the complete disappearance of the spirilla from the blood, found them present in the vascular papillæ of feathers and even in the pericardial exudate of geese, previously infected with *Sp. anserinum.*

The view expressed above is based upon a large number of observations made on rats and mice. When these receive injections of blood containing very small amounts of immune bodies the spirilla are retarded in their appearance and eventually are found in very small numbers, perhaps but one or two on a cover-glass. Rat 1/131, referred to above, will serve as an illustration. In that experiment the filtrate injected, as will be shown later, contained immune bodies as well as a small number of spirilla, and the result was a delayed and very mild infection, which, in spite of this fact, conferred an immunity lasting more than 66 days. The mild infection in such cases is comparable to the relapses mentioned above.

In view of the fact that mice, as well as man and monkeys, are subject to relapses, it might be expected that, after complete recovery has taken place, the immune bodies would be eliminated or oxidized more readily than in the rat, and hence the duration of such immunity would be shorter. To test this point a number of mice which had apparently recovered were injected with fresh, rich, spirillar blood on March 12, with negative result. The data are as follows:

Mouse 6/229, inoculated January 28; the second and last relapse occurred on February 15. Interval before reinoculation, 25 days.

Mouse, 7/229, inoculated also on January 28; the third and last relapse occurred on February 21; interval before reinoculation, 19 days.

Mouse 4/244, inoculated on February 7; the first and last relapse occurred on February 16. Interval before reinoculation 24 days.

The failure to infect these mice shows that the duration of the active immunity in these animals exceeds 25 days. Whether it is actually shorter than that of the rat we are not able to state, but in view of the rather long duration of passive immunity in mice it would seem that the active immunity would last for several months.

HYPERIMMUNITY.

This condition can be readily established in rats by repeated injections of spirillar blood. In our series about 30 rats have been thus immunized. The spirillar blood, drawn about 45 hours after inoculation and containing 10 to 50 spirilla per field, was injected in doses of 0.25 to 1.0 c.c., intraperitoneally, every other day. The majority of our rats have received from 26 to 32 of such injections, and hence have been under treatment for over two months. They tolerate these inoculations perfectly, and, so far as the danger from intraperitoneal injection is concerned, that is practically *nil*. Among the hundreds of rats and mice which we have injected in the course of this investigation, probably not more than one or two died as the result of this method of inoculation.

The repeated injection of spirillar blood results not only in an increase in the amount of agglutinating, germicidal, and immune bodies, but also in the production of precipitins or anti-complements, and possibly even anti-immune bodies. The so-called anti-complement action was particularly noted in the blood of rats which had received the maximum number of injections. As the effect of such action is to inhibit or lessen the preventive and curative power of a serum it obviously must be taken into consideration. The following will serve to illustrate this apparent variation in the strength of an immune blood.

Rat 5/145, fairly rich in spirilla, was given an injection of 1 c.c. of an immune blood, obtained after eight injections. Within five hours

the spirilla completely disappeared. A number of similar experiments with immune blood resulting from seven, eight, and thirteen injections caused the disappearance of the spirilla in from one to two or three hours. When therefore a rat, 2/127, which had received 26 injections of spirillar blood was bled, it was expected that its blood would accomplish the same result in a dose of 0.5 c.c. or even less.

Accordingly two rats, 4, 6/40, rich in spirilla, received 48 hours after inoculation an injection of 0.25 and 0.5 c.c. respectively of the blood of Rat 2/127. It was a matter of surprise to find that these injections had only a very slight effect on the spirilla, which persisted for more than 72 hours after the first inoculation. This surprising result was explained by assuming the presence of an anti-complement action. To determine this point, the above experiment was repeated with this difference, that the dose of 0.5 c.c. of the immune blood was added to about 5 c.c. of normal defibrinated rat blood and the mixture was set aside at 36° for about one hour.

Two rats, 2, 3/55, in the onset stage and containing spirilla about one per field, received each an injection of the above mixture, kept at 36°. From the second, the spirilla disappeared within one hour, and from the first within two hours. The only difference between this and the preceding experiment was, first, that the immune blood was injected in the former, mixed with salt solution, while in the latter, it was mixed with normal rat blood and digested at 36°, for one hour in order to neutralize the effect of the anti-complement or precipitin bodies; and second, that the rats in the former set were entering on the decline stage, while in the latter they were still in the onset stage. The cure in the onset stage is probably even a more severe test than if it had been attempted in the decline stage. This experiment may therefore be considered as demonstrating the presence of "anti-complements," the action of which can be neutralized by allowing the immune blood to stand with an excess of normal blood for one to two hours at 36°.

As a result of these observations the immune blood in most of the subsequent work was treated in the manner indicated. Normal blood has little or no action on the organisms, and hence can be injected in large amount without influencing the course of the infection.

For the purpose of comparison it is desirable to establish a standard

or unit of strength for the immune blood. The *unit* which we employ may be defined as that amount of immune blood or serum which on simultaneous, intraperitoneal injection with 0.1 c.c. of rich spirillar blood will just protect a 100 g. rat against infection. The strength is then expressed as so many units per cubic centimeter. The spirillar blood is always drawn about 45 hours after inoculation, and contains from 10 to 50 spirilla per field. The dilution of the immune blood may be made with salt solution, but it is better to dilute with normal rat blood allowing the mixture to stand at 36° for one hour. Obviously such a standard is not absolutely accurate, but it is sufficiently so for all practical purposes.

In applying this test it is important to remember that the immune blood possesses a powerful germicidal action, and, consequently, if the mixture of immune and spirillar blood is allowed to stand for any length of time, the organisms may be weakened or even killed. Any injurious action *in vitro* of this kind would necessarily give false values. To obviate this effect, we have always diluted the immune blood with 2 to 3 c.c. of salt solution before adding the spirillar blood. The resulting dilution was at once injected into the test animal. It may be advisable, in order to counteract this injurious action, which is more noticeable the stronger the immunity, to resort to separate injections of the immune and spirillar bloods in different parts of the body.

According to the definition given, if 0.5 c.c. of recovered blood, 3/77, protects, in the manner mentioned, it follows that it contains in 1 c.c. two immunity units. A hyperimmunized blood, such as that of Rat 2/127 used above, protects in a dose of 0.002 c.c. and hence it contains 500 units per cubic centimeter.

The example just given shows the comparative strengths of recovered and hyperimmunized bloods. It will be seen that while 1 c.c. of recovered blood has but about two units, that of a hyperimmunized rat is 250 times as active. It is safe to say that this by no means represents the maximum strength attainable. We hope to be able to have a blood which will contain twice as many units as that given above, and, if cultures of the organism were at one's disposal, there should be no difficulty in securing a blood having 100 or 1,000 times the strength of that used above. It should be noted that our figures refer to de-

fibrinated blood and not to serum. We have employed the former almost exclusively as a matter of economy in time and material. Obviously the strength of the respective sera would have been about twice that given above.

PASSIVE IMMUNITY.

This condition can be readily established in rats, mice, and monkeys. The methods of demonstrating the presence of an immune body, discussed above, may also serve to illustrate the production of passive immunity. Inasmuch as it is the basis of the important problem of prevention and cure of relapsing fever, further examples will be given under these heads.

Passive immunity, it will be seen, can be obtained by the injection of filtered, or dialyzed (possibly heated), blood; also, with such in which the spirilla have died out or become enfeebled. It may be objected that the immunity established by either of these procedures is an active one due to chemical products of the organism. This, however, hardly seems to be a correct explanation, for everything points to the presence of an immune body from the very onset of the infection. Hence, the immunity resulting from the use of such blood is in all probability a borrowed one. This is particularly seen when simultaneous injections of such blood and spirilla are made, for the immediate protection obtained under such circumstances can only be explained by the presence of an immune body.

Rats and mice can be passively immunized with ease by means of a hyperimmune blood. Much less than one unit will suffice for this purpose provided the immune blood is injected one or two days in advance of the spirillar blood. On the other hand, one unit may fail to protect if given four days previous to the injection of the spirilla (see p. 341). As will be shown later, passive immunity can also be conferred on monkeys, and there is no doubt but that the same result can be obtained in man.

The quantity of blood which is to be used to induce passive immunity obviously depends upon the strength of such blood, that is, the number of units it contains, and upon the duration expected of such immunity. Thus, in mice, monkeys, and man, owing to the peculiar

conditions which bring on relapses, it is necessary to inject a much larger dose of immune blood than what would seemingly be necessary to protect against a simultaneous injection of the spirillar blood. While the calculated dose will prevent the first paroxysm it will not be sufficient to hold in check the first relapse, which appears on the fifth to seventh day after the inoculation. Hence, the need of injecting much larger doses of immune blood than such as are calculated from the experiments on rats in which no relapse occurs.

The duration of passive immunity depends, therefore, primarily upon the dose of the immune blood which has been administered. As seen from the following experiment, it may persist in rats for more than 68 days, and in mice for about 65 days.

Rat 5/1 received on February 16 a simultaneous injection of 0.1 c.c. of spirillar blood plus 0.010 c.c. of immune blood (result of 13 injections). No infection. Tested on March 31 with another injection of 0.1 c.c. of spirillar blood it was found to resist. Duration, 43 days.

Rat 4/245 received on February 8 a simultaneous injection of 0.1 c.c. of spirillar blood plus 0.050 c.c. of immune blood (13 injections). No infection resulted. Tested on March 31 with same dose as preceding; result negative. Duration 51 days.

Rat 6/245 received on February 8 a simultaneous injection of 0.1 c.c. of spirillar blood plus 0.090 c.c. of same immune blood as above. No infection. Tested on March 31, the same as the preceding and with like result. Duration, 51 days.

Rat 4/214 received on January 22 a simultaneous injection of 0.1 c.c. of spirillar blood plus 0.2 c.c. of immune blood (result of six injections). No infection. Tested on March 31, the same as the preceding, it became infected. This shows that 0.2 c.c. of a weak solution protects for less than 68 days.

It should be noted that the above results pertain to the immunity which follows a simultaneous injection of spirillar and immune bloods. Such mixed immunity is probably more lasting than if only immune blood had been injected. No experiments with pure passive immunity have as yet been tried.

HEREDITARY IMMUNITY.

It is an interesting fact supported by a number of observations that the *Sp. Obermeieri* may pass from the mother to the fetus. Three such cases, reported by Albrecht in the early eighties, deserve special mention. In one, a seven-months-old fetus lived eight days after birth and on autopsy spirilla were found in the heart blood. In another case, the fetus, also seven months old, lived 76 hours

after birth and on autopsy likewise showed spirilla. In the third case, the fetus, seven and one-half months old, was born on the 17th day of the second apyrexia, but although it showed no spirilla it presented the characteristic enlarged spleen.

These facts are in striking accord with the numerous observations which have been made during the past year regarding the presence of *Sp. pallida* in congenital syphilis. The finding of *Sp. Duttoni* in the eggs of ticks is another illustration of a seemingly general fact that the actively motile spirilla are able to pass from parent to offspring. Not only is it possible to develop congenital or hereditary infection, but it becomes equally possible to acquire active, and probably even passive, immunity in this way.

The experimental evidence which we have to offer on this point pertains to the rat. It clearly establishes the existence of congenital or hereditary immunity and has, as will be readily seen, an important bearing upon the origin of the immunity with reference to tick fever which the native negro children apparently enjoy. The only explanation which Dr. Koch has been able to give for such cases is that they had been infected in early childhood. That a somewhat different explanation is possible will be seen from the following:

Experiment 1.—A female rat, 2 / 117, was inoculated with 0.25 c.c. of spirillar blood on November 29. On December 3 it received a second injection of 0.25 c.c. of spirillar blood. On December 7 it gave birth to several young. These were not examined for spirilla at the time but were allowed to grow up. On February 7, two months later, the three surviving young rats were inoculated, each receiving 0.1 c.c. of rich spirillar blood. They were then examined daily for five days in succession. Two of the young rats gave no evidence of infection. The third rat showed spirilla, about one per field, on February 9, but none before or after. Control rats developed the usual infection. Fully to appreciate this experiment it should be stated that young rats are extremely susceptible to infection. In these the spirilla appear early (within 15 hours) and in very large numbers (50 to 100 per field) and persist for four or five days. It is evident, therefore, that *two, out of the three, had complete immunity two months after birth*, and that a third developed but a very slight infection.

Experiment 2.—A rat, 5 / 145, was given an injection of 0.25 c.c. of spirillar blood on December 13 and on December 15 it was given a curative dose of immune blood (1 c.c.). As a result of the injection the spirilla disappeared from its blood within five hours. On February 16, 18, and 20 it was given 0.25, 0.5, and 0.5 c.c. respectively of spirillar blood. On February 22 it gave birth to young. Three of these were inoculated on March 22 (28 days later) with 0.1 c.c. of spirillar blood and did not develop an infection. *In this case, the immunity, active or passive, persisted for more than one month.*

Experiment 3.—Rat 3/157 received 5 c.c. of a Berkefeld filtrate on December 17, but no infection resulted. On February 10, it was given an injection of 0.33 c.c. of spirillar blood. This injection was repeated on February 13, 15, 18, and 20. On February 22 it gave birth to young. Three of these were tested on March 29, the same as the preceding. No infection followed. *The immunity, active or passive, lasted for more than 35 days.*

Experiment 4.—Rat 7/140 received on December 11 an injection of 5 c.c. of a Berkefeld filtrate and two days later it showed a very slight infection, only one spirillum being found. Unlike the preceding it received no other injection. On February 17, 68 days after inoculation, it had young. Of these four were tested on March 24 with 0.1 c.c. each of spirillar blood. In the blood of all four, the spirilla promptly appeared on the first day; on the second day they were 20 per field; on the third day, 50 per field; and in one of the four they were still present on the fourth day.

This experiment serves as a control on the previous ones in so far as it shows the severity of infection which the young rats undergo. It further shows that any passive immunity which they acquired from the mother, injected 68 days before their birth, had disappeared in the 35 days which followed.

The immunity of the mother, it may be added, was largely a passive one, as is seen from the very light infection which she exhibited.

The foregoing experiments furnish abundant proof that immunity may be acquired before birth and may persist for a month or more. The conditions of the tests, however, were such as to leave open two possibilities. First, the young may have had congenital infection *in utero* from which they recovered either before or after birth, in which case they possessed an active immunity. Or, second, they may have acquired passive immunity from the mother. In the first three experiments it may be assumed that the immunity was of the first type. A further study of this interesting problem is contemplated.

PREVENTION.

The prevention of spirillar infection is so easily accomplished in rats, mice, and monkeys as to leave no doubt about the equal efficacy of the method in man. An essential requirement is a very active immune serum. In all experiments made heretofore with the spirilla of man, geese, or chickens the process of immunization was not pushed very far. Usually either recovered blood was used or such as was obtained after several injections of spirillar blood. It will be seen from the following trial that in ordinary recovery the blood has a low immunity value, not over one or two units per cubic centimeter.

Experiment with decline blood.—Rat 4/243 was bled 60 hours after inoculation with spirillar blood, that is to say, at the close of the decline stage. In the blood, on standing, the spirilla died out in less than 30 hours. Six days later a young rat, 2/253, was given a simultaneous injection of 0.5 c.c. of this decline blood plus 0.1 c.c. of spirillar blood. The spirilla appeared on the following day; on the second day they were 20 per field; on the third day 100 per field; on the fourth day only dead spirilla, about 10 per field and in addition some small agglutination groups, were to be found. The decline blood used for this experiment clearly contained less than two units per cubic centimeter, and probably did not contain even one unit. While the blood of this rat was insufficient to protect against a simultaneous injection of spirilla it was effective in the same dose when given three days before the injection of spirillar blood.

Experiment with recovered blood.—The fact that this blood has a marked preventive action has been incidentally brought out heretofore on p. 341. In the latter case the recovered blood of Rat 3/77 was found to protect in a dose of 0.5 c.c. against a simultaneous injection of 0.1 c.c. of spirillar blood. A dose of 0.2 c.c. was almost sufficient to accomplish this result. In other words, this recovered blood contained more than two and less than five units per cubic centimeter. It therefore was considerably stronger than the decline blood mentioned above.

