THE RELAPSING FEVER OF PANAMA*

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Publication of this paper has been withheld by the writer in order that the animal reactions of the new world monkeys might be worked out, and also that further attempts to produce a polyvalent hyperimmune serum might be made, but it is thought that the paper should now be published as it is, as the production of an immune serum can be much better accomplished near a constant and larger supply of laboratory animals.

INTRODUCTION

As Novy,[1] Mackie[2] and others,[3] in part, have pointed out, it is possible with the means at present at our disposal to separate the spirochetes causing relapsing fever into four groups, the separation being based on animal reactions, specific characters, such as agglutination and immunity, and certain clinical features of the infection in man.

This paper deals chiefly with some of the characters of the spirochete causing the relapsing fever of the Isthmus of Panama. The work was done at Ancon Hospital during the period between July, 1907, and February, 1908.

Relapsing fever has been reported from time to time in the canal zone since the American occupation in 1904. Most of the cases have been from Colon Hospital, about twenty-five in number, and five from Ancon Hospital (Panama). They have occurred chiefly among white laborers and have been distributed among Italians, Spaniards, Turks, Germans, Scotch, Greeks and Americans. The patients have all been seen in the wards, where the diagnosis has been made by blood examinations, and they have all presented the classical features of relapsing fever with two or more relapses.

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*The greater portion of this investigation was read at the Canal Zone Medical Association, Ancon, C. Z., Feb. 8, 1908.

1. Novy, F. G., and Knapp, R. E.: Jour. Infect. Dis., 1906, iii, 291.
2. Mackie, F. P.: N. Y. Med. Jour., 1908, lxxxviii, 337.
3. Uhlenhuth and Haendel: Arb. a. d. k. Gsndhtsamte., 1907, xxvi, h. 1, 1.
   Manteufel: Arb. a. d. k. Gsndhtsamte., xxvii, h. 2, 327.
   Shellack, C.: Arb. a. d. k. Gsndhtsamte., xxvii, h. 2, 364.
   Marchoux, E., and Salimbeni, A.: Ann. de l'Inst. Pasteur, 1903, xvii, 569.

Carlisle[4] has given us an excellent summary of the geographical distribution of relapsing fever. With regard to America, a few sporadic cases and one epidemic have been reported in the United States, all of them being traced to recently arrived immigrants or sailors. Cases have been reported from Tuxpam, Mexico, a seaport between Vera Cruz and Tampico, and from Cuba, Peru, Chile and Bolivia. Hirsch[5] in 1881 stated that nothing was known of its existence in Central and South America. Dr. R. Franco[6] of Bogota, U. S. of Colombia, has discovered a febrile spirochetosis, the spirochete of which, it is said, can not be differentiated from *Sp. duttoni*. *Ornithodoros turichatus* is thought to be the species of tick which conveys the disease. It may be that the relapsing fever of Panama has been introduced from the United States of Colombia, where it appears to be endemic.

There are no records of the disease having been recognized on the Isthmus of Panama previous to 1905. If it appeared here before that date it was probably called "malarial fever" or "typhus fever." In the absence of an absolute diagnostic criterion many cases of relapsing fever in the past must have been confounded with typhus and malarial fevers.

It would be disappointing to find in a disease of so wide a geographical distribution as relapsing fever that Hippocrates[7] had not made some clinical observations, and we are, therefore, not surprised to learn of an epidemic occurring in the island of Thasus, off the coast of Thrace, described by Hippocrates, presenting many features of resemblance to relapsing fever. "The chief points of resemblance between the ancient and modern epidemics are the invariable occurrence of relapses, the marked character of the crises, and the frequent associations with the more ordinary events of the disease of copious perspirations, hemorrhages, particularly epistaxis, jaundice and splenic enlargements."[8]

From Carlisle's summary of the various reported epidemics and sporadic cases we learn that at one period relapsing fever was regarded as being indigenous to the British Isles. J. Warburton Begbie[8] gave as a synonym the term "epidemic fever of Scotland or Ireland."

4. Carlisle, R. J.: Jour. Infect. Dis., 1906, iii, 233.

5. Hirsch, A. (see Carlisle): Handbook of Geographical and Histological Pathology, i, London, 1883.

6. Blanchard, R.: Bull. Acad. Med., 1907, 5111. Abstr. in Jour. Trop. Med. and Hyg., 1908, xi, 58.

7. Hippocrates: Epidemiorum Hippocratis, Liber Primus, Sectio Secunda, Status Tertius.

8. Begbie, J. W.: Reynolds' System of Medicine, Lippincott, Philadelphia, edition 2, i, 456.

Relapsing fever has frequently been associated with typhus fever and with the insanitary conditions which favor the development of typhus fever. It was recognized in Scotland and Ireland as early as 1817 and after several epidemics was last seen there in 1871. The cases in the United States reported by Austin Flint occurred in 1850-51. Clymer at Philadelphia in 1844 recognized it among Irish immigrants there. It was at this time that relapsing fever was very prevalent in the British Islands, particularly at Edinburgh. The disease in the United States did not spread very far. A few cases occurred in Washington, Maryland, New Jersey and Connecticut. One case was noted in Boston. Flint's cases were observed in Buffalo, where he was practicing at that time. Philadelphia and New York City were visited by an epidemic in 1869.

There have been numerous epidemics in Europe, occurring in Russia for the first time in 1833 at Odessa, a seaport. There have been epidemics in St. Petersburg, Warsaw, Moscow, Novgorod and many other places in Russia. The United States Consul-General's office reports the total number of deaths in Russia from relapsing fever in 1901 as given by the Medical Department to be 2,466 out of a total of over 700,000 deaths from infectious diseases.

Many cases have been reported from India. H. Vandyke Carter[9] thought that relapsing fever was constantly present in Bombay.

Germany has been visited by epidemics from time to time. Obermeier[10] made his observations on relapsing fever during the epidemic of 1868-73 in Berlin. Germany has been free from an epidemic since 1880. One imported case was discovered in Hamburg in the person of a Persian emigrant on his way to the United States. In spite of the emigration from Russia at present and from Great Britain and Ireland in the past when epidemics of relapsing fever were present, it is to be noted that the disease has never gained a foothold in the United States. Relapsing fever patients are infectious for a very long period, four to seven weeks, throughout the entire course of the disease, giving a suctorial insect or acarid abundant opportunities to take on the spirochete, so that its failure to spread when introduced is strongly indicative that the conditions are unsuitable for the continued existence of the intermediary or alternate hosts—insects, acarid or rat.

The spread of relapsing fever during the past century may be related to the distribution of *Mus decumanus* over Europe and around the world.

9. Carter, H. V.: Spirillum Fever, London, 1882.
10. Obermeier, O.: Centralbl. f. d. med. Wissensch., 1873, p. 145.

From a study of the epidemiology of relapsing fever we would expect to find cases appear in seaport towns and those in the interior reached by emigrants or sailors from places where relapsing fever was endemic.

Thirty-one cases of relapsing fever have been recognized in the Commission hospitals in the canal zone during three years out of about 65,000 admissions, where blood examinations are made of every patient entering the medical wards. The disease here has not been confined to one race or nationality.

Table 1 records certain clinical data of interest in connection with seventeen cases occurring during 1907:

TABLE 1.—CLINICAL DATA IN 17 CASES

Whites (15)	
Americans	4
Germans	2
Spaniards	4
Scotch	2
Italians	1
Greek	1
Turk	1
Blacks (2)	
Martiniquan	1
Antiguan	1

Ages		Length of Residence on the Isthmus (2 months to 3 years)	
13 years	1	2 months	1
21 years	1	3 months	4
22 years	1	4 months	1
26 years	1	4½ months	1
27 years	3	5 months	1
30 years	2	6 months	1
32 years	2	6½ months	2
35 years	1	7 months	1
36 years	1	11 months	1
39 years	1	15 months	1
40 years	1	24 months	1
44 years	1	30 months	1
45 years	1	36 months	1

Occupations		Place of Residence on the Isthmus	
Laborers	11	Colon and Cristobal	5
Carpenters	2	Rio Grande	1
Cable operator	1	Ancon dredge (suction)	1
Sailor (dredge)	1	Gatun	7
Engineer	1	Empire	1
Unknown	1	Mindi	1
		Corozal	1

It is to be noted in Table 1 that there is a disproportionate number of cases among white employés—7 to 1—while the number of white to negro employés during the period from which the data was compiled was more nearly 2 to 7. The average number of white employés for the year 1907 was 10,709, while the average number of black employés for the same period was 28,634. The seventeen cases tabulated were distributed among natives of nine countries, who had lived on the isthmus from two to thirty-six months; thirteen of the seventeen patients, in all probability, developed the disease on the Atlantic or Colon side of the isthmus, where there appear to be two foci of infection, Colon and Gatun, the former a seaport and the latter a village six miles from Colon on the Pan-

ama railroad, on the site of the proposed locks, where a large number of laborers are quartered.

It is not intended to discuss, at any length, the clinical aspects of relapsing fever, but, more particularly, to give an account of some observations which it is hoped will throw some light on the nature of the micro-organism causing the fever found on the Isthmus of Panama.

Attempts to identify the spirochete of relapsing fever by means of morphological and staining characters are subject to grave inaccuracies, for there is a fairly wide limit to the variations of length, width, number of spirals, regularity of curvature and homogeneity in the same strain in different animals of the same species and at different periods during the infection.

The terms "spirillum" and spirochete" are used at present indiscriminately when applied to the micro-organism causing relapsing fever. With high magnification and better methods of staining and cultivation it may be possible to classify the spiral-shaped micro-organism in groups according to size, the number and arrangement of flagella; according to their shape—cylindrical or ribbon-shaped; according to their pathogenicity, according to their amenability to culture.

Spirochetes are almost as widespread as bacteria. In the blood stream they are associated with relapsing fever in men and the spirillosis of geese, goats, horses, rats, mice, fowl, sheep, cows and bats. Spirochetes have been found in non-specific ulcers on the external genitalia, ulcers on the legs or body, tropical ulcers, in stomatitis and in intestinal inflammation and ulceration. They have been found at autopsy here in Panama in most cases of gangrene of the lung and in other lesions of the respiratory tract. In a case of sprue, recently, smears from a parchment-like membrane at the margin of the teeth showed a pure culture of spirochetes and fusiform bacilli. Spirochetes are practically always found in inflammatory conditions of the mouth, particularly of the gums. It is not uncommon to find spirochetes associated with protozoa in infections of the intestinal tract of man and the lower animals.

Insects harbor spirochetes, which have been found in their intestinal tract, sexual organs and ova. Several varieties of spirochetes are found in natural waters, particularly in the surface pollicles of stagnant waters.

Schaudinn[11] directed the attention of the medical world to a group of spiral-shaped micro-organisms, more especially to one member of the group which he has designated *Treponema pallidum,* and with Hoff-

11. Schaudinn, F., and Hoffmann, E.: Gsndhtsamte., 1905, xxii, 527.

mann, in 1905, has shown to be almost constantly present in the tissues of persons suffering from syphilis. The same year Castellani[12] described the spirochetes found in yaws.

The spirochete of relapsing fever was discovered by Obermeier in 1873, who observed it for the first time in 1868 while studying an epidemic of relapsing fever in Berlin. Obermeier's observations were made with the spirochete of European relapsing fever, a disease belonging to the general class of relapsing fevers, but one which must be differentiated from the tick-fever of Africa and the fevers of Bombay and the Isthmus of Panama.

It is impossible at the present time to differentiate the varieties of spirochetes of recurrent fever by cultural methods, and it can not be said that morphology affords an accurate means of distinguishing them, for in the observations on the isthmian relapsing fever the spirochete has exhibited considerable variation in size, length, number of spirals and in staining qualities. It will be necessary then to adopt as a means of differentiation the effects of the micro-organism on man and susceptible animals—animal reactions and the effect of the serums and cells of susceptible animals on the micro-organism—agglutination, lysis and phagocytosis.

H. Vandyke Carter published some notes in 1877 and a complete description in 1882 of the spirillum fever of Bombay. The disease appeared in Bombay in 1877, being introduced by immigrants from famine districts. Carter successfully inoculated monkeys with the spirochete of Bombay fever. Rogers'[13] description of the epidemic is very much like that of Welsh[14] of an early epidemic in Scotland, the disease spreading through families and attacking clinical clerks and hospital attendants. According to Norman Chevers and Carter, relapsing fever has always existed in India. During periods of famine the mortality has been very high. It is to be noted that vermin are particularly active in debilitated persons.

Human tick-fever, or the relapsing fever of the Congo, was first observed by Livingstone[16] in 1857. The etiologic factor, *Sp. duttoni,*

12. Castellani, A.: Jour. Ceylon Brit. Med. Assn., 1905, ii, 54.

13. Rogers, L.: Fevers in the Tropics, London, 1908, Oxford Med. Publication: Henry Frowde.

14. Welsh, B.: A practical treatise on the efficacy of blood-letting, etc., Edinburgh, 1819.

16. Livingstone: Missionary Travels and Researches. John Murray, London, 1857. Chapters xix, pp. 283, 382; xxx, pp. 628, 629.

TABLE 2.—SOME OF THE RELATIONSHIPS OF THE SPIROCHETES AND THE RESPECTIVE DISEASES CAUSED BY THEM

	Panama	Carlisle	Africa	Europe	Asia
Febrile paroxysms in man:					
Number	3-4	3	5-6	2-3	2-3
Duration	2 days + —	2 days.	1 day + —	3-9 days.	?
Severity of disease	Mild.	Mild.	Severe.	Severe.	Severe.
Number of Spirochetes in blood during paroxysm.	Very few, 1 to 30 fields + —	Very few, 2 to coverslip + —	2 to 3 per field.	1 to field.	?
The infection in monkeys	Mild with relapses.	Mild with relapses.	Severe and fatal, with relapse.	Severe, with relapses. *	Very mild †
The infection in white mice	Mild, 2 relapses.	?	Severe, several paroxysms.	Mild, naturally not susceptible.	Mild, infected with difficulty.
The infection in white rats	Mild, 1 paroxysm.	Mild, 1 paroxysm.	Severe, several paroxysms.	Mild, naturally not susceptible.	Infected with difficulty.

* Uhlenhuth and Handel.
† Mackie.

was discovered by Ross[17] and Milne in 1904 and by Dutton and Todd[18] in the same year.

Dutton and Todd studied the disease in man and monkeys and produced the disease in monkeys by means of naturally infected ticks, *Ornithodoros moubata,* and in one experiment by means of young ticks, newly hatched in the laboratory, from eggs laid by infected parents.

In America there have been several epidemics and occasionally sporadic cases. In the earlier epidemics in the United States, the disease was introduced by Irish emigrants. More recently sporadic cases have been reported in the United States among Russians, Armenians, or Spaniards. The disease in most instances has been the European variety and is, undoubtedly, of this type in the two cases reported recently by Goldfarb.[19] There is, however, a distinctly American type of the disease, such as the cases seen in Panama and the two cases studied by Carlisle.

Fatal cases of relapsing fever were reported from Tuxpam, Mexico, during 1905. On the Isthmus of Panama, the disease was first observed and the diagnosis verified by blood examination at Colon Hospital in 1905, since when there have been recognized at Colon and Ancon hospitals 31 cases.

Carlisle reported two cases of the American type in 1905 occurring at Bellevue Hospital, New York City. The second case was derived from the first through the bite of an infected monkey. The first was that of a ship steward who had recently visited Galveston and Key West.

Among recent studies of relapsing fever are those of Breinl and Kinghorn[20] on *Sp. duttoni;* Norris, Pappenheimer and Fleurnoy[21] and Carlisle, and Novy and Knapp's "Studies in *Spirillum Obermeieri.*" Novy and Knapp fell into an error in calling the spirochete studied so thoroughly by them *Sp. obermeieri.* The clinical course of the disease in Carlisle's case and the animal reaction of the spirochete as determined by Norris and Novy are those of the relapsing fever of America, not that of Europe. Mackie has recently reported an epidemic in India in which the body louse was thought to be the transmitting agent.

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17. Ross, P. H., and Milne, A. D.: Brit. Med. Jour., 1904, ii, 1453.
18. Dutton, J. E., and Todd, J. L.: Memoir xvii, Liverpool School of Tropical Med. University Press of Liverpool.
19. Goldfarb, S. J.: Med. Rec., New York, 1908, lxxiii, 433.
20. Breinl, A., and Kinghorn, A.: Memoir xxi, Liverpool School of Tropical Medicine, University Press of Liverpool.
21. Norris, C., Pappenheimer, A. M., and Fleurnoy, T.: Jour. Infect. Dis., 1906, iii, 266.

The spirochetes of relapsing fever may be placed in four groups:

Group A.—The group causing a relapsing or recurring infection in man, monkeys, white mice and white rats, including *Sp. duttoni* and the tick-fever of Africa.

Group B.—The group causing a recurring infection in man, monkeys and white mice, but with a single paroxysm in white rats. This group comprises the relapsing fever of Panama and the two cases studied by Carlisle.

Group C.—The group causing a recurring infection in man and monkeys but failing to cause an infection in small rodents with blood direct from human sources, yet causing an infection in small rodents after a preliminary passage through the monkey. This group includes the relapsing fever of Europe, caused by *Sp. obermeieri.*

Group D.—The group causing a recurring infection in man and monkeys but only transient infections in white rats and white mice. This group includes the relapsing fever of Bombay caused by *Sp. carteri.*

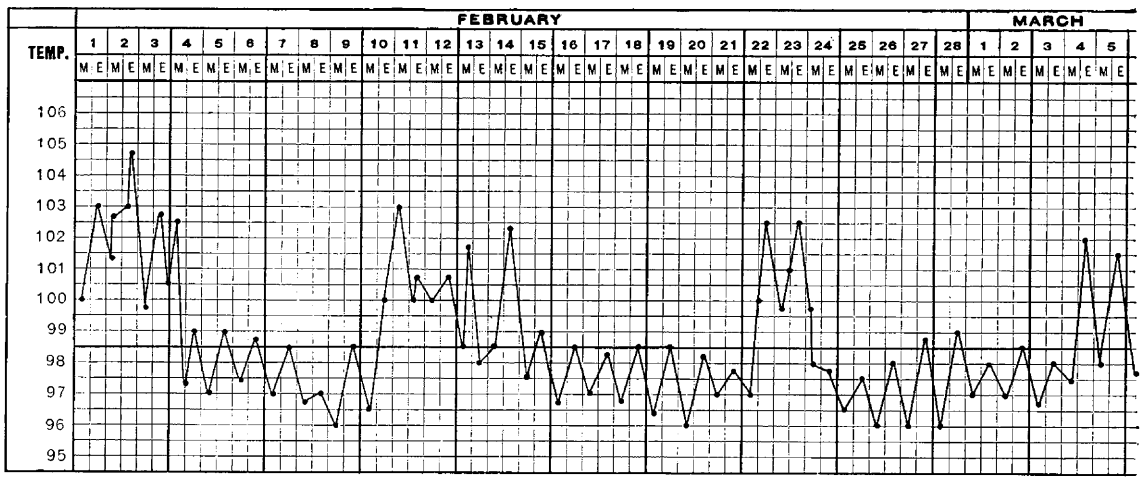


Fig. 1.—Course of case of relapsing fever of Africa. Note the remissions and intermissions during

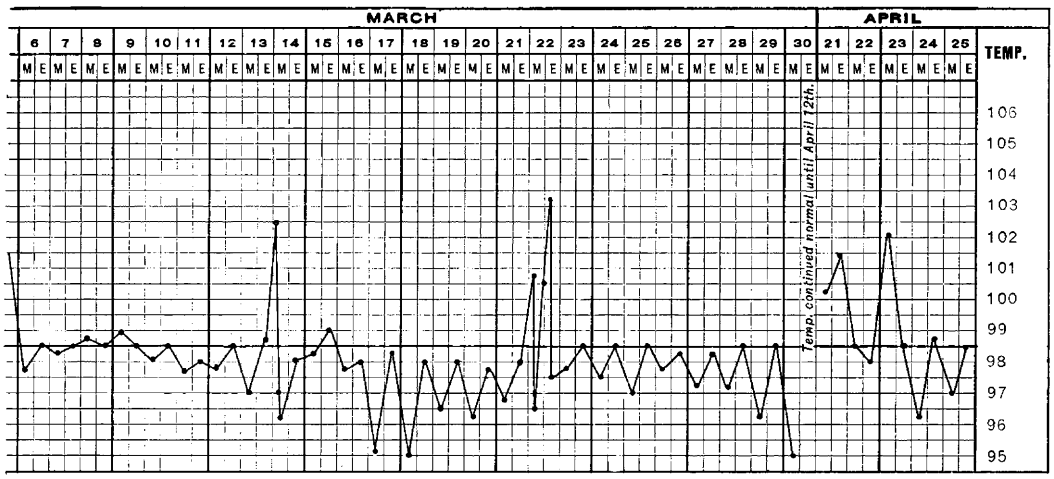
These groups are also separated by differences in the clinical course of the respective fevers in man. The relapsing fever of Africa (Fig. 1) is sharply differentiated from that of Asia, Europe and America by animal reactions, specific agglutination, immunity and clinical features.

The fever of Europe (Fig. 2) is different from that of Asia and America in the duration of the febrile paroxysm, which is longer in the European type than the others, and by animal reactions and other specific characters, such as agglutination and immunity.