Experiment with hyperimmunized blood.—The facts shown above in connection with recovered blood are brought out with remarkable clearness when the blood of hyperimmunized animals is used. Experiments of this kind have been made with the blood of rats which received 6, 7, 8, 13, 20, and 26 injections of spirillar blood. It will be sufficiently illustrative to give a series of tests with the latter blood.

Rat 2/127, after receiving 26 such injections, was bled and its blood was used for a large number of trials of which the following will be of interest. Of this blood 0.05 c.c. was added to 4.95 c.c. of normal defibrinated rat blood, and the mixture was set aside for two hours at 36°. Of this mixture, portions of 0.1, 0.2, 0.3, 0.4, 0.5 c.c., corresponding to 0.001, 0.002, 0.003, 0.004, 0.005 c.c. of the original immune blood, were measured out into Hitchens' syringes and diluted with salt-citrate solution. To each syringe, 0.1 c.c. of spirillar blood was then added and the whole at once injected into a rat.

Rat 8/58 received the above mixture containing 0.001 c.c. of immune blood.

The following day it showed spirilla, about $\frac{1}{10}$ per field; the second day, five per field; the third day only one could be found, and none on the fourth day.

Rat 5/61 received the mixture containing 0.002 c.c. of immune blood. It showed no spirilla on the first or second day after inoculation; on the third day only one spirillum was found and none on the fourth day.

Rat 6/51 received the mixture containing 0.003 c.c. of the immune blood. At no time did it show spirilla in its blood.

Rats 7/61 and 9/58 received the mixtures containing 0.004 and 0.005 c.c. respectively of the immune blood. No infection occurred in either of these rats.

From the foregoing, it is evident that 0.002 c.c. of the hyperimmunized blood was practically sufficient to protect against a simultaneous injection of spirilla. This blood, therefore, contained 500 immunity units, which represent a considerable increase in the immunizing value as compared with the strength of the recovered blood (two to five units per cubic centimeter).

Similar preventive experiments were made with white mice and the results were equally definite though the possibility of relapse must be taken into consideration.

Mouse 5/214 received on January 22 a simultaneous injection of 0.1 c.c. of spirillar blood plus 0.25 c.c. of immune blood (result of six injections). No infection.

Mouse 6/214 received on the same date a similar injection, except that 0.5 c.c. of the immune blood was used. No infection. When tested on March 28 with 0.1 c.c. of spirillar blood it failed to become infected, showing that the duration of the passive, or rather, mixed immunity exceeded 65 days. In a similar experiment, under identical conditions, a rat, 4/214, which received only 0.2 c.c. of the immune blood, became infected at the end of 68 days. (See p. 350.)

In these two experiments no attempt was made to ascertain the occurrence of relapses. It was realized, somewhat later, that in an animal subject to relapses, a preventive inoculation will prevent the usual first infection, but, strange to say, it will not ward off the relapses which come on about the same as if the first attack had taken place. This is true when small doses are used, but with very large ones it may be possible to protect even against the relapses. The prevention of relapses will be discussed at the end of this chapter.

Monkeys.—Only one experiment has been made thus far with the monkey. The details of this test, together with the control, will be given under the next heading. It can be stated, however, that 0.35 c.c. of an immune blood protected against a separate and immediate injection of 0.25 c.c. of spirillar blood. As in the case of mice, the dose of blood, however, was not sufficient to ward off the relapse.

The most active blood which we have obtained protects a 100 g. rat in a dose of 0.002 c.c., or in other words, one unit protects 100 g. body-weight. The protective dose for a 5 kg. monkey would be 50 units or 0.1 c.c. of this immune blood. Similarly, the protective dose for a 75 kg. man would be 750 units or 1.5 c.c. of this immune blood. If serum had been used instead of blood it probably would have been twice as strong, and hence a correspondingly smaller dose would have been effective.

It may be added that a smaller dose than the above would have been sufficient to protect if given a day or two before the injection of spirillar blood. This is seen in the fact, mentioned above, that a recovered blood, which does not protect against 0.1 c.c. of spirillar blood in 0.5 c.c. dose when given simultaneously, readily does so when given two or three days before. On the other hand, the injection of 0.5 c.c. of a recovered blood four days previous to inoculation with spirilla failed to protect a rat, whereas when given simultaneously it protected perfectly. Furthermore, experiments have shown that immune blood will protect, if given at any time previous to the appearance of the spirilla in the blood. After that occurs curative doses of the immune blood must be used.

The prevention of relapses is obviously a matter of some importance. One method by which this can be accomplished is to inject large *curative* doses of immune blood. From the work which we have done thus far this seems to be the only practicable procedure. Two other methods suggested themselves, namely, active and passive immunization with small doses of spirillar and immune blood. These methods have been tried on mice, but the result has not been as satisfactory as was anticipated. The details of this test are briefly as follows:

Six mice were injected on April 17 each with 0.1 c.c. of spirillar blood, 1 / 109. Two of these were reserved for controls. Two of the mice were given each two injections of spirillar blood after the disappearance of the spirilla from the first attack. These injections were made for the purpose of inducing, if possible, an active immunity during the apyretic stage. The other two mice were given each two injections of immune blood with the idea of conferring a passive immunity during the spirillum-free stage. Examinations were made daily until May 7.

Control Mouse 2 / 110 showed spirilla on April 18 and 19. On April 26, two spirilla were found in its blood indicating the first relapse.

Control Mouse 3 / 110 also showed spirilla on April 18 and 19. On April 26 it had the first relapse, only one spirillum, however, being found. Two spirilla were found on May 2.

Mouse 4/110 showed spirilla on April 18 and 19. On the 20th, and again on the 23d, it was given an injection of 0.25 c.c. of spirillar blood. It had a relapse on April 26, four spirilla being found, and again on April 28 when but one was detected.

Mouse 5/110 showed spirilla on the two days as before. It was then given injections of spirillar blood at the same time as the preceding. This mouse showed no relapse until May 3 when two spirilla were found.

Mouse 6/110 showed spirilla on the two days as before. On April 20 and again on April 23 it was given injections of 0.25 c.c. of immune blood. A relapse occurred on April 27 when 10 spirilla were found. Also on May 3 when two spirilla were found.

Mouse 7/110 showed spirilla on the two days as before. It was then given injections of immune blood, the same as the preceding. It showed no relapse.

From the above it will be seen that apparently one mouse was actively immunized and one passively immunized to the extent of preventing a relapse. The mildness of the first relapse in the other four mice, however, leaves open the possibility that a relapse occurred in the other two, but, owing to the scantiness of the spirilla, was overlooked. Further experiments are needed to determine the feasibility of preventing relapses by either of these two methods.

CURE.

In view of the rather weak strength of recovered blood, which, as shown under the preceding head, has a value of but two to five immunity units per cubic centimeter, it follows that such blood cannot be used to advantage as a means of prevention, much less as a cure, for relapsing fever. With hyperimmunized blood, however, things are entirely different since such blood, containing 500 units per cubic centimeter, can be readily obtained at present and, without doubt, under improved conditions this strength can be greatly surpassed. The corresponding serum would obviously have about twice the strength of the defibrinated blood.

The method of immunization heretofore employed has been to inject intraperitoneally, on alternate days, 0.25–1.0 c.c. of rich spirillar blood into a rat. Possibly more rapid immunization, together with a greater anti-bacterial strength of the blood, can be secured by giving larger injections (1–2 c.c.). Such experiments are now being conducted with the hope of increasing materially the immunizing value of the blood.

From results obtained it appears that when a large dose of blood is injected an appreciable interval must elapse before the next injection is made, in order to allow a full recovery of the phagocytes and a regeneration of the anti-bodies. When such injections are made in close sequence the strength of an immune blood may be actually decreased by more than one-half. Thus, in one test 20 rats were immunized, each receiving 26 injections of spirillar blood in doses of $\frac{1}{4}$–$\frac{3}{4}$ c.c. Twelve of the rats were then bled and the blood was found to have a value of 500 immunity units per cubic centimeter. The remaining eight rats were continued under treatment, but instead of receiving small doses they were given on alternate days 1.0–1.5 c.c. When bled, after several such injections, the blood was found to have a strength of about 100 units.

As yet no attempt has been made to prepare a curative or preventive serum from any other animal than the rat. It is our intention to immunize a horse at an early date, the chief drawback to this procedure being the large number of rats which must be employed in order to secure an efficacious serum.

Obviously, as soon as it becomes possible to replace the use of spirillar blood by a pure culture of the organism the last difficulty in the practical production of a curative and preventive serum will have been overcome. The larger animals can then be immunized with as much, if not greater, ease than is now done in the case of diphtheria. We know to what extent it is possible to immunize a horse against the cholera vibrio, it being not at all difficult to obtain a serum which will protect a guinea-pig in a dose of 0.0001 c.c., and the experience with rats justifies the belief that this result can be equalled with *Sp. Obermeieri*, for at present our serum protects a rat in a dose of 0.001 c.c.

Cure of injected rats.—The following experiments in the treatment of infected rats are selected for the purpose of demonstrating the efficacy of the method.

Experiment 1.—Rats 5 and 6/145, weighing about 150 g. each, were injected with spirillar blood on December 13. Forty-three hours later an examination showed that each rat had spirilla in the blood, about one-third per field. This, it should be stated, represented the onset and not the decline stage. The first rat was then given 1.0 c.c. and the second 1.5 c.c. of an immune blood, 3/97, 2/98 (result of

eight injections). Re-examined *five hours later not a single spirillum could be detected* in the blood of either rat. This shows that a relatively weak blood, in a dose of 1.0 to 1.5 c.c., is able to cure the disease in the onset stage within a few hours.

Experiment 2.—Rats 4/173 and 1/174 were inoculated with spirillar blood on December 26. On December 28 each of the rats had about three spirilla per field. The first was given an injection of 2 c.c. of an immune blood, 4/154 (result of seven injections). An examination made *half an hour after the injection failed to reveal a single spirillum.* The second rat was given an injection of 3 c.c. of the same blood, and in this also not a single organism could be detected one hour after the injection. This experiment, it may be added, was performed before the Society of American Bacteriologists which held its meeting in Ann Arbor on that day.

Experiment 3.—The following trial shows the effect of the immune blood when employed in a dose insufficient to effect a full cure. Rats 2 and 3/154 were inoculated with spirillar blood on December 16. Two days later they each showed spirilla, about 10 per field. Accordingly each one received 1.0 c.c. of immune blood, 7/104 (result of eight injections); the effect was about the same in each rat. At the end of two hours the spirilla clearly showed the injurious action of the immune blood. Most of the organisms were agglutinated in groups of 5 to 50 cells. The motion was very sluggish and apparently they were dying off. The number had decreased to about 0.5 per field. This condition persisted for several hours after which recovery from the effects of injected blood began to manifest itself. This was noticeable in the increased motion of the spirilla and by the lessened number of agglutination groups. When examined about seven hours later, the spirilla had apparently increased in numbers to about one per field; they showed a normal activity and scarcely any indication of agglutination.

This experiment teaches that an injection of a small amount of immune blood, insufficient to cure at once, may cause a notable decrease in the number of spirilla and by doing so may materially modify the course of the disease. This fact will, without doubt, find an application in the treatment of the disease in man.

Experiment 4.—Rats 4 and 6/40 received on March 12 an injection

of spirillar blood. When examined 48 hours later the former had 40 and the latter had 20 spirilla per field. The former was then given 0.25 c.c. and the latter 0.5 c.c. of an immune blood, 2/127 (result of 26 injections). The details of this test are given on p. 334. It will be sufficient to state here that the dose was not sufficient to cure at once. The effect of the immune blood was marked and was indicated by the presence of dead spirilla, and especially by the agglutination phenomena. The spirilla recovered from the effect of the anti-bodies in about three hours.

The blood employed in this experiment had a strength of 500 units per cubic centimeter, that is to say, it protected in a dose of 0.002 c.c. It follows that 125 to 250 units are apparently insufficient to cure an infected rat weighing about 150 g. Both rats showed spirilla on the following day, though in very small numbers.

It is true that the injection of the immune blood in the two preceding experiments produced a marked effect upon the spirilla and even caused some decrease in their number, but this result was offset by the unusual persistence of the spirilla on the following or third day. Taken as a whole, the result of the last experiment was not as favorable as in the other trials where 1.0 c.c. of immune blood derived from rats which had received one-half or even a less number of injections was used.

The explanation of this apparent failure was soon found to be due to an "anti-complement" action. On neutralizing this action of the immune blood by means of normal rat blood, as described on p. 347, it was possible to effect the cure of two rats, 2 and 3/55, in one and two hours respectively by injections of 0.5 c.c. of this same immune blood.

Inasmuch as the rats which weighed from 100 to 150 g. were cured by 0.5 c.c. of immune blood (=250 units) it will be seen that from 1.7 to 2.5 units per gram of body-weight represents the minimum amount needed to effect a cure in rats. It probably will hold true in general that about two units per gram of body-weight will effect an immediate cure. In a monkey, as will be seen, the disappearance of the spirilla was prompt after the injection of five units per gram of body-weight. On the two-unit basis a man weighing 75 kg. would require 150,000 units, which amount would be contained in 15 c.c. of a serum 10 times as strong as the one used in our experiments.

Cure of infected mice.—Unlike rats, as pointed out heretofore, the white mice are subject to a variable number of relapses, and in this respect they approach the monkey and man. It was therefore desirable to determine the effect of curative doses of immune blood on these animals. The number of such experiments has not been large, but it has been sufficient to show, first, that the spirillar infection can be cut short or "cured" within an hour; and, second, that the "cure" is not necessarily permanent, for relapses may eventually occur, the same as in untreated mice.

Experiment 1.—Mice 2 and 4/213 received an injection of spirillar blood on January 21. On the following day they showed spirilla; the former one, and the latter two, per field. They were then given an injection of 0.25 and 0.5 c.c. respectively of immune blood (result of six injections). In the former the spirilla decreased in an hour to about one-fourth per field; in two hours long spirilla or agglutinations were present and in 14 hours no organisms could be detected. On the other hand, the second mouse also developed agglutination groups and there was a temporary decrease during the first hour or two, but 14 hours later the organisms had actually increased to 10 per field and on the following day the mouse was dead.

At the time of this experiment the possibility of a relapse in a "cured" animal was not expected, and no examination of the first mouse was made until February 19, that is, 29 days after the original inoculation, when two spirilla were found in its blood. This, it will be seen, was the time for a fourth relapse. On the following day no organisms could be seen. The mouse was then examined daily, until March 7, but no other relapse was detected. On March 12 it received an injection of 0.1 c.c. of spirillar blood without becoming infected, thus showing that a condition of active immunity existed.

The following experiment shows conclusively the curative effect of an immune blood and the occurrence of relapse notwithstanding such treatment.

Experiment 2.—Mice 2, 3, and 5/244, received an injection of spirillar blood on February 7. When examined 28 hours later spirilla were present. The first and third had one per field; the second two per field; and the third one per field. They were then injected with 2, 1, 0.5 c.c. of an immune blood (result of 13 injections). The first

when examined one hour later showed no spirilla. It was not examined again until February 21, from which time until March 12 it was examined daily. On February 23 (time for second relapse) one spirillum was found. On February 27 again a single spirillum was met with.

In Mouse 3/244, examined one hour after the injection of immune blood, only two dead and two very sluggish ones could be detected. Re-examined two hours later and on the next day with negative result. The mouse died on February 10.

In Mouse 5/244, within one hour, no spirilla could be found and the examination on the next day was likewise negative. From February 21 to March 7, it was examined daily but no spirilla could be detected.

A control mouse, 4/244, was inoculated the same time as the above and was examined daily until March 7. It showed spirilla on February 8 (two per field); February 9 ($\frac{1}{10}$ per field); and again on February 16 ($\frac{1}{10}$ per field). It therefore presented but one relapse. This mouse as well as 2 and 5/244, used in the above experiment, were inoculated on March 12, each receiving 0.1 c.c. of spirillar blood. All three resisted infection, demonstrating that a condition of immunity had been established.

No experiment has been made to cure mice with stronger immune blood than that used above. It is possible that a more active blood would be effective in preventing relapses. Attempts at preventing relapses in mice, either by active or passive immunization, have been described.