The fever of Asia or Bombay differs from that of America, chiefly in the animal reactions of the spirochete and the clinical features of the disease; the Asiatic fever being more fatal.

Furthermore, as has been determined in this investigation, there is an individuality to the strains of spirochetes of the same species in the same locality, for an animal immunized to one strain is not immune to another strain from a different human source.[22]

In this investigation of the relapsing fever of Panama the animal reactions of white mice, white and wild rats and monkeys have been worked out. Experiments in immunity have been carried out, parallel infections with *Trypanosoma lewisi* have been studied, and the part played by the liver in the infection has been inquired into. The specific nature of stains has been investigated, and the effect of certain anilin dyes in lowering the resistance of inoculated animals to the infection has been noted.



the paroxysm and the subnormal oscillations of temperature during the afebrile period.

SOURCE OF THE SPIROCHETES USED IN THIS WORK

Animals have been inoculated from four patients, but only two of the strains so derived have been carried on for any length of time. The two strains used in this investigation will be designated as A and B.

ORIGIN OF STRAIN A

Patient.—L. G., hospital No. 33,107, from whom this strain was obtained, was a laborer, native of Spain, aged 39; length of residence on the Isthmus, three years; residence Gatun, a village in the Canal Zone, six miles from Colon, and one of the foci of infection. (See Fig. 3.)

22. I have since learned that Carl Fränkel (abstr. in Jour. Trop. Med. and Hyg., 1908, xi, 70, from Klin. Med., 1907, p. 928) has made a similar observation with regard to the spirochetes of the African fever.

Previous History.—The patient was twice in hospital; two weeks in Colon hospital and two weeks in Ancon hospital. He had not taken quinin.

Present Illness.—He has been ill four days. Onset with chill followed by fever, since when he has had several chills and much fever.

Clinical Notes (By D. Summersgill).—The spleen was enlarged and tender; lungs, glands, liver, skin, blood vessels, nervous system, urine and stool negative. The blood contained a few spirochetes. Blood was taken for animal inoculation Oct. 11, 1907, at 3:30 p. m., when the temperature was 102 F. Spirochetes were present in the peripheral blood at this time. Four cubic centimeters of blood from vein at elbow were inoculated intraperitoneally into a small monkey *cebus* and 2 c.c. intraperitoneally in two white rats and two white mice.

ORIGIN OF STRAIN B

Patient.—B. J. (See Fig. 4), from whom this strain was obtained, was a patient in Colon hospital; laborer, native of Turkey; age 27; residence, Gatun; length of residence on the Isthmus, six and one-half months.

Previous History.—The patient had been in hospital once; had had malaria many times; no dysentery.

Present Illness.—He had been sick three months; had swelling of feet with ulceration, and weakness.

Physical Examination (By Dr. Brem).—Abdomen pendulous. Movable flatness in flank—indistinct fluid. Mucosæ pale. Liver enlarged. Skin waxy. Tongue pale, flabby and coated. Lungs and glands negative. Spleen palpable 9 cm. below costal margin. Blood vessels soft. Pulse full. Heart: Presystolic murmur prolonged into systole, blowing and coarse, heard all over precordium with maximum intensity at apex; pause in aortic and pulmonary area; transmitted into vessels of neck, to axilla and towards sternum along costal margin. Feb. 5, 1908: Apex beat not visible. Palpable in the fourth and third interspaces, 12 cm. to the left of the sternum. Soft bruit accompanying the first sound all over precordium. Best at apex. Blood pressure 116.

Leucocytes, Jan. 21, 1908, 4,500; Jan. 22, 1908, 6,000.

Red blood cells, Feb. 3, 1908, 4,800,000; red blood cells, Jan. 8, 1908, 3,344,000.

Hemoglobin, Jan. 15, 1908, 70 to 75 per cent.; Jan. 16, 1908, 55 per cent.; Feb. 2, 1908, 69 per cent.

Urine and stools, negative.

Blood taken from patient's arm Jan. 21, 1908, and inoculated into two white rats intraperitoneally.

CHARACTERISTICS OF THE MICRO-ORGANISM

In human blood, in the relapsing fever of Panama, there are comparatively very few spirochetes seen during the paroxysm, one to forty or fifty fields, or perhaps only three or four to a cover-slip. In the period between paroxysms it is rarely possible to find a spirochete in the peripheral blood. In none of the cases studied here has the spirochete been present in what might be called considerable numbers. This observation is another means of differentiating the fever of Panama from that of Europe. Blood films taken from cases of the relapsing fever of Europe often show considerable numbers of spirochetes. Films from a case of European relapsing fever (Fig. 2), which I have studied through the kindness of Dr. Samuel J. Goldfarb, often show six spirochetes to

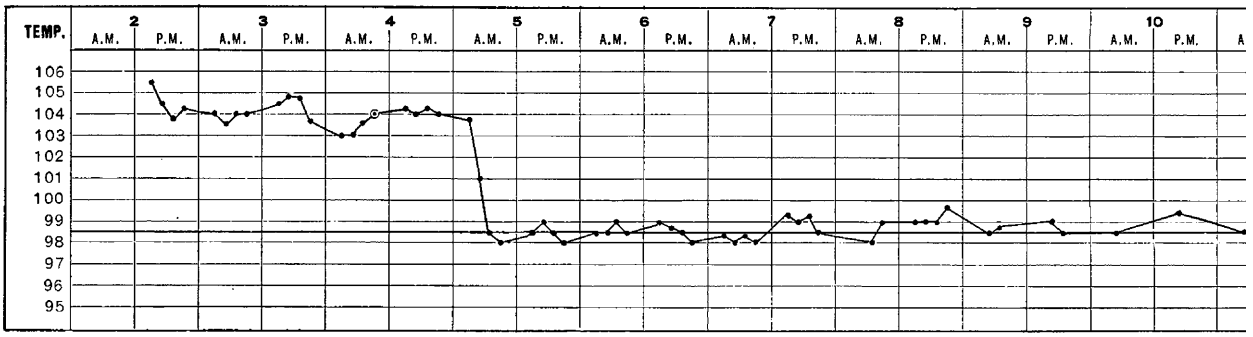


Fig. 2.—Course of case of relapsing fever of Europe. Note

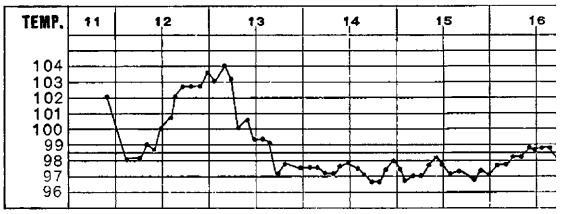


Fig. 3.—Course of case of r

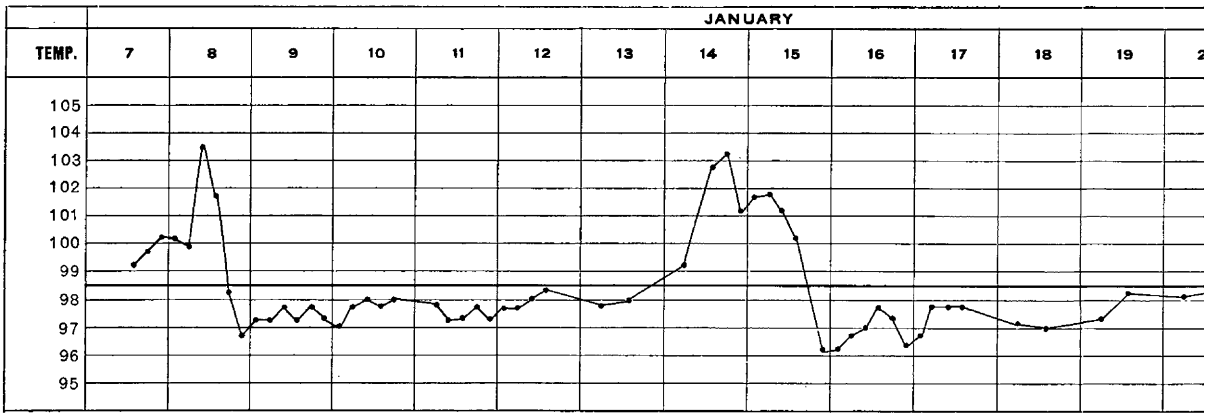
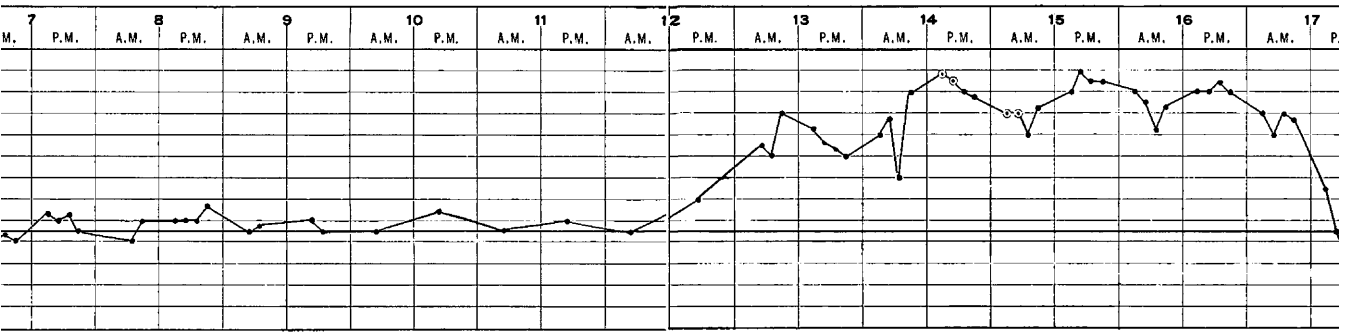


Fig. 4.—Course of case of re



Course of case of relapsing fever of Europe. Note the sustained temperature during the paroxysm and the prolonged duration of

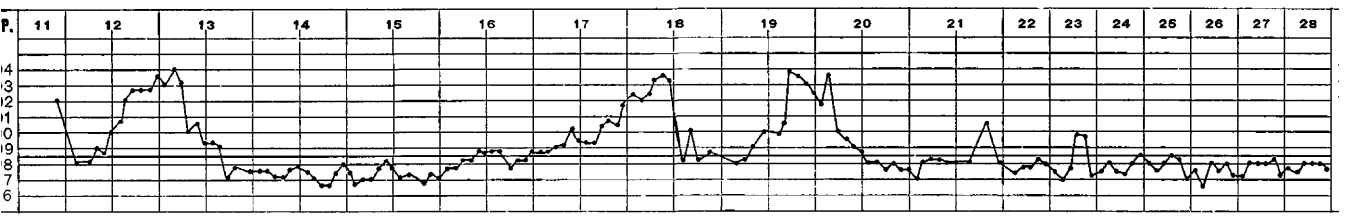


Fig. 3.—Course of case of relapsing fever of Panama from which strain A was obtained.