Cure of infected monkeys.—The following experiments serve to demonstrate the preventive and curative action of immune blood in these animals. The immune blood used for Monkeys 1 and 2 was the combined yield of six rats which had received 26 injections of spirillar blood. This mixture in a dose of 0.002 c.c. was almost sufficient to protect against a simultaneous injection of 0.1 c.c. of spirillar blood. The rat, 7/90, which received this injection showed only two very sluggish spirilla 48 hours after inoculation, and only one per field on the following day, after which they disappeared. Inasmuch as 0.003 c.c. protected perfectly, the blood clearly contained from 330 to 500 units per cubic centimeter. The curative value of

this mixture was not determined in rats, but it probably was about the same as in other tests where immune blood resulting from 26 injections had been used. In such trials from 0.5 to 1.0 c.c. (250–500 units) was usually sufficient to cure, in one hour or less, a rat weighing 100 to 150 g.

Macacus rhesus, No. 1.—This was a small female and weighed but 1,670 g. On account of her small size she was selected for the first cure experiment. Since, as pointed out above, rats which weighed from 100 to 150 g. were cured by 0.5 c.c. of immune blood containing 250 units it follows that from 1.7 to 2.5 units per gram body-weight represent the minimum amount needed to effect a prompt cure. It was therefore decided to give the monkey five units per gram of body-weight or a total of 8,350 units, which amount was contained in 16.7 c.c. of the immune blood. With the object of increasing the severity of the test, and at the same time approximating the conditions in treatment of man, the inoculations of the monkeys were made subcutaneously and not intraperitoneally as had always been done with rats. The fresh spirillar blood, 2/90, used for the inoculation, contained about 40 per field and the dose was 0.25 c.c.

On the day following the injection no spirilla could be detected in the blood of the monkey. They were present on the second day, about one-half per field. On the third day the number had increased from one to five per field. The spirilla probably had not as yet reached their maximum number, since only 24 hours had elapsed from the beginning of the attack, but as the duration of an attack, according to Norris and his co-workers, is from two to four days it was decided to proceed with the treatment. The monkey was therefore given subcutaneously 16.7 c.c. of the immune blood which amount as shown above represents five units per gram body-weight. Owing to the quantity of the fluid, the injections were made in several places so as to favor rapid absorption.

The first examination was made exactly one hour after the injection of the immune blood. *It showed a total absence of spirilla.* Not a single spirillum alive or dead could be detected in the fresh blood and no evidence of the organism could be obtained from stained preparations. The monkey was then examined every hour, as shown in the table, and to insure accuracy, at each interval four fresh blood prepara-

tions were examined (two by each one of us). Stained preparations were also made at these hourly intervals, but neither the quadruplicate examinations of the fresh blood or of the stained specimens revealed a single spirillum. Daily examinations made in dublicate during the next three weeks failed to discover any evidence of spirilla. A relapse, however, occurred on May 6 when 10 spirilla were found. The temperature, it will be seen, remained about normal.

On the day following the injection the monkey showed a diarrhea which persisted for two days. At the same time an urticaria-like rash or blotches appeared on the eyelids and especially at the outer and inner canthi. Two day later the rash had perceptibly subsided; no other effect of the treatment was noted.

The temperature, it will be noted, dropped promptly after the administration of the immune blood. It did not reach the normal level for some days. This undoubtedly was due at first to the blood injected, for a slight febrile condition may be expected to follow injections of whole blood, especially when given in large doses, as in this case, where the fluid introduced corresponded to 1 per cent of the body-weight.

As seen from Table 1 a rise in temperature occurred on the fourth day of the cure. This at first was interpreted as indicative of a relapse and although no spirilla could be found it was decided to give the monkey a preventive inoculation. Accordingly 1 c.c. of the immune blood was given subcutaneously. The persistence of the fever and the absence of spirilla led to a suspicion of sepsis. Owing to the large quantity of corpuscles injected, large nodules had formed at the several places and one of these masses was found to be infected. The abscess was opened on the fifth and again on the sixth day. The temperature promptly dropped to normal and has remained so for the remainder of the period of observation.

The number of leucocytes before the infection of the curative dose was about one per field. During the next three hours following the injection it had increased to about two to five per field, and this condition persisted for three or four hours after which the number became normal.

This experiment, therefore, shows that an attack of 24 hours' duration in a monkey can be cured by a subcutaneous injection of immune

blood. The evidence of the curative result is seen in the disappearance of the spirilla from the blood in less than one hour; in the prompt fall of temperature, and in the mildness of a very late relapse.

Macacus rhesus No. 2.—This was a female which weighed 2,910 grams. It was used to demonstrate the preventive properties of the immune blood. For this purpose it was given a subcutaneous injection of 0.25 c.c. of the spirillar blood, 2/90 (40 per field). Following this it was given, likewise subcutaneously, but on the opposite side of the body, an injection of 0.35 c.c. of the same immune blood as used for the above experiment.

The calculated dose of this immune blood, on the basis of 0.003 c.c. per 100 g. of body-weight, was 0.087 c.c. That is to say, since one unit is necessary for every 100 g. of body-weight, the monkey would therefore require 29.1 units as the minimum to afford protection against a simultaneous injection. Four times the calculated minimum dose, that is 0.35 c.c., was given, in order to insure a definite and positive result. This amount, it will be seen, contains 116 units on the 0.003 c.c. basis, or 175 on the 0.002 c.c. basis. The former corresponds to four, and the latter to six, units per 100 grams body-weight.

The test, as carried out, was perhaps more severe than usual, owing to the separate and subcutaneous injection of spirillar and immune bloods. In the previous work on rats and mice the spirillar and immune bloods were mixed together, and hence were injected simultaneously. As pointed out heretofore, this mixing of the two fluids *in vitro* may weaken and kill many of the spirilla. In the experiment as carried out, this injurious action was avoided, and consequently it may be regarded as a severe test of the efficiency of the preventive treatment.

Macacus No. 1, served as a control for this experiment and, as shown above, it developed spirilla in its blood 48 hours after the injection, and these persisted until they were destroyed by the curative injection.

The blood of *Macacus No. 2* was most carefully examined in duplicate (two observers) on each day after the injections were made. No spirilla were found on the first, second, third, and fourth days. *On the fifth day only one spirillum was found* after prolonged search, but none on the sixth day. On the seventh day the spirilla reappeared

and were fairly numerous, that is to say, they were about $\frac{1}{10}$ per field. The dose of immune blood administered clearly prevented the first attack, or at all events, delayed it for some days. It was insufficient, however, to ward off the retarded appearance or relapse of the disease.

It was decided to test the effect of a small curative dose of immune blood on this relapse. The monkey was accordingly given at 3 P. M. a subcutaneous injection of 7.3 c.c. of immune blood of two rats (result of 26 injections). This dose represents about 0.25 per cent of the body-weight, or more accurately, 1.25 immunity units per gram body-weight. In view of the fact, pointed out on p. 359, that 1.7 to 2.5 units per gram represent the minimum amount needed to effect a prompt cure, it is evident that the dose given in this case was too small to effect a prompt destruction of the spirilla. This actually proved to be the case. It will also be seen that this amount of immune blood was not sufficient to prevent the occurrence of a relapse.

As indicated in Table 2, at 9:30 A. M. the spirilla were about $\frac{1}{10}$ per field and the temperature was 40.6°. At 3 P. M., at the time the inoculation was made, the spirilla had all but disappeared, none being found in the fresh blood and but very few in the stained preparations, and the temperature had fallen to 40.2°. As this change really occurred before the injection was made it can in no wise be ascribed to the immune blood. When re-examined at 5:30 P. M., no spirilla could be found and the temperature had dropped to 39.6°. On the next morning, *although the temperature was normal*, 38.9°, five active spirilla were detected in the blood. The daily examinations, in duplicate, during the following 10 days proved negative. On the 14th day after the first appearance of the spirilla in the blood a severe relapse occurred. This is seen in the large number of spirilla (10 per field), although the temperature was not very unusual. From the table it will be seen that on the seventh and eighth days after the first appearance of the spirilla there was a marked rise in temperature, which might be regarded as indicative of an abortive relapse. At all events no spirilla could be detected in the fresh blood or in stained preparations. On these two days, it so happened that the temperature was taken in the afternoon and the elevation was regarded at the time as due to the tubercular condition.

A study of Table 2 will show that the immune blood has exerted

considerable preventive action. This is seen in the absence of a first attack, the mildness of the first relapse (April 11–14), and the absence of a second relapse on April 18–19. During the following week the anti-bodies apparently decreased considerably, for the relapse on the 25th was marked. In fact, the spirilla were more numerous than at any previous time.

The high daily temperature, especially that of the afternoon, together with a persistent diarrhea and some cough indicates a probable tuberculosis.

TABLE 1.

CURATIVE EXPERIMENT.

Macacus rhesus No. 1, Female, 1,670 Grams.

Date of Examination	Spirilla	Temperature	
April 6	..	....	Received 0.25 c.c. of spirillar blood subcutaneously
" 7	0	....	
" 8	⅛ p. f.	....	
" 9, 10:00	5 p. f.	41.0°	At 12:15 given s.c. injection of 16.7 c.c. of immune blood (5 units per gram body-weight)
1:15	0	40.2	Increased leucocytosis
2:15	0	40.2	" "
3:15	0	39.9	" "
4:15	0	39.7	Decreased "
5:15	0	39.5	Normal "
" 10	0	39.7	Diarrhea and rash
" 11	0	39.5	" " "
" 12	0	39.6	" absent, rash less
" 13 9:30	0	40.2	" slight
3:00	0	40.6	Injected s.c 1 c.c.of immune blood. Abortive relapse
5:30	0	40.5	
" 14	0	40.4	Abscess opened
" 15	0	40.05	" reopened. Slight diarrhea
" 16	0	39.3	No diarrhea
" 17	0	38.6	
" 18 P. M.	0	39.0	
" 19 "	0	39.0	
" 20	0	38.3	
" 21	0	38.3	
" 22	0	38.0	
" 23	0	38.95	Hemokonia present in variable amount from day to day
" 24	0	38.6	
" 25	0	39.1	Hemokonia common
" 26	0	38.6	
" 27	0	38.8	
" 28	0	38.3	
" 29	0	39.1	Hemokonia
" 30	0	38.4	
May 1	0	38.8	
" 2	0	39.0	
" 3	0	38.35	
" 4	0	38.1	
" 5	0	38.4	
" 6	Ten found	38.7	
" 7	0	39.1	

This experiment clearly shows that an immune serum in a dose of four to six units per 100 grams of body-weight was sufficient to prevent a first attack but not the relapse which came on in the usual time.

Furthermore, a dose of 1.25 immunity units per gram body-weight is not sufficient to insure a prompt destruction of the spirilla in a relapse. Neither is it enough to prevent the further recurrence of relapses. It is probable that the latter dose if given in the beginning would have been sufficient to prevent, not only the first attack, but also the relapse, and a dose approximately of this strength, will be desirable as a prophylactic in man.

TABLE 2.

PREVENTIVE AND CURATIVE EXPERIMENT.

Macacus rhesus No. 2, Female, 2,910 Grams.

Date of Examination	Spirilla	Temperature	
April 6, A.M.	.....	.....	Received separate s.c. injections of 0.25 c.c. of spirillar blood and 0.35 c.c. of immune blood (4–6 units per 100 grams body-weight)
" 7	0	.....	
" 8	0	.....	
" 9	0	.....	
" 10	0	39.7°	
" 11	One found	39.6	Fifth day
" 12	0	39.5	
" 13 9.30.	1/5 p. f.	40.6	
3.00.	One found	40.2	Received 7.3 c.c. of immune blood (1.25 units per grams body-weight)
5.30.	0	39.6	
" 14	Five found	38.9	Diarrhea
" 15	0	40.25	No diarrhea
" 16	0	39.4	" "
" 17	9	39.1	" "
" 18 P.M.	0	41.2	Abortive relapse? or tuberculosis?
" 19 " "	0	40.6	" " "
" 20	0	39.2	Chills and diarrhea
" 21	0	38.85	" " "
" 22	0	38.0	Slight diarrhea
" 23	0	39.15	" "
" 24	0	38.9	" "
" 25	10 p. f.	40.0	Diarrhea. Relapse on 14th day after first recognition of spirilla (April 11)
" 26	2 p. f.	41.1	
" 27	0	38.9	
" 28	0	40.7	Leucocytosis
" 29	0	39.15	
" 30	0	38.85	
May 1	0	38.2	
" 2	0	38.7	
" 3	0	38.9	
" 4	0	39.0	
" 5	0	38.8	
" 6	0	39.2	
" 7	0	39.1	

General considerations.—The foregoing facts clearly show that an attack of relapsing fever can be aborted or cured in rats, mice, and monkeys within a very short time, in an hour or less, provided a sufficiently active immune blood or serum is employed. The experiments on monkeys indicate that a single curative injection may not only cause the prompt disappearance of the spirilla but may practically prevent the occurrence of a relapse. When a relapse does

occur after treatment, as in mice and in the monkeys it is seemingly milder than would otherwise be the case. Moreover, it is obvious that such relapses can be cured as easily as, and perhaps more so than, the original attack.

The prophylactic experiments on the animals mentioned, also show clearly that it is possible, with a relatively small dose, to prevent an attack of the disease. They also show that this preventive action is of short duration, for unless the quantity of serum used be quite large, relapses are likely to occur, even in the absence of the original or first attack, which may be looked upon as having been aborted by the prophylactic injection.

The prevention of relapses, it has been shown, can be accomplished by the injection of a single large *curative* dose which not only will destroy most of the spirilla at once, but also will remain long enough in the body to prevent the growth of any surviving organisms. As seen from the experiment with Monkey No. 1, a dose of five immunity units per gram of body-weight is sufficient to cure and prevent the subsequent relapse for a period of 27 days.

It may be possible to prevent a relapse by the injection of one or two small curative doses of immune serum (about one unit per gram of body-weight) two or three days before the relapse is due. In this way passive immunization may be brought about, and to such an extent, that the relapses can no longer occur.

Another possible procedure for the prevention of relapses consists in an active immunization of the animal during the apyretic stage. This can be done by injecting a large dose of spirillar blood immediately after recovery from an attack. The greater part, if not all, of the organisms thus introduced are at once destroyed, and the active immunity should be correspondingly increased. A day or two later a second injection of spirillar blood should be given still further to increase the immunity. This treatment may be repeated once or twice more before the close of the apyretic stage in order to obtain as great a resistance as possible. This procedure, it will be noticed, is essentially a vaccination with living virulent organisms and may be compared to the well-known anti-rabic treatment, in which progressive inoculations with a living virus are made previous to the appearance of the disease.

It will be seen that the methods of passive and active immunization can be combined into a prophylactic measure which will assure man a perfect and lasting protection against the disease. This vaccination will be as positive as that against small-pox. To secure this protection it will be necessary to receive, first, an injection of the immune serum (one to two units per gram of body-weight), and then, on the second and fourth days injections of spirillar blood. The active immunity which is thus induced in experimental animals is known to persist for several months, and without doubt it could be increased so as to last for a year or more.

A combined curative and preventive treatment will undoubtedly be found to be efficacious in the human disease. If only rat serum is employed, at most a very unsatisfactory procedure on account of the smallness of the animal, the quantity would hardly be sufficient to cure an attack. Thus, the dose for a 75 kg. man, corresponding to that used for Monkey No. 1, would be 750 c.c. or about 375 c.c. of serum. While the use of so large an amount of fluid is out of question it should be remembered that the strength of the serum used by no means represents the maximum obtainable. It is quite certain that one can be obtained which will be 10 times as strong, and with such the dose would be but 37.5 c.c., a quantity which is easily tolerated. On the two-unit basis the dose of such a serum would be but 15 c.c. Even stronger sera than this are possible, which, of course, would still further decrease the dose needed to cure an attack and at the same time protect against a relapse. For this purpose, as shown heretofore, at least five units per gram of body-weight are necessary.

The dose as mentioned is sufficient to cause the almost immediate disappearance of the organisms from the circulation. As rapid a result as this may not be desirable in the treatment of man, owing to the possible danger of thrombosis due to the agglutination of large numbers of spirilla. Smaller doses, repeated every hour or two, will probably be preferable to the use of a single large dose. The injection of about two units per gram of body-weight, repeated once or twice in the course of the day will probably offer the safest procedure.

The preventive dose for a 75 kg. man, corresponding to that

given Monkey No. 2, would be 9.0 c.c. of blood or about 4.5 c.c. of serum (six units per 100 grams of body-weight). Obviously, with a more active serum this dose can be materially decreased, but even as it stands it compares favorably with the quantity of diphtheria antitoxin administered for preventive purposes. The protection acquired by such a dose is of but short duration.

For the present it will be necessary to resort to the immunization of horses by injecting the spirillar blood of rats. When the artificial cultivation of *Sp. Obermeieri* is once accomplished it will be possible to immunize such animals with ease and to obtain large quantities of an extraordinarily active serum. The use of the serum or immunized rats is hardly practicable, though it may be found useful in localities where the disease is prevalent.

The experimental work here given will, without doubt, serve also as a basis for the serotherapy of tick fever which, notwithstanding its clinical resemblance to relapsing fever, must be considered as etiologically distinct from the latter.

AGGLUTINATION.

The presence of specific agglutinins can be readily demonstrated in decline as well as in recovered blood. When, for example, the decline blood which is still rich in spirilla is drawn, defibrinated, and placed in a test tube, the corpuscles soon settle, leaving a clear serum above. In the course of 12 to 24 hours a whitish film or patches appear above the layer of corpuscles. When one of these patches or islands is taken up with a finely drawn-out tube it will be found to consist of enormous masses or agglutinations which may fill several fields of the 2 mm. objective. The border of each mass is fringed with radially arranged spirilla. The smaller groups of 50 to 200 cells form perfect radiating rosettes.

At first the spirilla exhibit a very active motion, and while in this state the appearance of the rosette is extremely interesting. Gradually, however, the motion decreases, and eventually all the spirilla become immobilized, and not infrequently it will be found that the spirilla have completely died out in 24 hours. In such blood, it may be added, there is no evidence of granulation.

In onset blood, under similar conditions, the spirilla remain single or nearly so, and, being specifically lighter, they form a light-colored deposit above the surface of the corpuscles. No patches or islands form, thus indicating the absence of an agglutinating action.

The blood of a recovered rat may be expected to have a more intense agglutinating action than in the decline stage. That such is the case can be readily seen from the following experiment. The blood of Rat 3/77 was drawn 12 hours after the disappearance of the spirilla. Two drops of this blood were mixed with a like amount of fresh spirillar blood, the latter containing about 40 spirilla per field. The mixture, examined at once, showed the very long compound spirals of 50 μ or more in length, also agglutination groups of 3 to 20 cells. At the end of five minutes not a single free spirillum could be found. They had all gathered up into tangles of 10 to 50 very actively motile cells. At the close of 20 minutes, many of the spirilla were dead and granular débris was noted. No living organisms were present at the end of 50 minutes, at room temperature. In a similar mixture placed at 37°, the spirilla at the end of 30 minutes were all found to be dead and agglutinated into groups of 10 to 100 cells. In the control blood the spirilla remained single and active.

Hyperimmunized blood.—When highly immunized blood is added to spirillar blood in the proportion of 1:100 agglutination occurs almost instantly, as will be seen on reference to the data presented on p. 333. With such blood, however, agglutination can be brought about in much greater dilutions. Thus, in one experiment the following three mixtures were used:

	No. 1 1–1,000	No. 2 1–2,000	Control
Normal rat blood	0.8 c.c.	1.8 c.c.	0.45 c.c.
Dilute immune blood (0.1. cc.=0.001 c.c. original blood)	0.1	0.1	....
Spirillar blood	0.1	0.1	0.05

Experiment 1.—With 1:1,000 mixture. This, when examined one minute later, showed hyperactive spirilla, also groups of three to four cells; five minutes later showed very long compound spirals, also loose tangles of 5 to 20 cells, likewise perfect rosettes; 20 minutes later showed in every field one to two small rosettes of five to 50 cells, all very active.

Experiment 2.—In this the 1:2,000 mixture was used. When examined one minute later showed hyperactive single and double spirals; five minutes later showed very long compound spirals, also loose tangles of 5 to 10 cells; 20 minutes later showed rosettes of 50 or more cells as in Experiment 1.

The control mixture even at the end of 30 minutes showed only single spirilla.

The foregoing tests merely demonstrate that the agglutinating action occurs *in vitro*. That a similar reaction may, and does occur *in vivo* can be easily shown. For this purpose all that is necessary is to inject a sufficient amount of an immune blood into a rat. Depending upon the amount injected, agglutination groups consisting of 10 to 100 or more cells promptly appear within a few minutes. If the amount of blood is insufficient to effect a cure these groups will persist for a greater or less length of time, two or three hours. The motion of the spirilla in such groups becomes sluggish and may cease altogether, so that it is not unusual to find groups of apparently dead spirilla.

An admirable demonstration of agglutination within the living body is seen when recovered, hyperimmunized, or passively immunized rats are given an intraperitoneal injection of rich spirillar blood. The results of such experiments are given under Pfeiffer's phenomenon.

It is worthy of note that irregular tangles of spirilla, living or dead, are also met with in the last stages of infection. These are not very common, and the perfect rosettes are only met with after the injection of immune blood or when decline blood is allowed to stand *in vitro* for some hours.

The agglutination phenomenon appears to begin with the union of two cells by means of their flagella. The cells may be brought close together forming a continuous long spiral or they may remain separated by the length of the whip (see Plate 9). Other cells may then attach themselves in like manner and thus give rise to a very long compound spiral. These may measure from 50 to 100 μ in length, and hence may consist of 10 or more cells. The tendency to form these long spirals is seen best when the amount of the agglutinins is small. When these are present in increased amount, either loose tangles composed of irregularly grouped cells or perfect, radiating rosettes result. It is probable that in the latter the flagella are directed centrally. Incidentally, it may be stated that rosette formation among bacteria has been described by Hefferan.

IDENTIFICATION OF SPIRILLA.

The question of the identity of the spirilla found in relapsing fever in different parts of the world is one that demands attention. In the absence of a cultivation method there remain but two other procedures, namely, animal inoculations and serum reactions. The animal reactions, particularly of rats and mice, can be made in almost any locality. Moreover, as shown heretofore, the viability of the spirilla in onset blood is such that the latter can be shipped to distant parts for further study and comparisons. Such onset blood, as has been shown in this instance, may contain infective spirilla for almost 40 days. It should be drawn under aseptic conditions and placed in a sterile test tube or in glass tubing which is then sealed in the flame. Presumably the blood or serum of hyperimmunized animals retains its specific properties for months and hence can be sent almost anywhere. Such a procedure is advisable when the living organism does not remain alive *in vitro* for any length of time.

The serum reactions which may be used for the purpose of identifying a spirillum will include the agglutinating, germicidal, and immunizing properties.

The agglutinating and the germicidal actions can be observed at the same time. The immune blood of known origin can be mixed in a watch-glass with a like amount of spirillar blood derived from man or an animal. Prompt agglutination and immobilization may be taken as proof of the identity of the organisms, especially when this occurs with dilute immune blood. This dilution can be made with normal blood or serum. Although we have made no experiments on this point, it is probable that killed spirilla may be employed for these tests in a manner analogous to the use of dead typhoid bacilli in the corresponding Widal test. Obviously onset blood must be used for such trials to avoid the auto-agglutination which otherwise is likely to occur.

The above tests which are made *in vitro* can be supplemented by a Pfeiffer's reaction. For this purpose the immune blood mixed with spirillar blood, is injected into the peritoneal cavity of a rat and from time to time some of the liquid can be removed by means of a capillary tube and examined for agglutinations and

dead spirilla. For final and complete identification it will be desirable to test the preventive and curative action of the known immune blood against the suspected spirillum. It is clear that the serum reactions are of the utmost importance in the identification of the pathogenic spirilla, and the suggestions here outlined are for the purpose of stimulating inquiry into the subject.

DIAGNOSIS OF RELAPSING FEVER.

The detection of spirilla in the blood is an easy matter when they are fairly numerous. At other times it requires a very careful search and a trained eve. This is particularly true when a living preparation is examined. The great motility of the organism and its extreme thinness renders it difficult of perception. Their presence is very often indicated by the sudden start of a corpuscle as a result of collision. The Welsbach light is much preferable to electric illumination or even to day-light, and in our work we have used it almost exclusively.

When very few spirilla are present it may be possible to detect them by a process of "enrichment." For this purpose the defibrinated blood is placed in a sterile tube and set aside for a day or two. The spirilla being lighter settle in a layer just above the corpuscles. .With a capillary tube this layer can be withdrawn and examined. All the spirilla are thus concentrated in a small volume, and hence can be more readily detected. Centrifugation may be employed to accomplish this same result.

The staining of the spirilla is easily effected with any of the modifications of the Romanowsky method. The MacNeal method, in which pure Bernthsen insoluble methylene violet is used, gives an exceedingly heavy stain in a couple of minutes. The intensity is such that the organisms can be seen even with a No. 3 objective. Several of the photographs accompanying this paper are of preparations stained by this method.

The inoculation of rats, mice, and perhaps monkeys should be resorted to in order to secure material which will serve for a more complete identification of the organism. The blood used for this purpose should be drawn before crisis has taken place, owing to the

possibility of the immune bodies being present in the later stages in sufficient amount to prevent infection. The failure of some experiments in the past may possibly be due to the use of such blood.

The serum diagnosis may be resorted to, especially in cases which have recovered from the first attack or from a relapse. The blood should be drawn immediately, or shortly after an attack, for at that time it is richest in agglutinating, germicidal, and immune bodies. These may be notably decreased in amount just before the onset of a new attack and for that reason the blood should not be used at that stage. The test for agglutinating and germicidal agents can be carried out as given above. The immunizing action of the suspected blood can be tested in like manner, but for this purpose a relatively large dose of the blood must be used. At least 5 c.c. of such blood should be injected into a rat about 24 hours previous to the inoculation with the known spirillum. If the preventive action is to be tested on a monkey a much larger amount, about 50 c.c., should be used.

It will be seen that the serum diagnosis of the disease hinges upon tests with living spirilla which at present can only be maintaned in a living animal. When artificial cultures are realized the diagnosis will be as easy as in the case of typhoid fever.

FILTRATION EXPERIMENTS.

The filtration of spirillar blood was suggested by previous experiments with *Tr. Lewisi* and *Tr. Brucei.* In the cultures of the former Novy and MacNeal noted very small forms, from 2 to 5μ in length, and on filtering such material, previously diluted with salt solution, through a small Berkefeld filter under a pressure of five pounds, a perfectly clear liquid was obtained, which when injected into white rats caused a typical infection with *Tr. Lewisi.* Of nine experiments made with *Tr. Lewisi* three were positive, five were negative, and one was uncertain, since the control animal failed to develop an infection. Similar experiments with cultures of *Tr. Brucei* and with suspensions of the organs of animals infected with the latter gave negative results.

It will be shown in the following experiments that the spirilla pass quite readily through the small Berkefeld filter, especially under

the conditions which obtained. Owing to the importance of these experiments it is deemed best to give the details as fully as possible.

The spirillar blood was drawn from the rats two days after inoculation, at which time it was rich in very actively motile organisms. A new infected rat and a new filter were employed for each experiment. The defibrinated rat blood (2 c.c.) was diluted with 20 c.c. of a salt-citrate solution (0.5 per cent each), and this mixture was at once placed in the small brass cylinder which held the Berkefeld bougie. The cylinder was then closed with a screw cap which was connected with a tank containing air under a pressure of 50 pounds. The lower end of the bougie was connected by means of a short rubber tube, provided with a Hoffmann screw clamp, to a sterile receiver. This clamp was screwed tight before the liquid was poured into the cylinder and it was not opened until one or two minutes after the compressed air had been turned on. A clear reddish liquid slightly discolored by dissolved hemogoblin, instantly came through. The pressure was allowed to remain on for about three minutes, or until it had dropped to about 40 pounds.

The filtrate of each experiment was injected at once into three rats. At the same time it was examined in duplicate for corpuscles and for spirilla. In all cases these examinations were negative. The cylinder was then opened and a drop of the fluid on the filter was examined. This always contained an abundance of living, very actively motile spirilla.

The small Berkefeld bougie was used in this work. The filter is 35 mm. long and 15.5 mm. in diameter. As the central canal is 7 mm. in diameter this leaves about 4.2 mm. for the thickness of the wall. As stated above, a new bougie was used for each experiment. For the first five experiments the bougies were carefully shaved with with sandpaper. That used for Experiment 1 was reduced to a diameter of 9.7 mm., which therefore left the wall with a thickness of only 1.4 mm. In the next four tests, the bougie was reduced to only 12 mm., leaving the wall about 2.5 mm. thick or a trifle over one-half the original thickness. In the last five experiments unshaved filters were used. All the filters before use were placed in distilled water and sterilized in an autoclav. The total duration of an experiment, from the time the mixture was made until the rats were injected, averaged about 10 minutes.

The results of these experiments are brought together in the accompanying table:

TABLE 3.

RESULTS OF FILTRATION.

	c.c. Filtrate Injected	No. of Spirilla Found and Day	Spirilla Absent on	
Exp. 1, Bougie 9.7 mm.—				
Rat 1	5	+1 3d.	4d.	Immunity lasts 66 days
Rat 2	5	+4 2d.	3d.	
Rat 3	4	+2 3d.	4d.	
Exp. 2, Bougie 12 mm.—				
Rat 1	5	+2 3d.	4d.	Immunity lasts 108 days
Rat 2	5	+2 3d.	4d.	" " " "
Rat 3	7	+1 3d.	4d.	" " " "
Exp. 3, Bougie 12 mm.—				
Rat 1	5	+1 2d.	3d.	
Rat 2	5	+1 2d.	3d.	
Rat 3	5	+1 2d.	3d.	
Exp. 4, Bougie 12 mm.—				
Rat 1	5	+2 2d.	3d.	
Rat 2	5	+2 2d.	3d.	
Rat 3	10	+2 2d.	3d.	
Exp. 5, Bougie 12 mm.—				
Rat 1	5	+2 2d.	3d.	
Rat 2	5	+2 2d.	3d.	
Rat 3	7	+2 2d.	3d.	
Exp. 6, Bougie 15 mm.—				Reinoculated 5 days later with 0.25 c.c.
Rat 1	5	0		=+3d. ½ per field. Absent 4d.
Rat 2	5	0		=+3d. ⅓ " " " "
Rat 3	7	0		=0
Exp. 7, Bougie 15 mm.—				Reinoculated 4 days later with 0.25 c.c.
Rat 1	5	0		=3d. +¼ per field Absent 4d.
Rat 2	10	0		=+3d. 1 " " " "
Rat 3	5	0		=+3d. 1/10 " " " "
Exp. 8, Bougie 15 mm.—				Reinoculated 4 days later with 0.25 c.c.
Rat 1	5	0		=+4d. 2 spirilla
Rat 2	5	0		=+4d. 2 "
Rat 3	10	0		=+4d. 3 "
Exp. 9, Bougie 15 mm.—				Reinoculated 4 days later with 0.25 c.c.
Rat 1	5	+1 3d.		=0
Rat 2	5	0		=0
Rat 3	7	0		=+2d. 1 spirillum
Exp. 10, Bougie 15 mm.—				
Rat 1	5	+1 2d.	4d.	
Rat 2	5	+2 2d.	3d.	
Rat 3	8	+5 2d.	4d.	

A study of the foregoing table shows that, under the conditions of the experiment, spirilla passed through all of the shaved filters. It is noteworthy that the organisms did not appear in the rats until on the second and even third day after inoculation, and that none were present on the following day. The number of spirilla in the infected rats was very small, usually but one or two being found in the whole specimen. Fresh blood preparations were examined in this work. This slight infection is clearly due to the passage of immune bodies through the filter. The duration of the immunity, notwithstanding the slight infection, was quite considerable since, as shown, the rats resisted infection 66 and 108 days respectively.