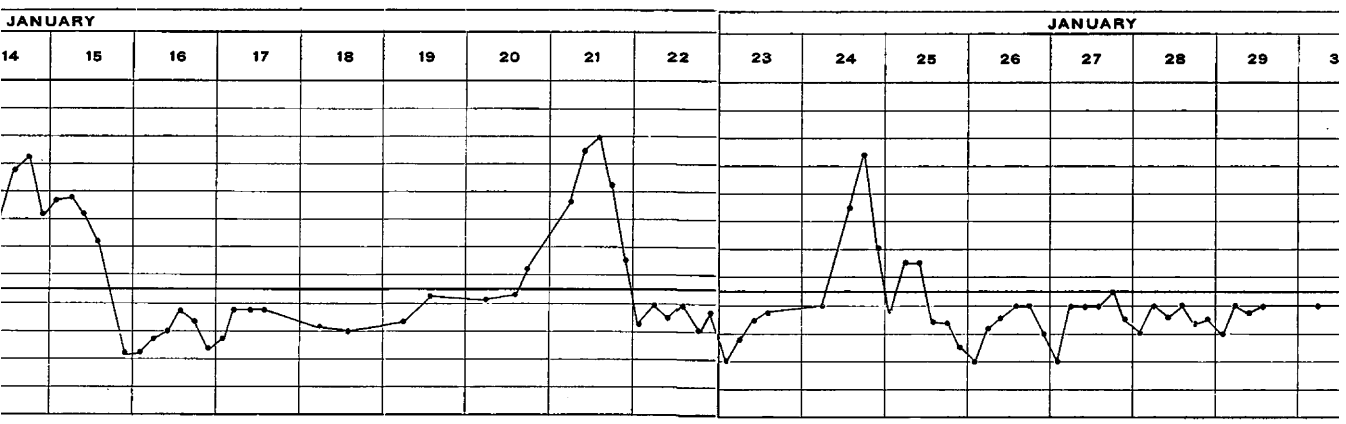
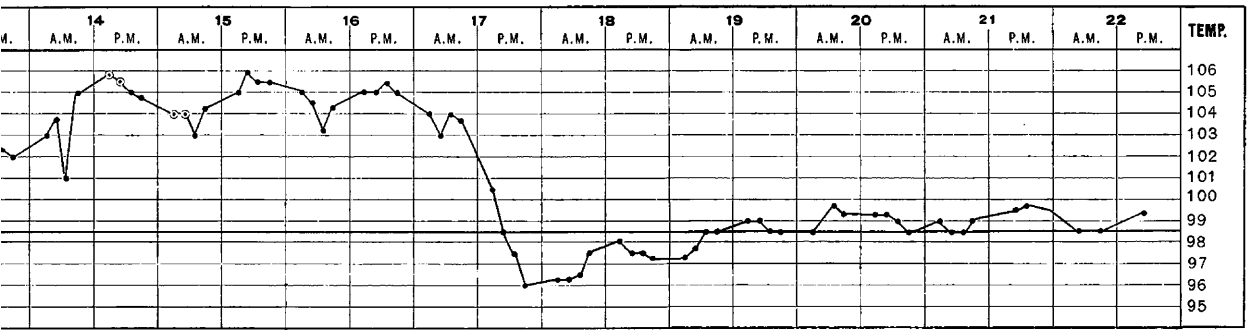
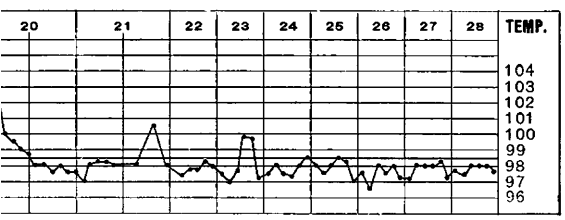


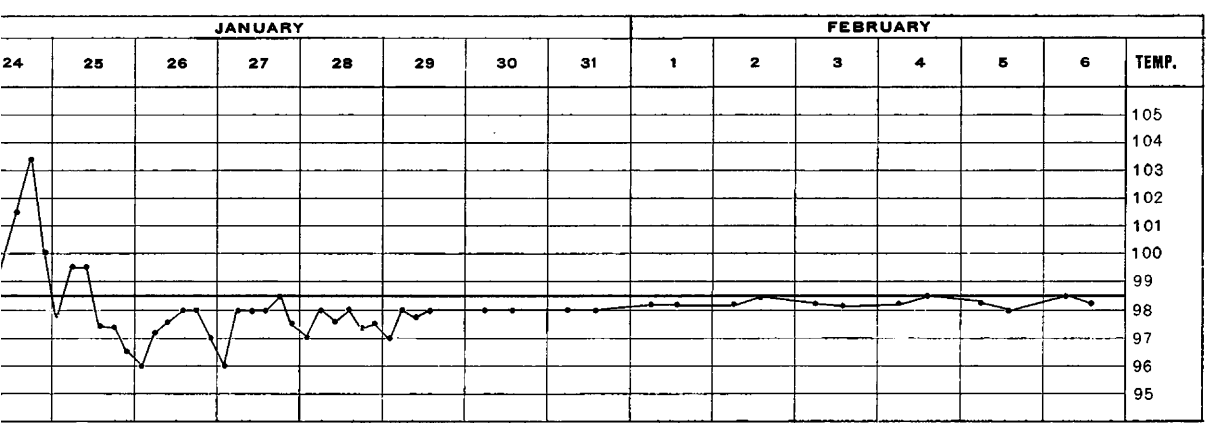
Fig. 4.—Course of case of relapsing fever of Panama from which strain B was obtained.



ing the paroxysm and the prolonged duration of the same.



which strain A was obtained.



which strain B was obtained.

one oil immersion field. Such a picture is never seen in blood films from cases of the fever met with here.

The description of the spirochetes is based on observations made with richly infected blood of white mice and white rats.

MORPHOLOGY

The movements of the spirochete are very rapid, except just before its disappearance from the blood stream. At this time observations of the nature and character of the movements may be made, or the motions may be slowed down by treating a drop of blood with two or three drops of citrated saline solution and making a cover-slip preparation. At first the motion of the spirochete is violent; in two or three hours it can be made out with great ease. Red blood corpuscles become segregated and in the clear spaces spirochetes will have gathered and can be seen moving

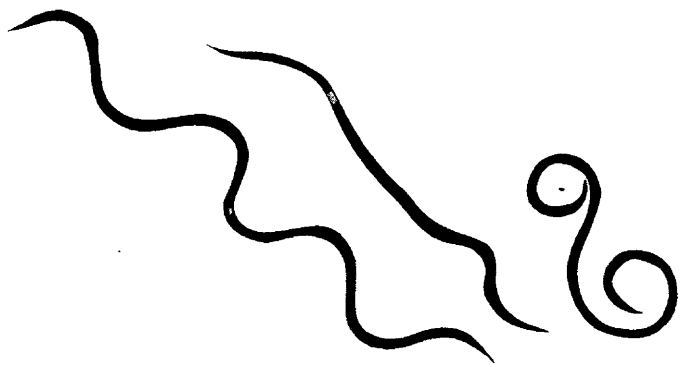


Fig. 5.—Spirochetes from blood of Rat 31, seen at height of the infection, showing one normal double form A, one stretched out form B, and one recurved form C, in one cover slip preparation.

in various directions. Frequently a spirochete may be seen to be attached to a red blood corpuscle by an invisible flagellum; the margin of the red blood corpuscle then becomes pulled out and the whole cell distorted, even dragged away down-stream with a spirochete evidently attached, yet always keeping nearly a red cell's diameter away. When the red cell is fixed, the attached spirochete may be seen to rotate to the right, apparently trying to go forward, then after a pause the rotatory movement is apparently reversed; this reversed movement frequently carries the spirochete away from the red blood corpuscle a short distance, though it still remains attached to it. During the pause in the rotation there is an opportunity to observe its outline and to determine whether the spirochete

is a spiral cylinder or ribbon. This question is difficult to determine when the spirochete is met in cross-section. During the pause just mentioned some spirochetes appeared to be spiral ribbons. One appeared to be curved in one plane only—on the flat. In fresh citrated blood preparations the observations were made with artificial light, Zeiss 2 and 3 mm. objectives and 6, 8, 12 and 18 oculars. Under these conditions, while the various motions of the spirochetes were made out with ease, the impression received as to the topography of the spirochete was probably illusory. Any one who has watched the polished balls on the governor of a stationary engine, when illuminated by artificial light, knows how by a slight effort of the will the balls may be seen to rotate to the right or to the left; just so with the spirochetes. One is not absolutely sure that it is rotating to the right or to the left, but one feels sure that it is rotating. Perhaps the physicists may throw some light on this matter by telling us what the optical effect on the observer will be under, say, two hypothetical conditions: light from an incandescent lamp reflected by a mirror through substage condenser, glass slide, blood serum, through the transparent body of a spirochete having a refractive index of $1 + x$ and a diameter of 0.3 micron under the following conditions: (a) the body of the spirochete rotating in a plane transverse to the rays of light, the body being a spiral ribbon; (b) the body being a spiral cylinder. I believe the question involved insoluble by a mere inspection of fresh or stained specimens.

At the end of three hours in citrated preparations the spirochetes frequently appeared attached to a red blood corpuscle at one end. The movements of the attached spirochete cause the red blood corpuscle to change its outline to a very marked degree, frequently pulling it out into a pear-shaped body. The spirals or curves of the spirochete push and pull the red blood corpuscles out of contour, giving one the impression that the spirochete is possessed of great rigidity.

Besides the rotary movement, there is an undulating movement or tremor; the spirochete does not always move rapidly away from the field, but rather rotates and trembles in one spot and then darts off a short distance within the field.

In citrated blood preparations, at the height of an infection many spirochetes may be seen in a prefragmenting stage; their motion is slow and there is a tendency to wreath or ring formation, their two extremities becoming attached and they may be seen to shake and tremble, the body of the spirochete apparently breaking up, while the fragments are apparently held in apposition by a sheath or envelope. The effect is very much like that of an agitated chain.

In citrated blood preparations, the spirochetes, after a short time, are commonly found in the clear spaces formed by the segregation of the red blood corpuscles.

The undiluted fresh blood preparation at room temperature, 76 to 82 F., presented on one occasion, at the end of sixteen hours, about the same picture that the citrated blood did at the end of four hours.

Aerotactism, such as is observed in fresh water flagellates, was never noticed. Aerotactism refers to the air-hunger observed in water flagellates from surface pellicles when studied in cover-slip films. These protozoa may be found in large numbers close to the margin of air bubbles.

Agglutination is seen in fresh preparations at the height of an infection; the parasites may adhere to one another end to end or side by side; the bodies of the spirochetes are probably fastened by entangled flagella.

When fresh and stained preparations are made from the same drop of blood, the fresh specimen frequently shows all regular forms, while

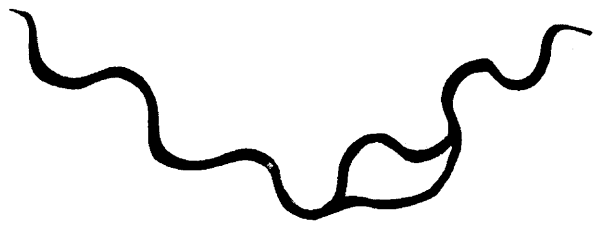


Fig. 6.—Blood from Mouse 8. The clear space in the middle is suggestive of transverse fission and the loop is suggestive of longitudinal fission.

the stained specimen may contain many bodies curved or straightened out. Drying and staining processes, therefore, may alter the regularity of contour of the spirochetes.

In stained preparations, the length, contour, number of spirals, the presence of flagella and staining qualities of the spirochetes were noted. Great variation in the regularity and number of curves was noted. In one film coarsely and finely curved forms were seen, as well as coarse and fine curves in one organism. Irregular curvatures, straightened and recurved forms were frequently encountered.

Toward the end of an infection achromatic spaces in the spirochete, or the prefragmenting phase, were frequently seen; sometimes as many as four spaces could be counted in one spirochete, the length of which was 8 or 10 microns. These achromatic spaces are not to be confounded with the achromatic space which is present in the double forms, which

appears just before transverse fission, and is always centrally located and wider than the former.

The act of subdivision was not observed. The centrally located achromatic space in the double form is strongly suggestive of transverse fission, while a very few loop forms with an apparent longitudinal split were suggestive of longitudinal fission. The achromatic zones are well demonstrated in films stained with acetone gentian violet. In these preparations, curiously, there is never the least semblance of a periplast, while in the films stained with some modification of the Romanowsky stain a faint line, bridging over the achromatic zone, is noted. Some films which were stained with Muir's flagellum stain showed spirochetes with a single, terminal, faintly staining flagellum, its diameter decreasing toward its free extremity. Occasionally, in a film stained with acetone gentian violet, a ring form could be seen. In these instances, in which the free extremity of a flagellum was in relation with the opposite end of the spirochete, a little more than one-third

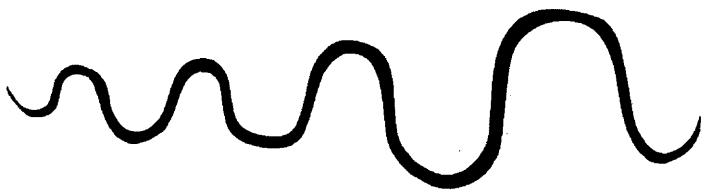


Fig. 7.—Spirochete from blood of Rat 66, showing large and small curves in one individual.

of the circumference of the ring would be more faintly stained than the rest and tapering, corresponding to a flagellum. In several of the acetone gentian violet preparations the appearance of spiral ribbons seemed unmistakable. In double infections of spirochetes and trypanosomes films were stained in various ways; yet, whenever the chromatin filament of the trypanosome was in evidence, nothing at all like this structure could be made out in the spirochetes. *A priori,* why should the spirochete of relapsing fever be provided with a chromatin filament? The spirochete is a rigid spiral, staining homogeneously, in every way like a bacterium, never like a protozoon. Its peculiar motion is probably due to rotation caused by a lashing contracting movement of the flagellum inducing a rotation of the body of the spirochete, the rotation being favored by its flat or ribbon-shaped contour. Its body is rigid and not flexuous to the degree observed in trypanosomes. The spirochete stains homogeneously, except just before its disappearance from the peripheral blood, when achromatic spaces or zones appear—the presegmenting

phase. It is stained purple with polychrome blues as bacilli are. Blue vacuolated cytoplasm with chromatin granules, such as one sees in protozoa, is never noticed, and it is decolorized by Gram's method. A very striking characteristic of spirochetes stained by gentian violet is the decolorization of the spirochete, either at once or after a few days, on exposure to diffuse sunlight. Films from Rat 31 colored with acetone gentian violet stained intensely and showed the achromatic zones beautifully. A few days later the spirochetes were completely decolorized and could be made out only by their sinuous transparent bodies; the red blood corpuscles and leucocytes, however, retained the gentian violet perfectly. A similar result was observed whenever a film was stained with Muir's too old flagellum stain, even when it had been previously stained with a polychrome blue stain.

SIZE

Measurements of length were made from stained preparations of the blood of rats and mice. Single spirochetes in which no achromatic zone could be detected measured from 7.2 to 13.2 microns. Double spirochetes having a central achromatic zone measured from 13.2 to 17 microns. The number of complete S-forms in single spirochetes having no achromatic zone varied considerably in the same film. The above measurements were made from spirochetes seen in the blood of Mouse 1, at the first remove from man.

ANIMAL REACTIONS

In man the disease is characterized by recurring paroxysms of fever lasting, usually, from twenty-four to forty-eight hours. The temperature rises slowly or rapidly to 103 or 104 F., occasionally to 105 F. There is always a chill and the access can not be distinguished by inspection from a malarial paroxysm. The duration of the first paroxysm is not definitely known, but is probably three days. The temperature falls slowly or suddenly to normal with the subsidence of symptoms, when, after a period of from three to eight days, oftenest five or six days, there is another paroxysm resembling the first and lasting about the same length of time. The usual number of paroxysms is three. There may be abortive paroxysms in which the temperature rises a degree or two above normal for two or three hours. During the febrile paroxysms a a few spirochetes appear in the peripheral blood. Very rarely a spirochete may be detected during the afebrile period. The spleen is always enlarged and tender; the tongue, pale and coated. Epistaxis occurs occasionally during febrile paroxysm (during the first relapse in case of F. S.). The pulse and respirations are accelerated during the febrile

paroxysm. Ten or twelve hours after the drop in temperature there is profuse sweating. Several cases were complicated by diarrhea and colitis with pus and blood in the stools. There have been no deaths in uncomplicated cases.

ANIMAL REACTIONS: WHITE MICE

Among animals the white mouse is the most susceptible. Mouse 3 was infected with blood containing not more than one or two spirochetes. As it was found that a drop or two of infected mouse-blood was sufficient to infect another mouse, and as it was desired to observe the progress of the disease without unnecessary sacrifice of the limited number of animals at my disposal, blood from the tail of an infected mouse was expressed into a Petri dish containing citrated saline solution to prevent clotting; this was injected into the peritoneal cavity of the next mouse in the series. Fifteen mice were infected in this way.

The disease in white mice is very much like that in man. There were three paroxysms, during which spirochetes appeared in the peripheral blood. The temperature of the smaller animals was not taken on account of the wide normal daily variation and the influence of various undetermined factors in causing irregularities in the temperature curve and making its record valueless. The temperature of the larger animals—goats, monkeys and dogs—was found to vary similarly.

The number of paroxysms, number of spirochetes in the blood and the period of incubation depend on a number of factors. The period of incubation by the method of inoculation used varied from twenty-four hours to six days. Mouse 3, inoculated from Mouse 1 with five drops of tail blood containing one spirochete to 500 fields, showed one spirochete in 100 fields after a period of twenty-four hours. Mouse 13 was inoculated from Mouse 12, when the blood from No. 12 showed no spirochetes in 500 fields. The period of incubation in No. 13 was five days and the infection was a typical one with three paroxysms.

Mouse 1 was inoculated directly from the patient during his second paroxysm. No spirochetes were seen until the third day, when there were two to a field. Two c.c. of uncitrated blood were used to infect Mouse 1. The infection was unusual, inasmuch as the period of incubation, in spite of the large amount of blood used, was prolonged, yet the spirochetes remained constantly in the animal's blood, without intermission, for ten days, when death occurred. Continuous infections have been observed in two animals, a white mouse and a black rat. The issue was fatal in each case. During the course of the infection in Mouse 1 there was a high degree of polychromatophilia, basophilic granular degeneration of the red blood cells, leucocytosis and a marked diminution in the number of red blood cells. Dyspnea and convulsions occurred before death. At autopsy the spleen was greatly enlarged and there were two encysted larval tapeworms (*Echinococcus murinum*) in the liver. The day before death spirochetes were rapidly increasing in the peripheral blood, there being 10 per field, with considerable agglutination. The mouse, therefore, died at the end of a paroxysm. (See chart of Mouse 1, Fig. 8.)

Mouse 2, inoculated intraperitoneally with 2 c.c. uncitrated blood directly from patient, had five and possibly six paroxysms with recovery. There was a marked remission during the first paroxysm, and as the blood was not examined on October 15 it is not known whether spirochetes were absent on that day or not. (See chart of Mouse 2, Fig. 8.)

Mouse 3, inoculated from Mouse 1, had a period of incubation of twenty-four hours and four paroxysms.

Mouse 4, inoculated from Mouse 2, had a period of incubation of forty-eight hours, only two paroxysms, and died six days after the disappearance of spirochetes from the peripheral blood. At autopsy the spleen was not much enlarged, and spirochetes were absent from the peripheral blood. During the progress of the infection there was much polychromatophilia and basophilic granular degeneration of the red blood cells. This basophilic granular degeneration has probably been mistaken for the resting forms of spirochetes. (See chart of Mouse 4, Fig. 8.)

Mouse 6, inoculated from Mouse 1, had three paroxysms and died three days after the disappearance of spirochetes from the peripheral blood. (See chart of Mouse 6, Fig. 8.

Mouse 9, inoculated from Mouse 3, had a period of incubation of 48 hours, two paroxysms, and was accidentally killed thirteen days after second and last paroxysm, during which period paroxysms were absent from the peripheral blood. (See chart of Mouse 9, Fig. 8.)

Mouse 12, inoculated from Mouse 6, had a period of incubation of forty-eight hours and three paroxysms, while spirochetes remained absent thirteen days after the last paroxysm. (See chart of Mouse 12, Fig. 8.)

The amount of blood used to inoculate these animals was about five drops. This was caught in a sterile Petri dish containing 5 c.c. of sterile, citrated, normal saline solution, and injected immediately intraperitoneally.

The spirochete counts were made from smears on cover-slips stained with Leishman's or Hasting's stain. A field was that which was obtained by a Zeiss 2 mm. objective and No. 6 ocular. When spirochetes were sparse at least 300 fields were counted; frequently 500 or a thousand; sometimes, three or four films.

It will be noted that in the initial paroxysm there is a greater number of spirochetes per field per day and the duration of the paroxysm is somewhat longer than the subsequent ones. The first paroxysm lasts about three days. The period between the first and second paroxysm is from four to five days. The second paroxysm lasts from two to three days; this may be followed by an intermission of four or five days, and followed by a third relapse lasting one, two or three days.