The experiments with the unshaved filter are not as clean cut as the preceding, owing to the thicker walls. They show, however, an infection of four out of 15 rats employed in the set. Of the 11 rats which showed no spirilla in their blood, on subsequent inoculation with a large dose of spirillar blood two did not become infected, in other words they were immune, which indicates that these were probably infected by the filtrate, but that, owing to the small number of organisms, these were overlooked. This would indicate that six out of the 15 rats, if not more, had become infected by the filtrates.

Of the nine which became reinfected on subsequent reinoculation one showed the spirilla on the second day, five showed them on the third day, and three on the fourth day. The number of spirilla found in each specimen was small, usually about two or three, and none were found on the following day. In control rats inoculated with the same material the organisms invariably appeared on the second day and were numerous, 10 to 20 per field.

Here again, it is evident that an immune body passed through the filter, and with this fact in mind it will readily be seen that a small number of spirilla may have traversed at the same time, but owing to the presence of the immune body they were unable to multiply. That such inhibition exists is actually seen in Experiments 9 and 10, where in the three positive cases the spirilla were very scarce. Owing to the extreme difficulty in detecting the spirilla when present in such small numbers it is quite probable that they were present in some of the other rats.

The conclusion to be drawn from these observations is that, under the conditions of the experiment, spirilla can pass through the filter, but that, owing to the simultaneous passage of an immune body, they are unable to multiply or do so only to a very limited extent. Hence, the failure to infect a rat with the filtrate does not necessarily mean that the spirilla were held back by the filter.

Furthermore, in view of the fact that *Tr. Lewisi* and *Sp. Obermeieri* do pass through a bougie, it follows that the filtration test is not a proof, as it is usually regarded, of the ultramicroscopic size of the organisms which traverse such filters. Another example of an organism which can pass through a filter is the *Micromonas Mesnili* of Borrel, who also has noted the interesting fact that spiril-

lar forms, resembling but shorter than *Sp. Obermeieri*, are present in tap-water and also traverse the filter. These organisms multiply in the filtrate at 20° but not in broth at 37°.

TICK FEVER.

The demonstration by Ross and Milne, Dutton and Todd, Koch, and others that the tick fever of Africa is due to a spirillum raises the question of the identity of this organism with that of *Sp. Obermeieri*. In our preliminary note of January 13, we pointed out the probability that tick and relapsing fevers were two distinct diseases, due to different species of spirilla.

This view was based upon the behavior of the two spirilla in experimental animals. Thus in rats the organisms of the former were found by Dutton and Todd to persist in the blood for from three to nine days after inoculation, a condition quite different from that noted with our spirillum. Moreover, they showed that the spirillum of tick fever was usually fatal to monkeys. Thus in five out of seven monkeys death was clearly due to the infection produced by the ticks, while in the remaining two it may have been due to other causes. In the infected monkeys, the spirilla were constantly present or nearly so. In these respects the course of tick fever in monkeys is quite different from that of relapsing fever, where recovery is the rule, and, moreover, the spirilla persist for but a few days although relapses may occur. In a young rabbit the tick-fever spirilla were present for 10 days after inoculation, but in an adult none could be found. In a guinea-pig spirilla were found for two days after inoculation. In our own experiments with *Sp. Obermeieri* we were unable to infect two young rabbits by intraperitoneal inoculation. Norris, Pappenheimer, and Flournoy, however, on intravenous injection were able to find spirilla in the blood for one and two days respectively in two rabbits, while in two others none could be found. Similarly in guinea-pigs we have not been able to find spirilla in the blood for more than one day after inoculation, whereas the experiments of Norris and his co-workers with these animals were negative. The natural immunity of the guinea-pig to *Sp. Obermeieri* was well established by the work of Sawtschenko and Melkich.

It will be seen, therefore, that the tick-fever spirillum differs

markedly from that of relapsing fever in its effects on monkeys, rats, rabbits, and guinea-pigs.

Through the courtesy of Dr. J. L. Todd of the Liverpool School of Tropical Medicine we received some slides of the tick-fever spirillum, made from the blood of rats and monkeys. A comparison of these preparations with the corresponding ones of *Sp. Obermeieri* show conclusively that the two organisms are entirely distinct. In view of these facts we have reached the definite conclusion that, notwithstanding the clinical similarity, the two diseases are etiologically different. Accordingly, as mentioned in the beginning of this paper, we propose to designate the organism of tick fever as *Spirillum Duttoni.*

It will be seen that the view that these diseases are distinct entities is not in accord with that of Dr. Koch. Although he points out that tick fever is characterized by shorter attacks (less than three days) than the European relapsing fever; that the spirilla in the blood of the former are exceedingly scanty as compared with the number found in the latter, he concludes that the diseases are not different, and that at most one can speak of an African variety of relapsing fever.

The morphological characteristics and the effects on animals offer at present the only evidence of the distinctness of these organisms. They are, however, sufficient to justify the conlusion reached and a belief that a full confirmation will be afforded when cross-experiments are made with the sera of animals immunized against these two spirilla.

In their preliminary note published March 10, Breinl and Kinghorn present important evidence bearing upon the individuality of tick fever. They show that the organism multiplies enormously in rats, and may produce death, the duration of the disease varying between one and 45 days; three or four relapses were noted before the rats died. Of six mice inoculated four died on the following day and the remaining two after 48 hours. *Sp. Obermeieri* is certainly not fatal to mice and rats, and in the latter the infection is short and without relapses.

Guinea-pigs showed only a temporary presence of spirilla, possibly due to a survival of the injected organisms. The injection of large

quantities of spirillar blood into rabbits caused death in from 3 to 10 days. In a dog a large dose caused a temporary infection, the spirilla being present for three days. A similar result was obtained with a pony. In one monkey inoculated with 1 c.c. of spirillar blood the organisms appeared within 24 hours, but disappeared after three days; subsequent relapses were noted.

As a result of these experiments on animals Breinl and Kinghorn reach the conclusion, the same as the one which we published two months before, that the two spirilla are different.

Morphologically, as stated, a very great difference exists between our *Sp. Obermeieri* and the spirillum of tick fever. The single individual or cell of *Sp. Duttoni* measures 16 μ, and is hence about twice as long as that of *Sp. Obermeieri.* Similarly the pair in process of division or agglutination measures 32 μ in the case of *Sp. Duttoni,* while it is 16 μ long in the case of *Sp. Obermeieri.* Obviously in taking measurements of spirilla, care must be taken to discriminate between a single cell and an agglutination or compound spiral consisting of 2 to 10 cells. The ordinary statement that *Sp. Obermeieri* varies from 10 to 40 μ and more in length gives the erroneous impression that such measurements refer to a single cell,which is far from being the case. The illustrations accompanying this paper will show spirals, single and in pairs, also a composite consisting of many cells. Forms such as the latter may be found to measure anywhere from 50 to 100 μ and more.

The number of turns is likewise given in an indefinite way, as ranging from 6 to 20, and for that reason such figures can hardly serve as a basis of comparison. It is evident that the number of turns stand in relation to the length of the cell, that is, whether it is single or elongated prior to or in the act of division. The short cell resulting from division is about 8 μ long and has but two or three turns, whereas the dividing form averages 16 μ in length and has four to six turns. Our photographs will permit verification of these figures.

The single cell of *Sp. Duttoni,* on the other hand, measures 16 μ in length, and, notwithstanding that it is twice as long, it has but two or three turns, the same number as the shorter *Sp. Obermeieri.* It follows that the distance between the turns in the former is from 4 to 5 μ, while in the latter it is but 1.5 μ.

A further important characteristic is afforded by the width of the turn or of the whole spiral. In the case of *Sp. Obermeieri* this width measures quite constantly 1.0 μ, whereas in *Sp. Duttoni* it amounts to 2.0 to 2.7 μ. This fact is referred to by Wellman when he says that the spirillum studied by him in West Africa "dies in rounder and more flowing curves than are generally described and pictured." His photograph, it may be added, is perfectly typical for *Sp. Duttoni* except in one regard, and that is the large number of spirilla which are shown in one field. Dr. Koch also speaks of the tick spirillum as appearing to be a trifle longer than the European spirillum. He regards the whiplike forms with long turns as the result of stretching incidental to the rapid drying of the very thin smears.

According to Dr. Koch, the spirillum of tick fever shows very little progressive motion, and hence remains at the same place for a long time. This certainly is not true for our organism, which moves about at great speed, especially in onset blood.

The number of spirilla which are present in the blood of man affords another distinction of importance. In the European and Asiatic relapsing fevers the spirilla are usually present in very large numbers, several being found in each field of the 2 mm. objective. In tick fever the spirilla are usually rare, and particularly is this true after the first attack. This scarcity of the spirilla was noted by the discoverers, Ross and Milne. In some films they found but two or three spirilla, while in one case, in which they mention the organisms as being "fairly numerous" the preparation showed but one spirillum in 30 fields.

Another feature to which attention should be called is the marked tendency of *Sp. Duttoni* to curl up thus giving rise to figure-8 forms or even perfect circles (Plate 12, Fig. 5). This tendency does not seem to exist in our spirillum.

A further distinction is afforded by the presence of several divisional zones in *Sp. Duttoni*, as a result of which this organism apparently yields a number of comma or S-shaped segments. This feature is shown in Plate 12, Fig. 4. Nothing of this kind can be observed in the *Sp. Obermeieri* which we have studied, but it should be noted that similar divisions are present in the spirillum of relapsing fever from Bombay.

The facts deduced above show conclusively that *Sp. Obermeieri* and *Sp. Duttoni* are two distinct organisms, and hence that the tick fever of Africa is distinct from the ordinary relapsing fever.*

BOMBAY RELAPSING FEVER.

The important question arises as to the identity of the spirillum studied by us with that described by Obermeier. The description given by him is necessarily meager and in only one respect is it possible to make a comparison. This is in the matter of length, which he gives as varying from the diameter of a corpuscle to 10 to 30 μ. As mentioned above, the length of our single cell, is 8 μ (about the diameter of a corpuscle, as can be seen in some of the photographs), while the pair is 16 to 18 μ long. The compound threads may, of course, be much longer. Obermeier gives no details as to the number of turns, and in the absence of illustrations it becomes difficult, if not impossible, to identify the species from his description.

We have been unable to make any direct comparison with European material, but through the kindness of Capt. W. S. Patton, I. M. S., we have received several blood smears from cases of relapsing fever occurring in Bombay.

A study of these slides revealed the interesting fact that the Bombay spirillum was quite different from our organism and that in some respects it resembled that of tick fever. It differs, however, from each of these to such an extent as to give rise to the belief that we are dealing with three distinct organisms. In other words, the evidence on hand points to the existence of three relapsing fevers in man. As to the existence of two of these there can be no doubt, but with regard to the third, that of Bombay, there may still be some question. In the absence of fresh material we are not in a position to settle this point, and such observations as we have made are here given with the object of directing attention to a possible plurality of the pathogenic spirilla. It becomes, therefore, very desirable to examine closely into the nature of the relapsing fever as met with in Russia, Asia, and Africa. It is not unlikely that a fair-sized group

*Since the writing of this paper one additional and clinching proof of the distinctness of these organisms has been supplied by Zettnow. In a short note of March 8 he shows that *Sp. Duttoni* has diffuse flagella. This observation effectually differentiates it from *Sp. Obermeieri*, which, as seen from our photographes has but one single terminal flagellum, and places it in the same group with *Sp. gallinarum*.

of spirillar infections will eventually be recognized. The necessity of resorting to the serum reactions, previously discussed, for the identification of spirilla will be readily seen.

The measurements of the Bombay spirillum, as seen from the subjoined table, agree fairly well with those of *Sp. Obermeieri.* There are, however, several notable differences as will be observed on comparing Plates 8 and 13. It will be seen that the Bombay spirillum is apparently thinner, that there is greater flexibility, a marked absence in the regularity of the turns, and considerable variation in their width. The agglutination into a long continuous spiral is not met with; the long forms, such as they are (Plate 13, Fig. 5), consist of several cells which are rather loosely apposed. This fact may be taken to indicate the presence of diffuse flagella which presumably would not permit the perfect union seen in *Sp. Obermeieri* (Plate 9, Fig. 3) which is due, as has been pointed out, to the presence of a single end flagellum.

Another striking feature is the tendency of the spirillum to form loops, either circular or figure-8 forms, similar to those of *Sp. Duttoni.* This is brought out clearly in the agglutination (Plate 13, Fig. 6) which, it will be seen, consists largely of looped spirals. This tendency is also seen in the curled ends which Fraenkel and Pfeiffer aptly compare to a "Schweineschwänzchen." We have never noticed this peculiarity in the organism studied by us.

A further and very important characteristic is that of multiple transverse division, much as in the case of *Sp. Duttoni.* The long spirals not only show a median clear space, but each half in turn may show a similar clear zone. The resulting segments or units, as shown in Plate 13, Fig. 3, have an elongated S-shape. In some instances (Plate 13, Fig. 4) evidence of further division is presented resulting in comma or vibrio forms. The presumption is that these transverse bands represent division but it should be stated that no free short forms of the vibrio or S-type have been found in the blood. It is of interest to note that Carter as early as 1877 described the Bombay spirilla as dividing in two or three parts.

SPIRILLUM GLOSSINAE, N. SP.

This organism was met with in smears of the stomach contents of two out of six tsetse flies (Nos. 6 and 8, *Glossina palpalis*) which were received from Lieutenant A. C. H. Gray of the Sleeping Sickness Commission. The spirilla were present in small numbers. They were found as single short forms, 8 μ in length, or as double cells which measure about 15 μ. It will be seen from the measurements given in the table, as well as from the photographs shown in Plate VII, that this organism is shorter and narrower and has more turns than *Sp. Obermeieri*. It will be noticed that the ends become indistinct and tapering. In some instances the spirals are drawn out into rather straight forms. In this species, as in the other spirilla studied, evidence of transverse division may be obtained (Plate 14, Fig. 4).

This occurrence of true spirilla in an insect is a matter of considerable interest. Up to the present time but three or four other instances are known. These are (1) *Sp. gallinarum* in the tick *Argas miniatus;* (2) *Sp. Duttoni* in the tick *Ornithodoros moubata;* and (3) *Sp. Obermeieri* in the common bed-bug *Acanthia lectularia*. Recently (February, 1906) the Sergents reported finding spirilla in a preparation, made in 1901, from the gut of a larva of *Anopheles maculipennis*. They were found in considerable number, at times were agglutinated, showed 1.5 to 4 turns and measured 8.5 to 17 μ. It is barely possible that this organism is the same as our *Sp. glossinae*.

TABLE 4.

MEASUREMENTS OF SPIRILLA.

	Length in μ	Width of Filament	Number of Turns	Distance of Turns	Width of Turn or of Spiral
Sp. Obermeieri—					
Single cell	8.0	0.25	2–3	1.5	1.0
Double cell	16–20	....	4–6	2.5	....
Sp. Bombay—					
Single cell	8.0	0.2	2–3	2–3	1.0–1.3
Double cell	16–20	....	4–5	....	....
Sp. Duttoni—					
Single cell	16	0.2	2.5	4–5	2.0–2.7
Double cell	30	....	5–6	....	....
Sp. Glossinae—					
Single cell	8.0	0.2	4.0	1.3	0.6
Double cell	15.0	....	....	....	....
Sp. pallida	13–15	0.2	11	1.3	0.6
Sp. gallinarum	10–12	0.25	4.0	2.0	1.0

The measurements given in this table are made from photographs magnified 1,500 or 3,000 times.

The *Spirochaete Ziemanni*, which Schaudinn reported as a stage in the life-history of the intracellular parasite *H. Ziemanni*, as has been shown, is not a spirillum but a trypanosome. In our paper on bird trypanosomes we questioned the relation of this trypanosome to the cytozoon of the owl. Since then we have shown that flagellate infection of mosquitoes is not an uncommon occurrence, and, in addition, we have obtained evidence which conclusively demonstrates that the organisms in question are in no wise related to *H. Ziemanni*.

SUMMARY.

The main results of this extended study can be condensed into the following statements.

1. *Spirillum Obermeieri* belongs to the bacteria and not to the protozoa.

2. In onset blood, kept *in vitro*, it may be maintained alive for 40 days, whereas in decline blood, owing to the presence of a germicidal agent, it rapidly dies out.