In severe infections the period of intermission between relapses may be shortened to one day. During the relapses the mice are slightly indisposed and take little food. After taking the blood films or removing blood for inoculation the tails of some infected mice bled profusely and required an application of collodion.

Mouse 1 lost considerable blood, which probably helped to cause some of the blood changes noted above and may have influenced the character of the infection and the fatal issue.

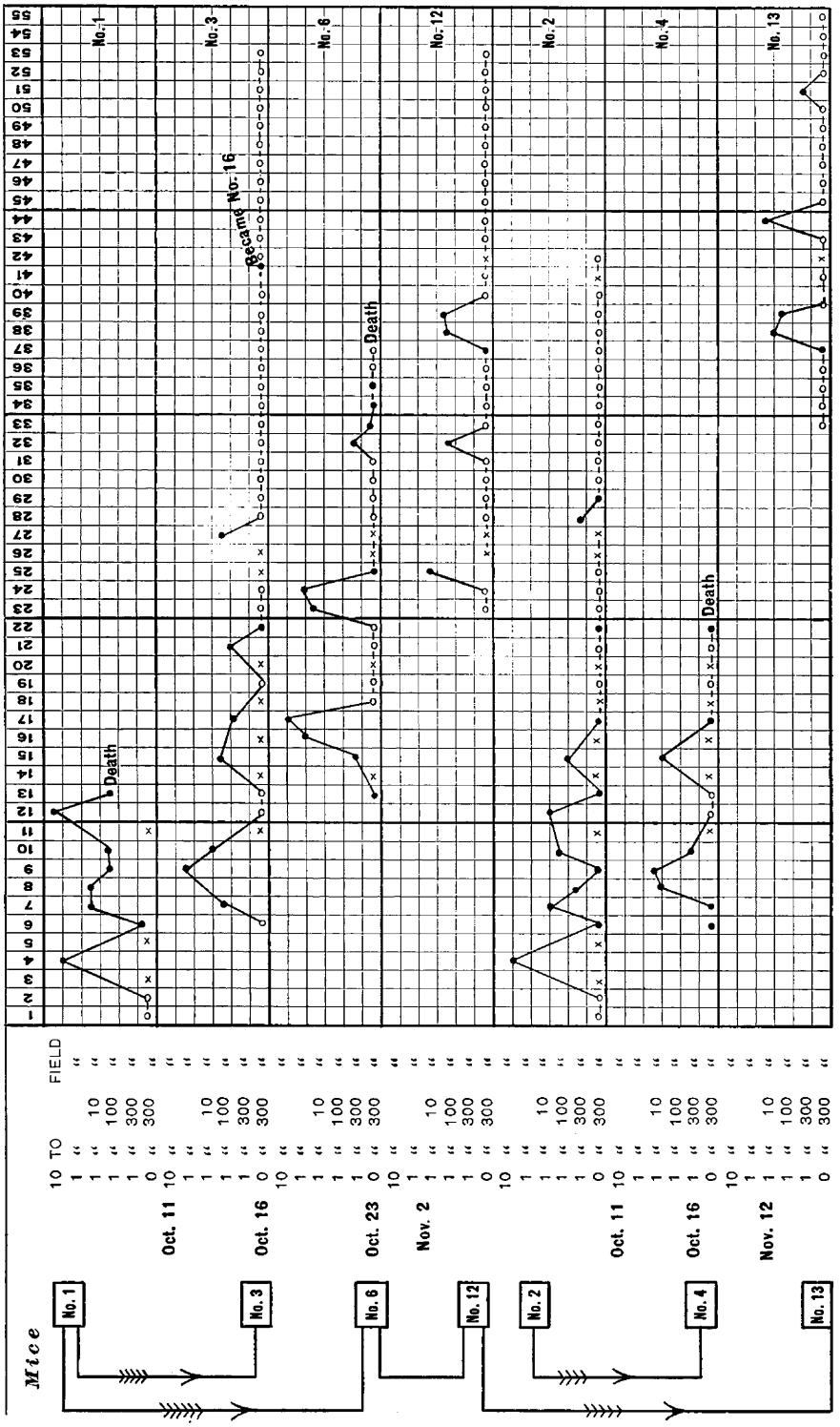


Fig. 8.—Chart of group paroxysms in white mice, showing the numbers of spirochetes in the peripheral blood. Circles (O) signify no spirochetes in 300 or more fields; crosses (×), blood not examined on this day.

Of the deaths in mice all but two occurred several days after spirochetes had disappeared from the peripheral blood. One of these had, in addition to the heaviest infection noticed in mice, a profound anemia. Most of the dead mice had encysted larval tapeworms (*Echinococcus murinum*) in the liver.

An attempt was made to ascertain if there was a definite or constant periodicity in the relapses which might be correlated with a life cycle of the parasite, the period of which might be suggested if the paroxysms appeared on similar days in the original animals and in those subinoculated from them; there was, however, no conformity between such paroxysms. (See Fig. 8, chart of group paroxysms.)

ANIMAL REACTIONS; WHITE RATS

The infection in white rats is characterized by a single paroxysm lasting two or three days followed by the rapid disappearance of spirochetes from the blood stream. The disappearance is not complete, as will be shown later, but it is rarely possible to demonstrate spirochetes in the peripheral blood twenty-four hours after the height of the infection.

The period of incubation in white rats depends on the number of spirochetes injected, among other factors. When minimal amounts of tail blood are injected the period of incubation is from two to five days. When larger amounts of heart's blood are used spirochetes may be demonstrated sixteen to eighteen hours afterward.

Peripheral (tail) blood uniformly requires a longer period than heart's blood to produce an infection, and it may be possible that bodies antagonistic to the multiplication of the spirochetes are present in larger quantities in peripheral blood than in heart's blood.

Several rats were examined during periods of 14, 15 and 16 days after the paroxysm for the presence of spirochetes, but none were ever demonstrated, save in one case—Rat 37—when one atypical, straightened-out, swollen form was seen on the third day after the height of the infection.

When minimal amounts of infected peripheral blood were injected spirochetes would appear, usually after two or three days, in small numbers—viz.: one spirochete to from 13 to 500 fields. On the following day one spirochete to two fields might be counted, after which they would immediately disappear.

TABLE 3.—COURSE OF INFECTION IN RAT 22

Inoculated Dec. 4, 1907, a. m., from the tail blood of Mouse No. 17, when this blood contained one spirochete to 100 fields, about 15 drops of blood being used.

12/ 5—0 spirochetes in 300 fields	12/11—0 spirochetes in 300 fields
12/ 6—0 spirochetes in 300 fields	12/12—0 spirochetes in 300 fields
12/ 7—1 spirochete in 300 fields	12/13—0 spirochetes in 300 fields
12/ 8—1 spirochete in 2 fields	12/14—0 spirochetes in 300 fields
12/ 9—0 spirochetes in 300 fields	12/15—0 spirochetes in 300 fields
12/10—0 spirochetes in 300 fields	

The injection of larger quantities of heart's blood, or more severely infected blood from rats, was followed by the appearance of one spirochete to 25 or 100 fields within twenty-four hours, and after forty-eight hours from 25 to 100 spirochetes in 100 fields. At the end of seventy-two hours there was either complete disappearance or the presence of spirochetes in numbers of from 1 to 7 or more per field. On the fourth day, or at the end of ninety-six hours, it was extremely rare to find spirochetes in the peripheral blood. Rat 20 had one spirochete per 100 fields on the fourth day of the infection. Ordinarily the paroxysm lasted three days. (See Table 4, showing course of infection in Rat 20.)

The duration of the paroxysm and the number of spirochetes appearing in the peripheral blood of the inoculated rat do not depend entirely on the number of spirochetes injected, as the following experiment shows:

TABLE 4.—COURSE OF INFECTION IN RATS 19 AND 20

Inoculated 11/26/07 from heart's blood of Rat 18. Rat 18 had one spirochete to 500 fields when examined in the a. m. When examined six hours later, at time of death and subinoculation of Rats 19 and 20, there were two spirochetes to 100 fields in heart's blood.

Amount inoculated 0.3 c.c.	Amount inoculated, one-third as much as Rat No. 19, or 0.1 c.c.
11/27—1 spirochete to 50 fields	11/27—1 spirochete to 33 fields
11/28—1 spirochete to 25 fields	11/28—1 spirochete to 6 fields
11/29—2 spirochetes to 1 field	11/29—1 spirochete to 1 field
11/30—0 spirochetes to 300 fields	11/30—1 spirochete to 100 fields
12/ 1—0 spirochetes to 300 fields	12/ 1—0 spirochetes to 300 fields
Fourteen daily successive examinations were made without finding spirochetes.	Fourteen daily successive examinations were made without finding spirochetes.

In this experiment Rat 20, receiving at time of inoculation one-third as much infected blood as Rat 19, had a slightly severer infection and a longer paroxysm.

White rats may appear very slightly indisposed for half a day during the paroxysm. Only one death occurred; this was after the disappearance of spirochetes from the peripheral blood and was due to pneumonia.

White rats were infected directly from two different human sources, and in both instances the period of incubation was forty-eight hours, although large amounts of blood were used—2 c.c. and 5 c.c. of undiluted blood.

It is to be noted that during the course of the infection there are from thirty to fifty times as many spirochetes in rat's blood as in man's blood.

ANIMAL REACTIONS: WILD RATS

The gray rat (*Mus decumanus*), when kept in small cages for convenience in handling, does not survive captivity and the rather rough handling necessary in making inoculations. Most of the rats of this variety brought to the laboratory and confined in small cages died within a few days; consequently they were not suited for a study of this infection.

The black rat (*Mus rattus*) may be kept for weeks and months in small cages. Several black rats have been inoculated, and, as these rats were harboring *Trypanosoma lewisi,* an opportunity was offered for studying the parallel infection of trypanosomes and spirochetes.

Wild Rat 5 was inoculated intraperitoneally with one drop of blood from Mouse 5 and died on the third day. Spirochetes were not found at any time during the three days.

Wild Rat 25 was inoculated from Rat 24 when the latter had 8 spirochetes per 100 fields, using citrated heart's blood. This rat had a mild continuous in-

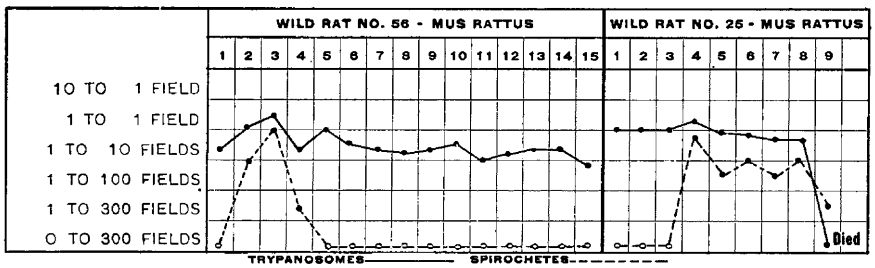


Fig. 9.—Parallel infection of spirochete and trypanosomes.

fection lasting six days, after an incubation period of seventy-two hours. Death occurred at the end of the sixth day. This observation may have some importance on account of the possibility of a suctorial intermediary host conveying the spirochete from a rodent having a slight continuous infection to man.