3. Man, monkeys, white mice, and rats, tame and wild, are subject to infection. The first three are subject to relapses, the latter are not.

4. All attempts at cultivation have proved unsuccessful.

5. A powerful specific germicidal body is present in decline and in recovered blood, notably in blood of hyperimmunized rats. This body does not originate after the blood is drawn, but exists within the living animal.

6. An immunizing body is also present and is probably distinct from the germicidal agent.

7. Pfeiffer's phenomenon can be demonstrated *in vitro* and *in vivo*. In the peritoneal cavity of hyperimmunized rats, the spirilla are killed almost instantly, after which they are taken up by the macrophages or large mononuclear cells.

8. Active immunity follows recovery from the infection. By successive injections of spirillar blood this immunity can be increased to a remarkable degree.

9. Passive immunity can be imparted by injections of recovered or hyperimmunized blood.

10. Both active and passive immunity may last for months.

11. Hereditary immunity can be obtained and is probably the result of infection *in utero*.

12. Preventive inoculations can be successfully made in rats, mice, and monkeys.

13. Infected rats, mice, and monkeys can be promptly cured by injection of hyperimmunized blood. Subsequent relapses, if any, can be prevented by curative doses of blood.

14. The preventive dose should be about 10 immunity units per 100 grams body-weight.

15. The curative dose is about five immunity units per gram body-weight.

16. A solid basis is thus established for the prevention and cure of relapsing fever in man.

17. Agglutination of spirilla occurs *in vitro* and *in vivo* under the influence of recovered or hyperimmunized blood. To a slight extent it occurs during crisis.

18. The agglutination, germicidal, and immunizing properties of recovered blood can be used in the sero-diagnosis of relapsing fever. Also for the identification of spirilla.

19. *Sp. Obermeieri* can be made to pass through a Berkefeld filter.

20. The tick fever of Africa is distinct from the relapsing fever of Europe. Its cause is *Sp. Duttoni*.

21. The spirillum of the relapsing fever of Bombay is apparently different from *Sp. Obermeieri* and *Sp. Duttoni*.

22. The evidence points to the existence of a group of relapsing fevers.

23. True spirilla occur in the stomach and intestines of insects.

24. The demonstration that *Sp. Obermeieri*, *Sp. Duttoni*, and *Sp. gallinarum* are bacteria and not protozoa means that many, if not all, of the other spirochetes belong to this same group.

25. The difference in number and arrangement of flagella may lead to a division into sub-genera.

26. The transmission of spirillar diseases by insects, and the congenital infection of mammals and eggs of insects are properties which up to the present have been regarded as characteristic of protozoa. These properties are now known for the first time to be shared by this group of bacteria. Yellow fever presents a marked analogy to the

spirillar infections and it is not improbable that the cause of this disease will be found to belong to this group of organisms.

Lastly, we desire to express our obligations to Dr. Charles Norris for the very great courtesy shown in placing the *Spirillum Obermeieri* at our disposal. Also we desire to acknowledge the kind assistance rendered by Dr. J. L. Todd, of Liverpool, Captain W. S. Patton, I. M. S., of Madras, Lieutenant A. C. H. Gray, of the Sleeping Sickness Commission, and by Dr. W. J. MacNeal. Our thanks are also due to the Board of Directors of the Rockefeller Institute for Medical Research for financial assistance rendered.

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EXPLANATION OF PLATES.

The accompanying microphotographs are reproduced at a magnification of 1,500 diameters, with the exception of a few which are magnified 3,000 times and are so indicated. The preparations are stained by the Romanowsky method, unless otherwise stated.

PLATE 8.

Spirillum Obermeieri in blood of rat, showing divisional forms.

FIG. 1.—A group showing spirilla of different lengths. The two long ones above show transverse division.

FIG. 2.—The dividing form in the upper part of preceding figure, reproduced at a magnification of 3,000×. Note the clear space in the middle of the pale central zone.

FIG. 3.—A small group of spirilla. The longer one shows a pale central portion indicating transverse division. Note the fading away of the tips.

FIG. 4.—A dividing form at a magnification of 3,000×. Note the pale central portion and pale free ends.

FIG. 5.—A dividing form showing same features as preceding.

PLATE 9.

Sp. Obermeieri in blood of rat, showing agglutination forms.

FIG. 1.—An agglutination of two spirals which might be mistaken for longitudinal division. Note the pale tips and granular stains. Magnification, 3,000×.

FIG. 2.—An irregular agglutination or tangle of many spirals. Preparation stained by MacNeal's method (Bernthsen's insoluble methylene violet).

FIG. 3.—A long spiral thread the result of agglutination by means of the flagellum.

FIG. 4.—Agglutination of two cells by means of the flagellum. Note the delicate flagellum connecting the two cells. Löffler's flagellar stain.

FIG. 5.—Same as the preceding but with longer connecting flagellar bridge.

PLATE 10.

FIGS. 1–4 are of *Sp. Obermeieri* in blood of rat. They show a long free flagellum at one end and a rudimentary one at the other. Löffler's stain. The relatively greater thickness of the cells, as compared with those shown in the other photographs, is due to the intense staining.

FIG. 1.—Magnification, 3,000×. Note the pale appendage at end opposite from flagellum.

FIG. 2.—Flagellum as long as the cell.

FIG. 3.—Magnification, 3,000×. Note that the flagellum seems to be given off from the side of the tip.

FIG. 4.—Cell showing flagellum similar to that in Fig. 2; a marked stub at the other end.

FIG. 5.—*Sp. gallinarum*. Agglutination group in blood of chicken.

PLATE 11.

Showing phagocytic destruction of *Sp. Obermeieri* in the peritoneal cavity of an hyperimmunized rat. 6/132. MacNeal's stain. Mononuclear macrophages.

FIG. 1.—Free agglutination group of spirals; also phagocyte with fringe of spirals, some of which are almost completely absorbed.

FIG. 2.—Phagocyte with tangle of spirilla.

FIG. 3.—Phagocyte showing comma-shaped remnants of spirilla within its plasma.

PLATE 8.

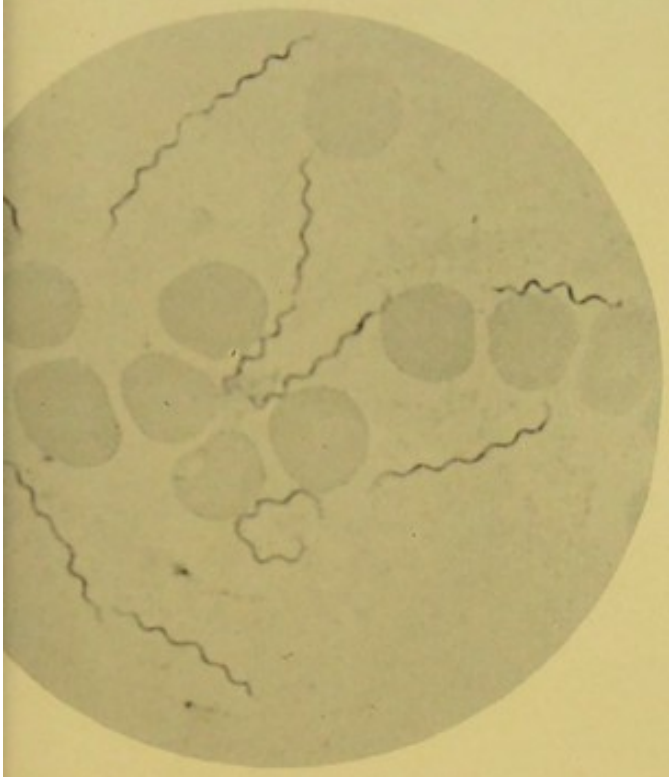


FIG. 1.

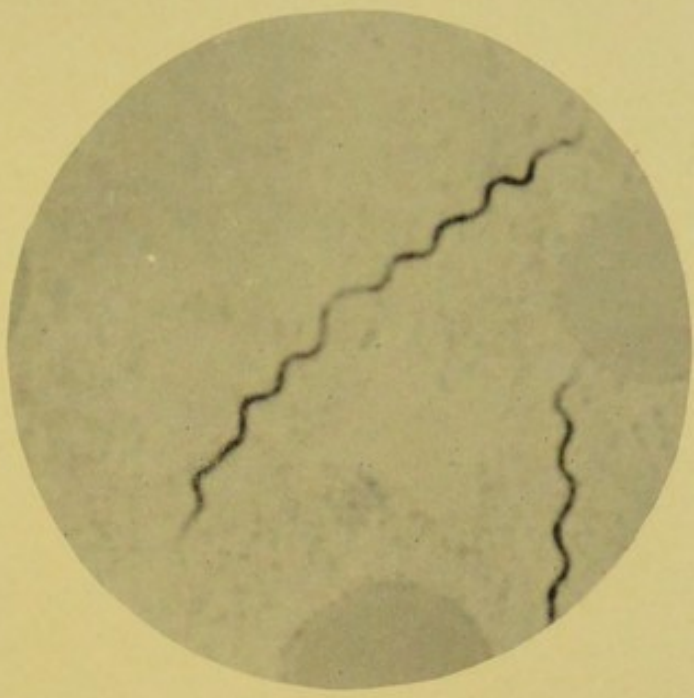


FIG. 2.

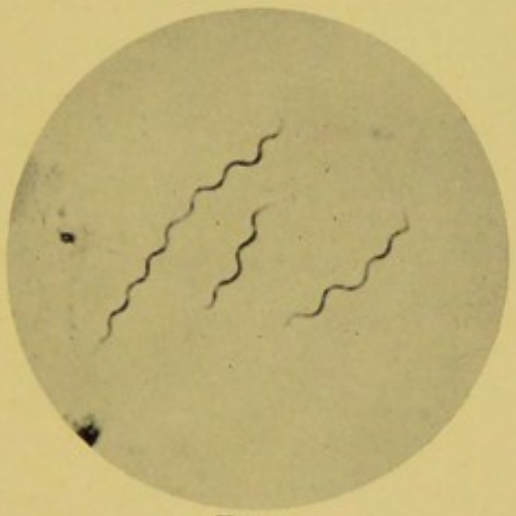


FIG. 3.

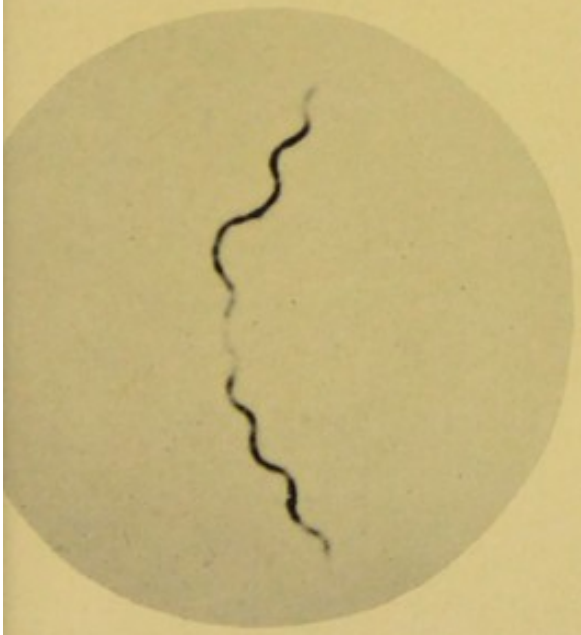


FIG. 4.

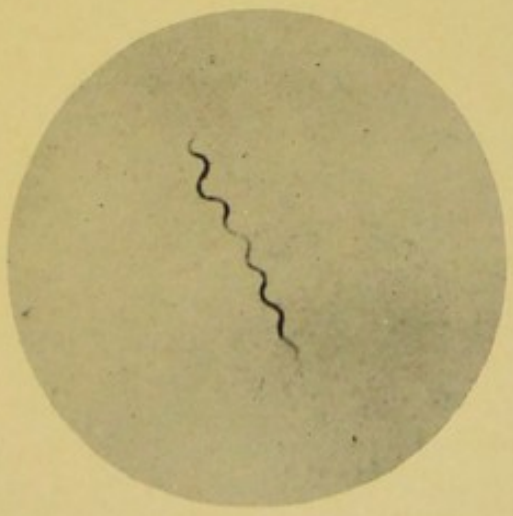


FIG. 5.

PLATE 9.

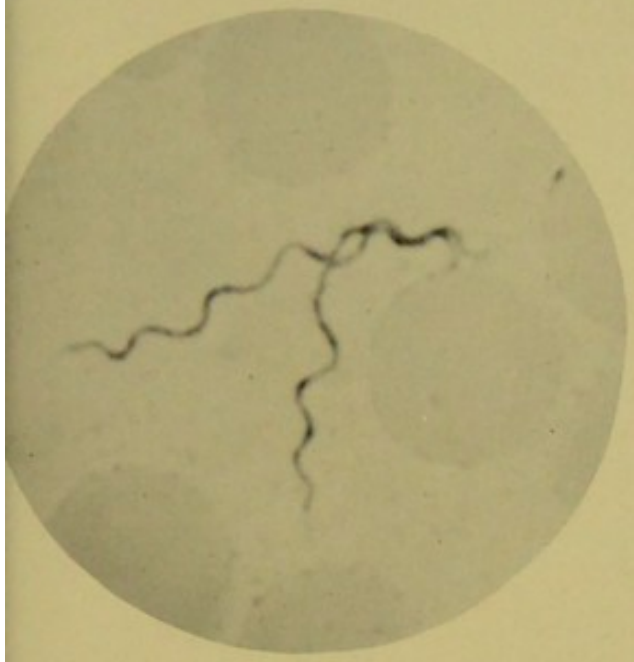
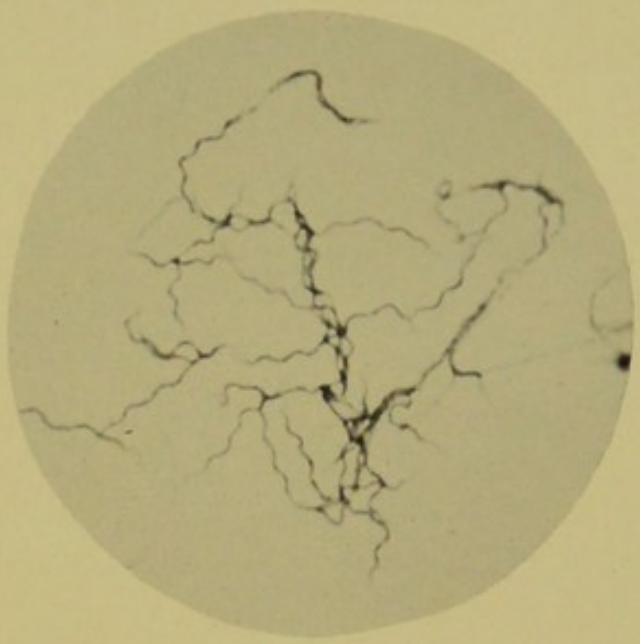


FIG. 1.



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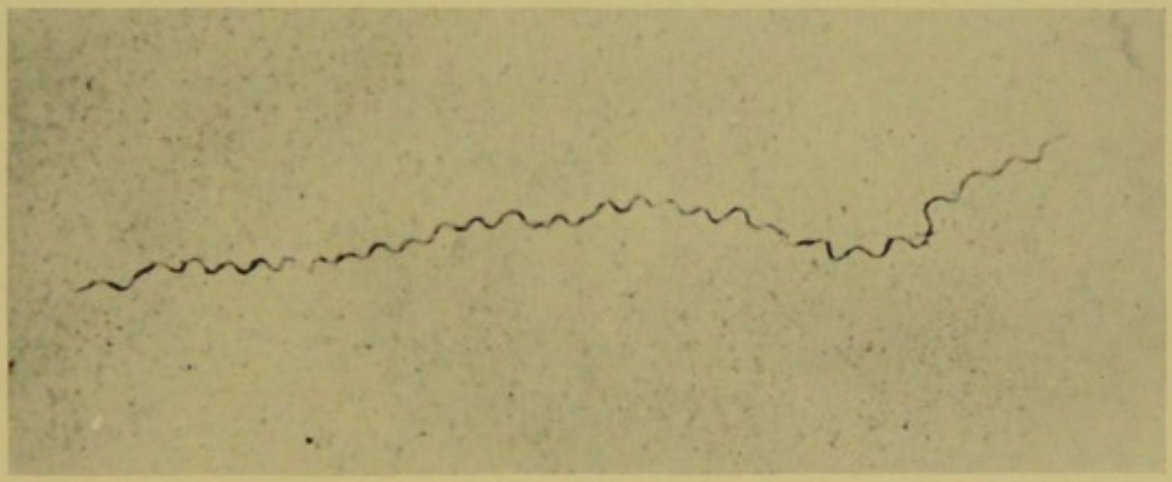


FIG. 3.

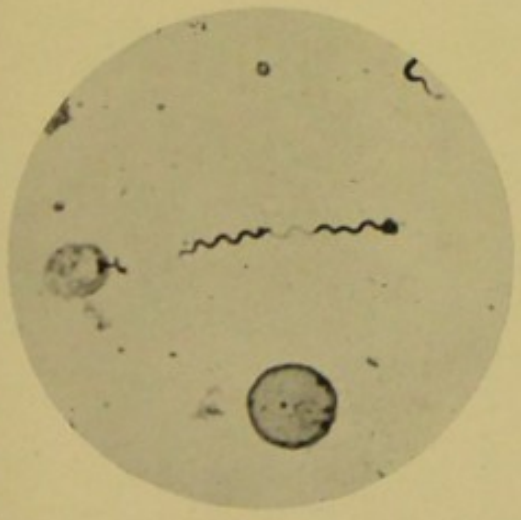


FIG. 4.

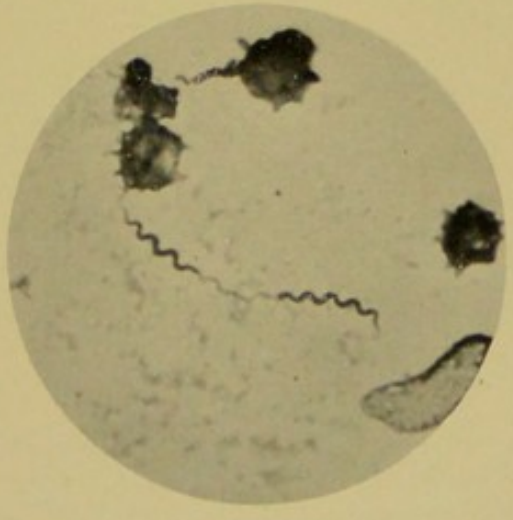


FIG. 5.

PLATE 10.

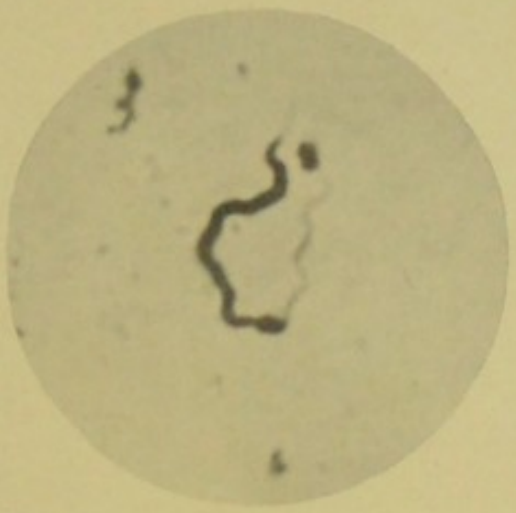


FIG. 1.

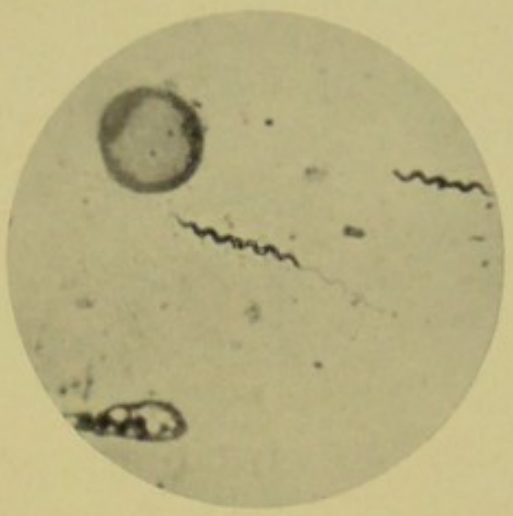


FIG. 2.

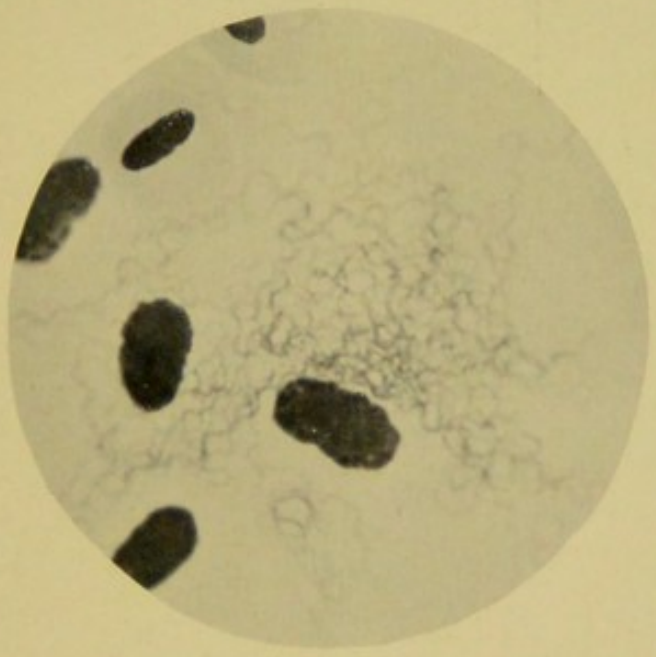


FIG. 5.

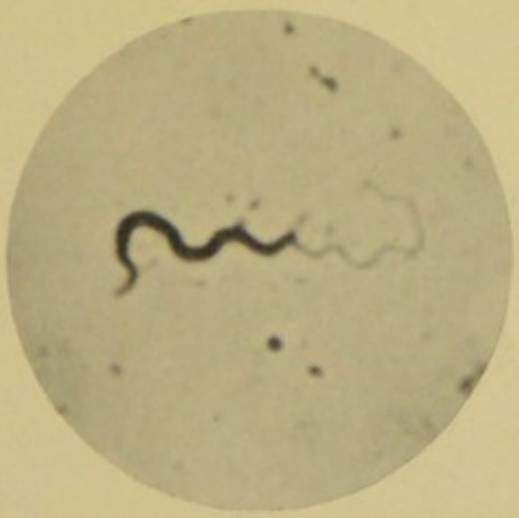


FIG. 3.

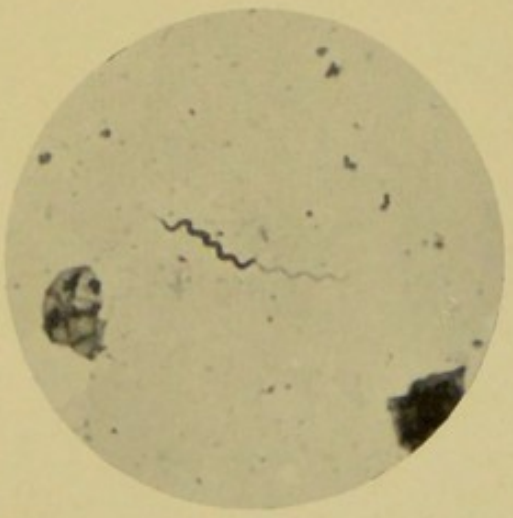


FIG. 4.

PLATE II.

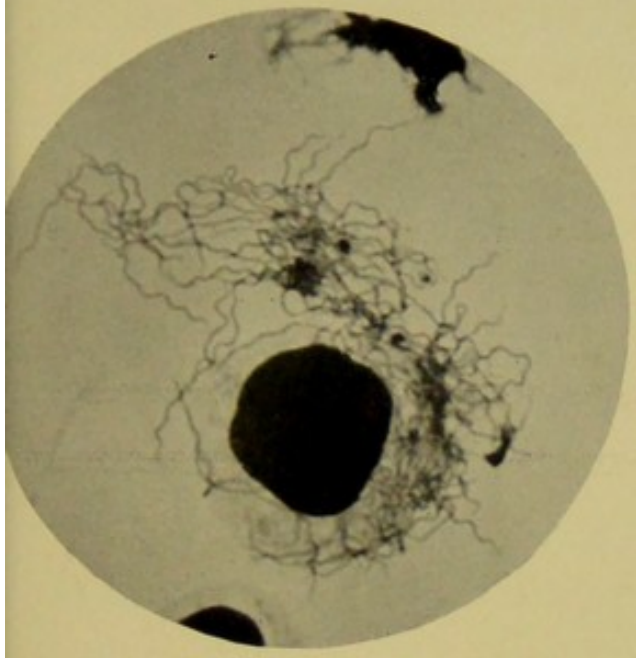


FIG. 1.

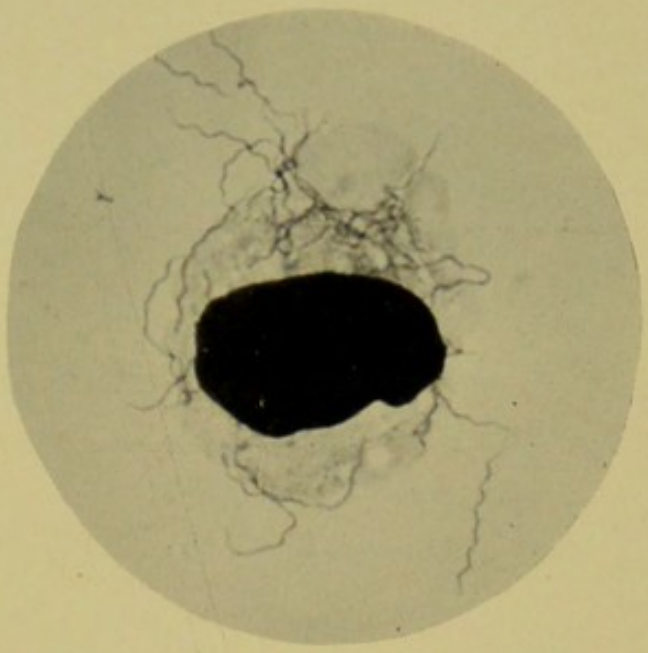


FIG. 3.

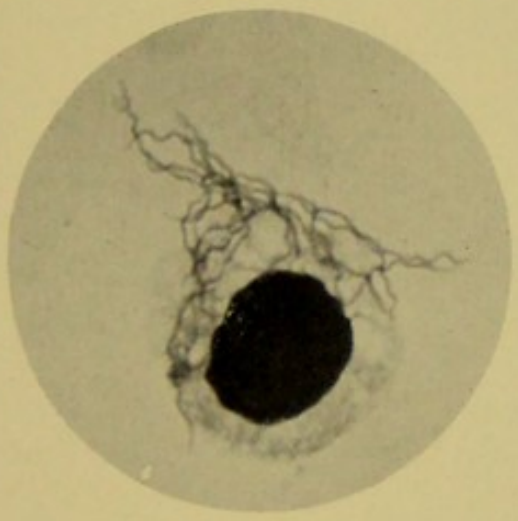


FIG. 2.

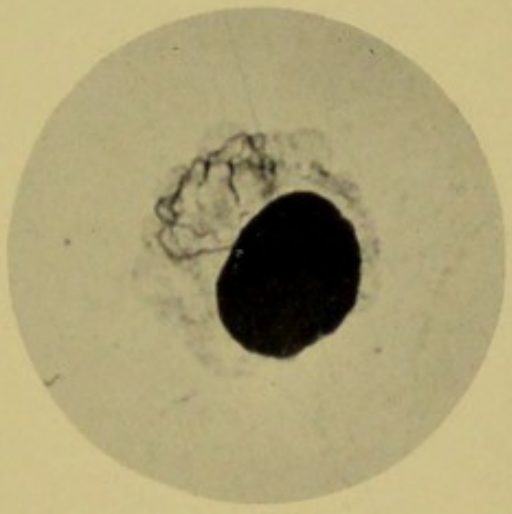


FIG. 4.

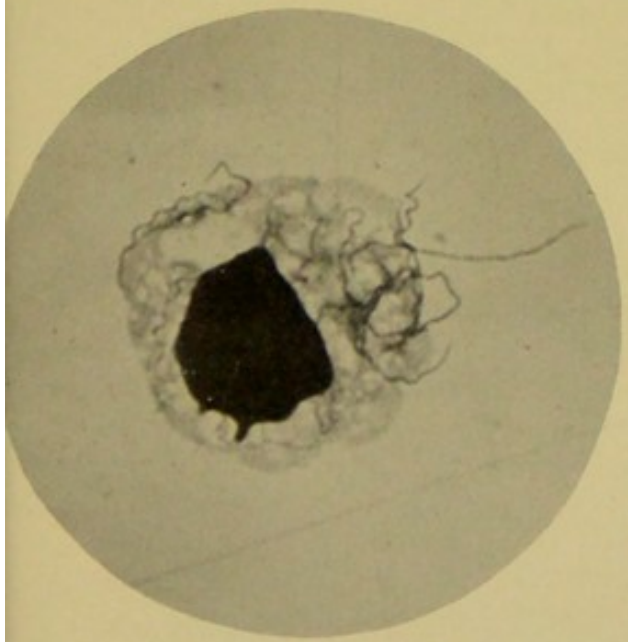


FIG. 5.

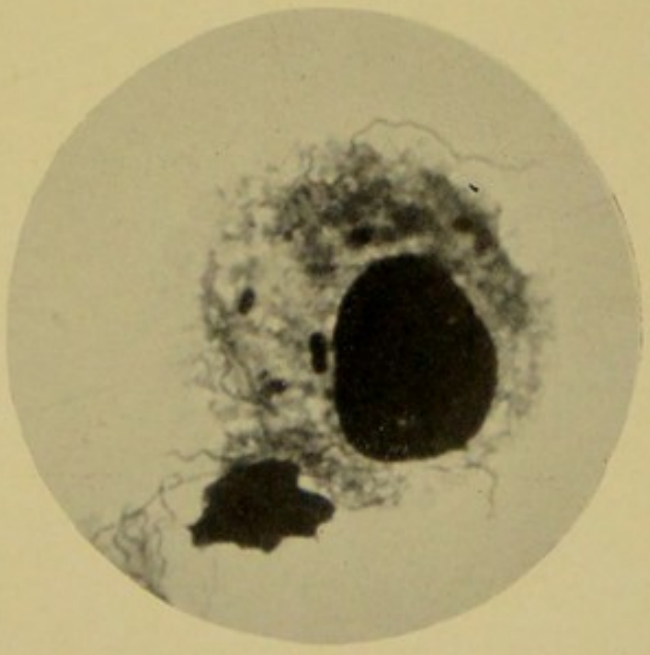


FIG. 6.

PLATE 12.

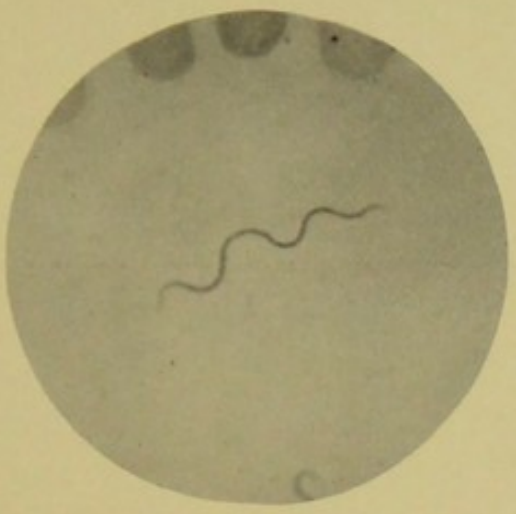


FIG. 2.