Wild Rat 56 was inoculated from Rat 51 when the latter had 250 spirochetes to 100 fields, using citrated heart's blood injected subcutaneously at the base of the tail. This rat had an infection lasting three days after an incubation period of twenty-four hours.

ANIMAL REACTIONS: MONKEYS

The infection in monkeys of the old world, genus *Macacus,* is similar to that in man and white mice, inasmuch as there is more than one paroxysm.

Macacus 65 was inoculated on February 4 from Rat 63 when No. 63 had 219 spirochetes to 100 fields, using citrated heart's blood. One c.c. of blood was inoculated intraperitoneally. Spirochetes appeared after twenty-four hours and remained in the peripheral blood three days, disappearing for five days and then reappearing for three days in about the same numbers as in the first paroxysm. There was a slight diarrhea noticed after the second paroxysm. During each

paroxysm there was some bleeding from the gums near two carious, upper, middle incisors. This blood from the gums contained spirochetes. The animal did not appear sick at any time during the course of the disease.

The blood changes were slight polychromatophilia of red blood cells with some fine and coarse basophilic granular degeneration. These were noticed after the first paroxysm.

At the beginning of the second paroxysm there was a slight drop in the temperature followed by a rise of 4 degrees above normal or to 106.4 F. The temperature was only slightly altered during the first paroxysm and was not greater than the variations noted during health. (See Fig. 10.)

ANIMAL REACTIONS: VARIOUS

A turtle, pigeon, frog and guinea-pig resisted an infection by infected rat's blood, which would have been sufficient to produce the disease in white rats. Two dogs and a goat were inoculated from human sources with blood obtained during paroxysms, but no infection resulted. It might have been possible to infect these animals if larger amounts of

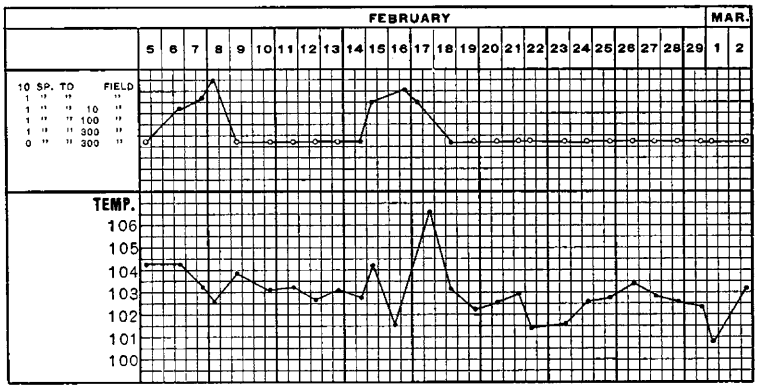


Fig. 10.—The spirochetal and temperature curve in Macacus 65. Note that there were only two paroxysms and that the temperature was not disturbed during the first one.

severely infected rat's blood could have been used, but the number of white rats at my disposal was so small that the greatest economy had to be exercised in subinoculations.

A raccoon has also resisted infection.

Novy and Knapp have shown that in the reactions of spirochetes and their hosts certain substances are produced which help to cause the disappearance of the spirochete from the blood and establish a qualified immunity against subsequent infections.

At the height of an infection it is evident that some change is taking place in the spirochete. Stretched out and irregularly curved forms are seen; many of these have achromatic zones. The movement of the

spirochete is less rapid, prefragmentation and chain formation are noticed and agglutination always occurs. Agglutinins and lysins are undoubtedly formed, but there is not a complete dissolution of the spirochete at this time. The sheath or periplast always appears to be intact in organisms seen in the blood stream. It is the internal substance of the spirochete that become fragmented (Fig. 11). Besides this, the spirochete apparently is not completely broken up in the peripheral blood stream, for fragments are never seen there, either free or in any type of leucocyte. If, however, as was observed by Levaditi and Manouélian,[23] with the spirochete of tick-fever, we sacrifice an animal at the height of an infection or a day or two later, it is possible, by Levaditi's silver impregnation methods, to demonstrate that the disappearance of the spirochetes from the blood stream has been due to the fact that

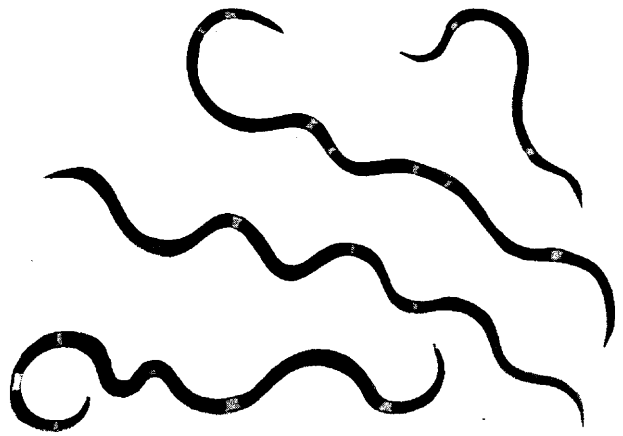


Fig. 11.—Spirochetes from blood of Rat 31 seen at height of infection, showing fragmentation of the protoplasmic substance, and an intact sheath on periplast.

they have been engulfed by endothelial cells lining the liver capillaries. Throughout the liver large, swollen endothelial cells may be seen, their cytoplasm dotted with spirochetes in all stages of fragmentation. From the appearance of the spirochetes within the endothelial cells it is probable that they are engulfed whole and that separation of the fragment occurs in the phagocytic cells by a solution of the periplasts.

23. Levaditi and Manouélian: Ann. de l'Inst. Pasteur, 1907, xxi, 295. Uhlenhuth and Haendel: Arb. a. d. k. Gsndhtsamte, 1907, xxvi, H. 1, 1. Manteufel Arb. a. d. k. Gsndhtsamte, xxvii, H. 2, 327. Schellack, C.: Arb. a. d. k. Gsndhtsamte xxvii, H. 2, 364.

The spirochetes disappear very rapidly from the peripheral blood after the height of the infection, but the disappearance is not complete, for on three occasions it has been possible to infect rats when no spirochetes could be demonstrated in films of the infective blood. In these instances the blood used was taken about twenty-four hours after the apparent disappearance of parasites from the peripheral circulation.

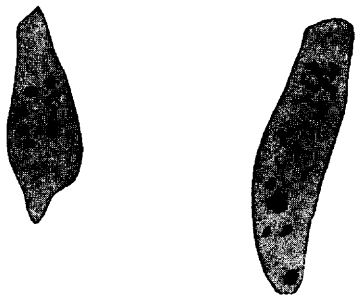


Fig. 12.—Endothelial cells from capillaries of the liver showing phagocyted spirochetes from Rat 26 killed at height of infection which had lasted three days. Levaditi pyridin preparation.

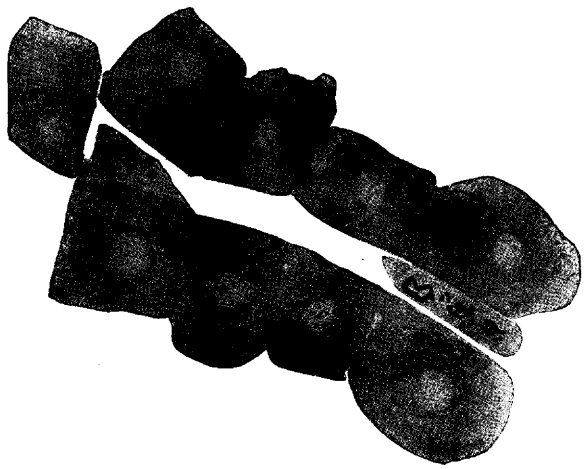


Fig. 13.—Section of liver of Rat 26, showing two endothelial cells of a capillary, containing phagocyted spirochetes. Levaditi pyridin preparation.

The following experiments illustrate this point:

Rat 57 was inoculated from Rat 55 when the latter had no spirochetes in 500 fields. Spirochetes appeared in the blood of No. 57 four days after inoculation.

Rat 47 was sacrificed when spirochetes had disappeared from the peripheral blood, which was injected into Rat 48, spirochetes appearing in the blood of Rat 48 on the sixth day, at the same time another rat, 49, was inoculated with an

emulsion of a portion of No. 47's liver in citrated saline solution; spirochetes appeared in No. 49's blood after an incubative period of four days, or two days less than in No. 48.

In this last experiment it is to be noted that the liver emulsion, although containing but traces of blood, the animal having been previously bled to furnish heart's blood for the inoculation of Rat 48, was more infectious than heart's blood from the same animal.

This experiment is interesting in view of the phagocytosis of spirochetes by liver endothelial cells. These experiments, besides showing the infectiousness of decline blood or of postdecline blood, show that phagocytosis plays an important part in the bodily defence.

If suitable emulsions containing liver endothelial cells can be prepared, it might be possible to demonstrate the production of specific opsonins during the infection.

IMMUNITY

During the course of the infection in white mice, white rats and wild rats a protective mechanism is developed which brings the infection to a close and confers a qualified immunity against a subsequent attack. The protective mechanism is one largely of phagocytosis, aided, as shown by Novy and Knapp, by specific agglutinins and lysins. The actively acquired immunity in white rats has lasted as long as forty-one days in individuals of the same strain. White mice have remained immune to the same strain sixty days; in fact, all attempts to reinfect animals with the same strain as that from which they were infected have failed, as the following experiments illustrate:

Active Immunity: Mice

Mouse 16 (inoculated December 20 from Mouse 14 with blood containing 1 spirochete to 38 fields) was formerly Mouse 3; spirochetes had been last seen in the blood November 7 or thirteen days previously. The immunity was sufficient to protect, spirochetes not being found in the blood during thirteen daily examinations.

Mouse 61 (inoculated January 28 from Rat 59 when No. 59 had 18 spirochetes to 10 fields, using citrated saline heart's blood intraperitoneally) was formerly Mouse 13, having had spirochetes in its blood for the last time on November 30. The immunity was complete, as No. 61's blood failed to show any spirochetes during subsequent daily examinations continued for nine days.