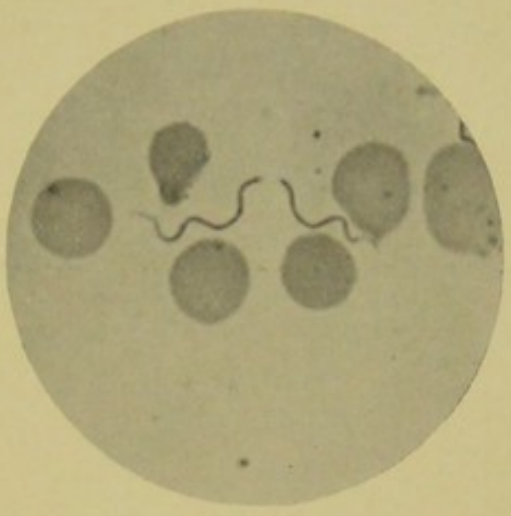


FIG. 3.

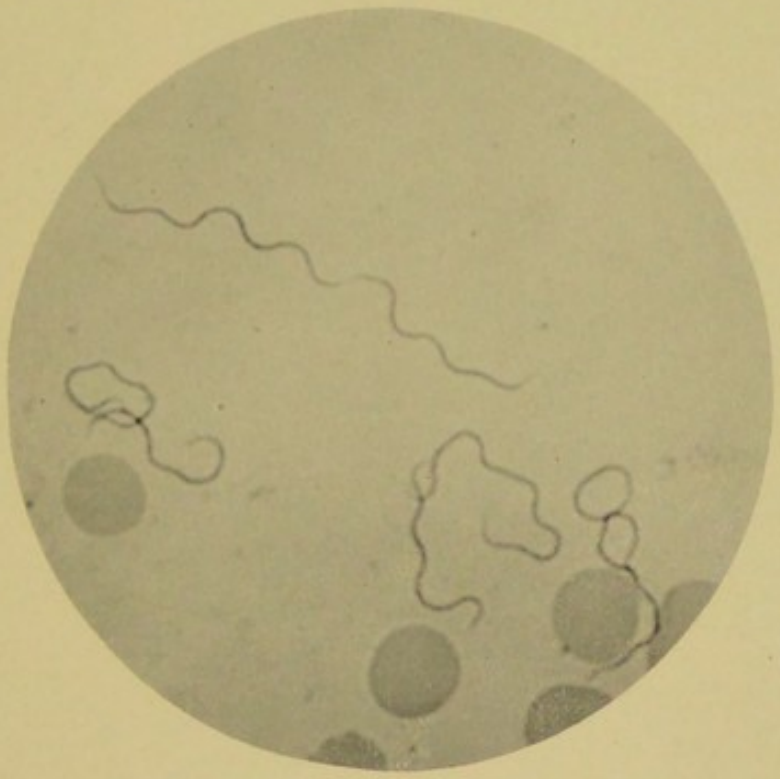


FIG. 1.

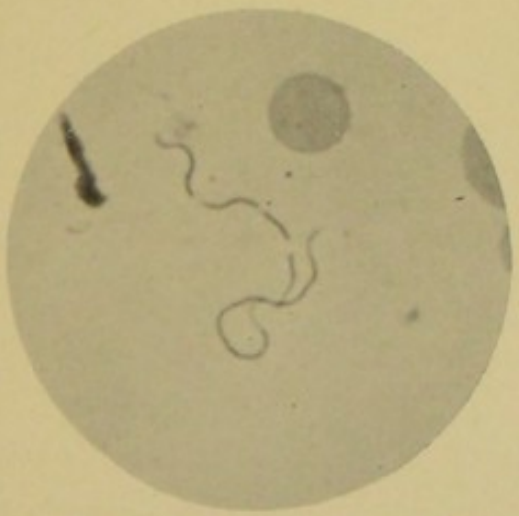


FIG. 4.

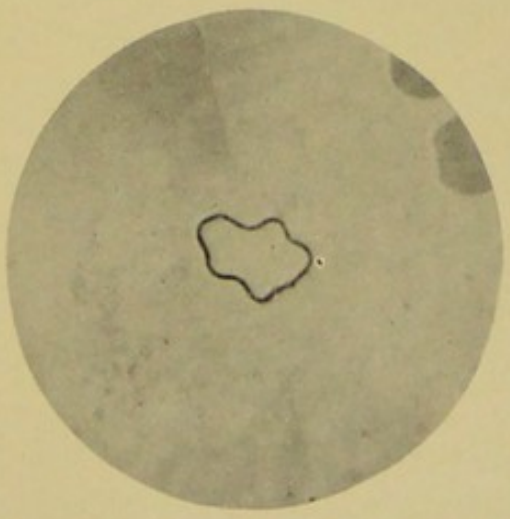


FIG. 5.

PLATE 13.

FIG. 1.

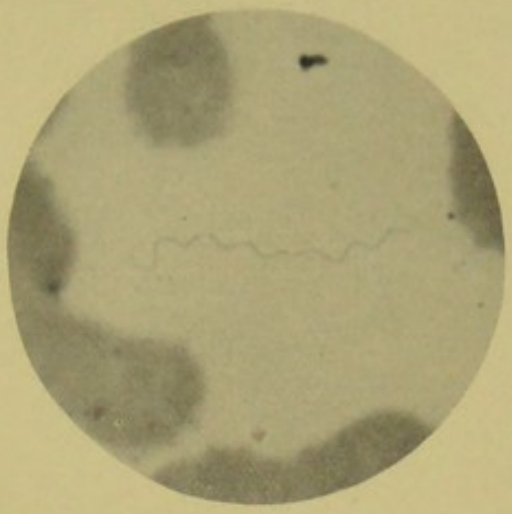


FIG. 2.

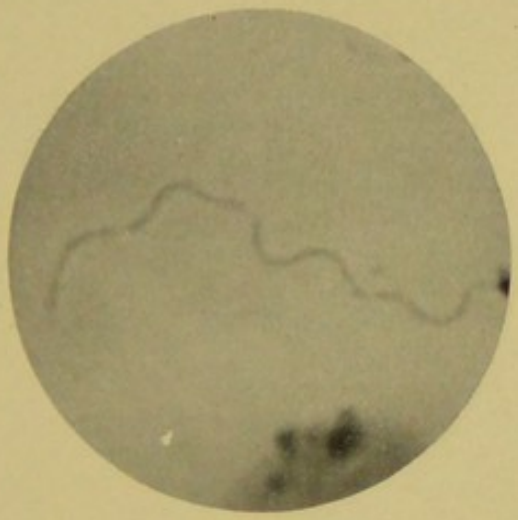
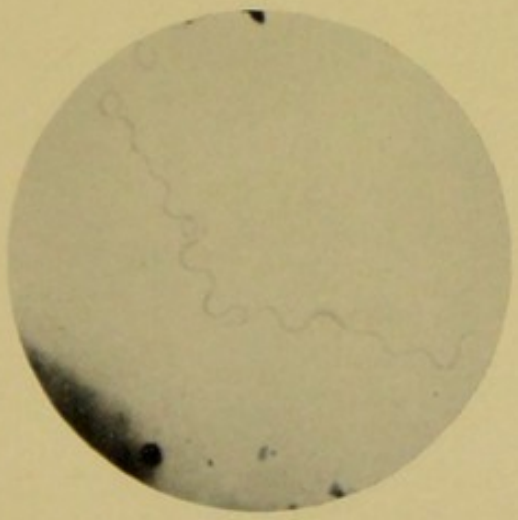


FIG. 3.

FIG. 4.



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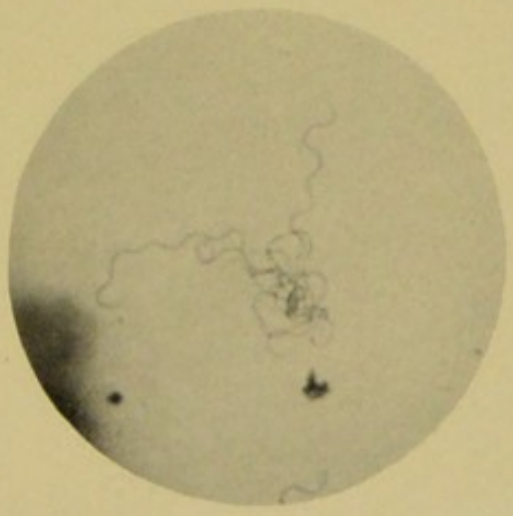


FIG. 6.

PLATE 14.

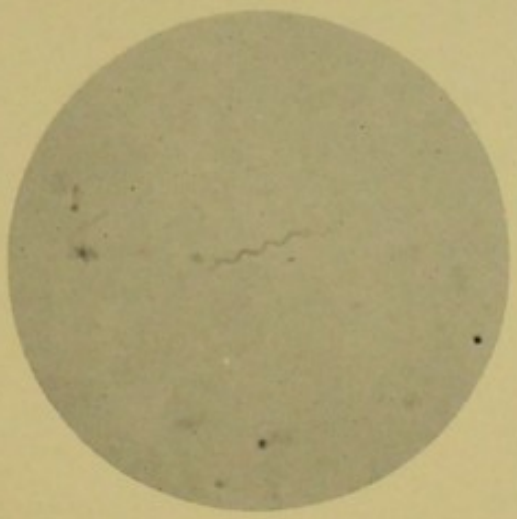
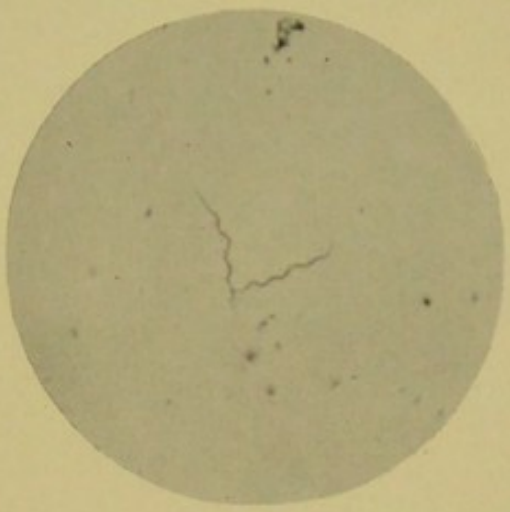


FIG. 1.



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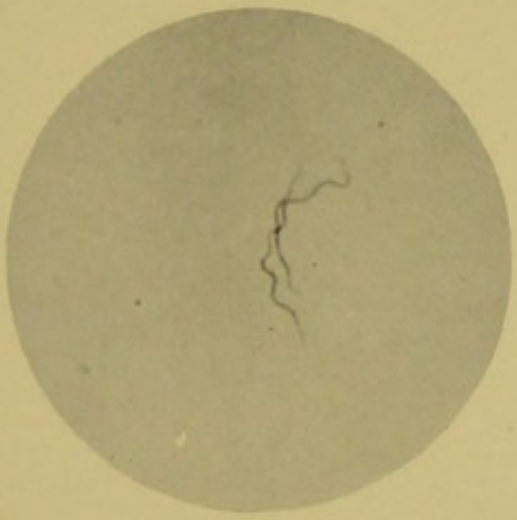

FIG. 3.

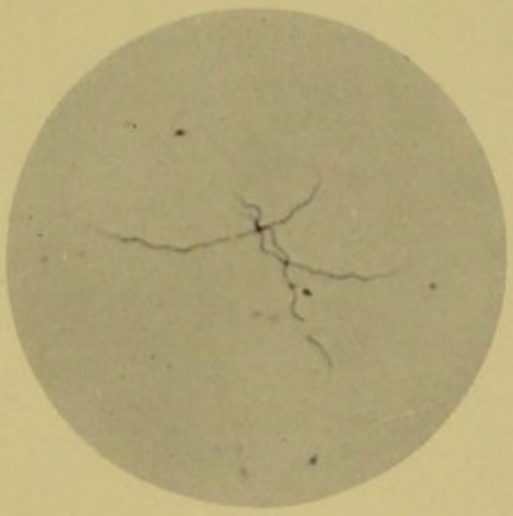

FIG. 4.

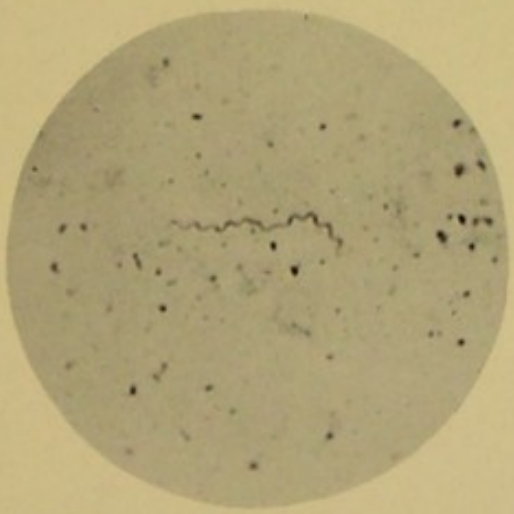

FIG. 5.

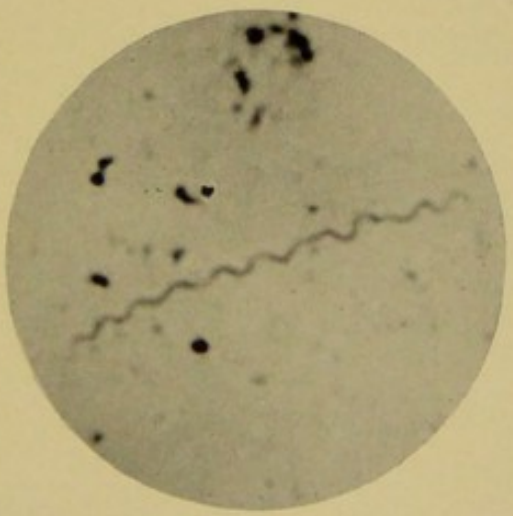

FIG. 6.

FIG. 4.—Phagocyte showing complete inclusion of several spirals.

FIG. 5.—Note the straight form of spiral showing transverse division. The phagocyte shows large numbers of comma or S-shaped remnants within its plasma.

FIG. 6.—Large phagocyte, the plasma of which is filled with chromatin particles; hence the dark portions in the plasma. The granules are probably the result of an enormous destruction of spirilla. Some comma-shaped remnants and three bacilli can be seen within the cell.

PLATE 12.

Spirillum Duttoni, or tick-fever spirillum from blood of a rat. Preparation of Dr. J. L. Todd.

FIG. 1.—Group of spirals. The long form shows a pale central portion indicative of transverse division. Note the pale free tips; the greater length of the spirals and the large, deep wavy bends, as compared with *Sp. Obermeieri* (Plate 8, Fig. 1, etc.); also note the tendency of the spirals to form circles and figure-8 forms.

FIG. 2.—A cell with typical large bends and pale tips.

FIG. 3.—A spiral showing clear central zone, evidently dividing.

FIG. 4.—One of the spirals here shown has three clear zones; division into vibrio form?

FIG. 5.—A spiral coiled in form of a circle; a very common condition.

PLATE 13.

Spirillum of relapsing fever from blood of man, Bombay case. Preparation obtained through Dr. W. S. Patton.

FIG. 1.—Perfect form with slight evidence of division into segments.

FIG. 2.—Very long form showing curled pale ends and some evidence of segmentation.

FIG. 3.—A spiral showing four S-shaped segments. The division in the middle gives two short spirals, which in turn divide into the S-form. 3,000×.

FIG. 4.—This shows a tendency to break up into comma-shaped segments. 3,000×.

FIG. 5.—Long spirillum resulting from the agglutination of three short or medium forms. Note the curling ends on account of which the cells do not coincide as perfectly as in Plate 9, Fig. 3.

FIG. 6.—An agglutination tangle. Note the tendency of the spirals to form loops or knots.

PLATE 14.

Spirillum glossinae, n. sp., and *Sp. pallidum*.

FIG. 1.—*Spirillum glossinae*, single cell; showing perfect spiral with faint tips. From *Glossina palpalis*, No. 8.

FIG. 2.—Two short spirals from the same preparation.

FIG. 3.—Two double length spirals from the same slide.

FIG. 4.—Three long spirals, like preceding. Note the clear space, showing transverse division, in the straightened out spiral.

FIG. 5.—*Treponema* (*Spirillum?*) *pallidum* from liver of a case of congenital syphilis. Preparation of Dr. F. Schaudinn.

FIG. 6.—Another specimen from the same preparation. Magnification, 3,000×. Note the faint tips and a light transverse zone in the middle.