Active Immunity: Rats

Rat 43 (inoculated December 24 from Rat 38, when No. 38 had 420 spirochetes to 100 fields, using citrated heart's blood intraperitoneally followed by 10 drops saline solution of orcein) was formerly Rat 27, having had spirochetes in his blood for the last time December 16. The immunity was complete, spirochetes failing to appear in No. 43's blood during the nine days subsequent to the last inoculation.

Rat 58 (inoculated January 25 from Rat 57 which had 3 spirochetes to 100 fields, using citrated saline heart's blood intraperitoneally) was formerly Rat 45,

having had spirochetes in his blood from December 29 to 31 inclusive. The immunity was complete, spirochetes failing to appear during thirteen days.

Rat 60 (inoculated January 28 from Rat 59 when No. 59 had 18 spirochetes to 10 fields, using citrated heart's blood intraperitoneally) was formerly Rat 33, having had spirochetes in his blood from December 16 to 18 inclusive. The immunity was complete, spirochetes failing to appear during ten days.

SPECIFIC CHARACTER OF STRAINS

The immunity conferred by one strain of spirochetes is not sufficient to protect against a second strain of the same species having a different origin. For example, I found that, while it was impossible to reinfect white mice or white rats with strain A, the one used to infect them originally, it was possible to infect them by using strain B. The history of these two strains has been given above. Strain A was obtained from Case 3 Oct. 11, 1907, during the antepenultimate paroxysm, passed through 16 white mice and about 50 white rats. Strain B was obtained more recently from a case occurring in Colon Hospital. Blood was drawn during the penultimate paroxysm and has been passed through 8 rats and one mouse. The chief differences between the strains consist in the fact that B was more recently obtained from its human host and that it has had a shorter residence in animals. Clinically the patients supplying these two strains had the isthmian type of fever. Morphologically there are no differences between the two strains of spirochetes. Parallel inoculations of strains A and B into animals immunized to A have been made, with the result that animals which had recovered from an infection by A could be infected by B, yet would be immune to A. The period of incubation was sometimes prolonged and the number of spirochetes few; yet, on the other hand, the infection by B in two animals immunized to A was prompt and severe, and in one—Rat 67—the infection by A in an animal immunized to B was also prompt and severe.

In this experiment a rat infected by and presumably immune to strain B was promptly infected by strain A nine days after the disappearance of strain B. This proves that the difference in the activity of the strains is not one of relative virulence, but is due to there being specific strains with specific immunizing powers. This is the most important fact developed in the course of the investigation and is of the greatest value in connection with any attempts to derive a curative or preventive serum; it is suggestive in explanation of the fact mentioned by Vandyke Carter and others that one attack of relapsing fever does not confer a high degree of immunity, for an individual becoming infected by one strain in a given locality might well be non-immune with regard to other strains in that locality.

TABLE 5.—IMMUNITY AND INFECTION IN MICE 17 AND 13

Mouse 17, inoculated Nov. 23, '07 from Mouse 13, when No. 13 had 1 spirochete to 10 fields. Immediately after the inoculation 5 c.c. blood was drawn from Rats 1 and 7, which had an infection, and injected subcutaneously into Mouse 17. (Passive Immunity Experiment.)

11/24—	No	spirochetes	in	300 fields
11/25—	No	spirochetes	in	300 fields
11/26—	No	spirochetes	in	300 fields
11/27—	1	spirochete	in	5 fields
11/28—	1	spirochete	in	1 field
11/29—	No	spirochetes	in	300 fields
11/30—	No	spirochetes	in	300 fields
12/ 1—	No	spirochetes	in	300 fields
12/ 2—	No	spirochetes	in	300 fields
12/ 3—	No	spirochetes	in	300 fields
12/ 4—	1	spirochete	in	100 fields
12/ 5—	1	spirochete	in	300 fields
12/ 6—	1	spirochete	in	100 fields
12/ 7—	No	spirochetes	in	300 fields
12/ 8—	No	spirochetes	in	300 fields
12/ 9—	No	spirochetes	in	300 fields
12/10—	No	spirochetes	in	300 fields
12/11—	1	spirochete	in	69 fields
12/12—	1	spirochete	in	20 fields
12/13—	8	spirochetes	in	100 fields
12/14—	No	spirochetes	in	300 fields
12/15—	No	spirochetes	in	300 fields
12/16—	No	spirochetes	in	300 fields
12/17—	No	spirochetes	in	300 fields
12/18—	No	spirochetes	in	300 fields
12/19—	No	spirochetes	in	300 fields
12/20—	No	spirochetes	in	300 fields
12/21—	No	spirochetes	in	300 fields

Mouse 13, inoculated Nov. 12 from Mouse No. 12 when No. 12 had fewer spirochetes than 1 to 500 fields.

11/13—	No	spirochetes	in	300 fields
11/14—	No	spirochetes	in	300 fields
11/15—	No	spirochetes	in	300 fields
11/16—	No	spirochetes	in	300 fields
11/17—	1	spirochete	in	14 fields
11/18—	1	spirochete	in	75 fields
11/19—	No	spirochetes	in	300 fields
11/20—	No	spirochetes	in	300 fields
11/21—	Not examined.			
11/22—	No	spirochetes	in	300 fields
11/23—	1	spirochete	in	10 fields
11/24—	No	spirochetes	in	300 fields
11/25—	No	spirochetes	in	300 fields
11/26—	No	spirochetes	in	300 fields
11/27—	No	spirochetes	in	300 fields
11/28—	No	spirochetes	in	300 fields
11/29—	No	spirochetes	in	300 fields
11/30—	1	spirochete	in	300 fields
12/ 1—	No	spirochetes	in	300 fields
12/ 2—	No	spirochetes	in	300 fields
12/ 3—	No	spirochetes	in	300 fields
12/ 4—	No	spirochetes	in	300 fields
12/ 5—	No	spirochetes	in	300 fields

TABLE 6.—INFECTION AND IMMUNITY IN MICE B-8 (17) AND 61 (13)

January 28, Mouse 17 (which became Mouse B-8) inoculated from B-5 when B-5 had 14 spirochetes to 100 fields. using citrated heart's blood intraperitoneally.

1/29—	No	spirochetes	in	300 fields
1/30—	No	spirochetes	in	300 fields
1/31—	No	spirochetes	in	300 fields
2/ 1—	4	spirochetes	in	100 fields
2/ 2—	7	spirochetes	in	100 fields
2/ 3—	2	spirochetes	in	100 fields
2/ 4—	No	spirochetes	in	300 fields
2/ 5—	No	spirochetes	in	300 fields
2/ 6—	No	spirochetes	in	300 fields
2/ 7—	1	spirochete	in	200 fields
Sacrificed				

In this experiment the mouse, while having an actively acquired immunity to Strain A, was infected by Strain B. Controlled by Mouse 61 (13).

January 28, Mouse 13 (which became Mouse 61) inoculated with citrated blood from the heart of No. 59 when 59 had 18 spirochetes to 10 fields.

1/29—	No	spirochetes	in	200 fields
1/30—	No	spirochetes	in	200 fields
1/31—	No	spirochetes	in	200 fields
2/ 1—	No	spirochetes	in	200 fields
2/ 2—	No	spirochetes	in	400 fields
2/ 3—	No	spirochetes	in	200 fields
2/ 4—	No	spirochetes	in	200 fields
2/ 5—	Not examined.			
2/ 6—	No	spirochetes	in	200 fields

In this experiment the mouse having an actively acquired immunity to Strain A was still immune after 59 days to Strain A. Control to Mouse B-8 (17).

Rats 36 and 37, immunized to strain A, having had spirochetes in their blood on December 21 for the last time, were inoculated on January 24 by strain B. The animals inoculated by strain B showed a prompt and severe infection.

TABLE 7.—COURSE OF INFECTION IN RATS 36 AND 37

Rat 36, inoculated December 18, p. m. from Rat 30 when No. 30 had 148 spirochetes to 100 fields, using citrated heart's blood intraperitoneally.

12/19—	13	spirochetes	to	100 fields
12/20—	220	spirochetes	to	100 fields
12/21—	360	spirochetes	to	100 fields
12/22—	No	spirochetes	to	300 fields
12/23—	No	spirochetes	to	300 fields
12/24—	No	spirochetes	to	300 fields
12/25—	No	spirochetes	to	300 fields
12/26—	No	spirochetes	to	300 fields
12/27—	No	spirochetes	to	300 fields

Rat 37, inoculated December 18, p. m., from Rat 30 when No. 30 had 148 spirochetes to 100 fields, using citrated heart's blood intraperitoneally.

12/19—	8	spirochetes	to	100 fields
12/20—	140	spirochetes	to	100 fields
12/21—	120	spirochetes	to	100 fields
12/22—	No	spirochetes	in	300 fields
12/23—	No	spirochetes	in	300 fields
12/24—	1 atypical spirochete		in	300 fields
12/25—	No	spirochetes	in	300 fields
12/26—	No	spirochetes	in	300 fields
12/27—	No	spirochetes	in	300 fields

TABLE 8.—COURSE OF INFECTION IN RATS B-3 (36) AND B-4 (37)

January 24, Rat 36 (which became Rat B-3), inoculated with Strain B from Rat B-1 when B-1 had 8 spirochetes to 1 field, using citrated heart's blood intraperitoneally.	January 24, Rat 37 (which became Rat B-4) inoculated from Rat B-1 when B-1 had 8 spirochetes to 1 field, using citrated heart's blood intraperitoneally.
1/25—18 spirochetes to 10 fields	1/25—43 spirochetes to 10 fields 1/26—10 spirochetes to 1 field 1/27—No spirochetes to 300 fields 1/28—No spirochetes to 300 fields; died.

Furthermore a rat (B-2) immunized to strain B was inoculated eight days after the disappearance of spirochetes from his peripheral blood, with spirochetes from Rat 53, strain A. The rat became infected within twenty-four hours and experienced a moderately severe infection.

TABLE 9.—COURSE OF INFECTION IN RAT B-2

Rat B-2, inoculated Jan. 24 from No. B-1 when the latter had 8 spirochetes to 1 field, using citrated saline heart's blood intraperitoneally.

1/25— 2 spirochetes to 1 field	1/31—No spirochetes to 300 fields
1/26— 20 spirochetes to 1 field	2/ 1—No spirochetes to 300 fields
1/27—No spirochetes to 300 fields	2/ 2—No spirochetes to 300 fields
1/28—No spirochetes to 300 fields	2/ 3—No spirochetes to 300 fields
1/29—No spirochetes to 300 fields	2/ 4—No spirochetes to 300 fields
1/30—No spirochetes to 300 fields	

TABLE 10.—COURSE OF INFECTION IN RAT 67 (B-2)

2/4—Inoculated Rat B-2 (which became Rat 67) from Rat 63, Strain A, when the latter had 210 spirochetes per 100 fields, using citrated neck blood intraperitoneally.	2/5— 25 spirochetes to 100 fields 2/6—340 spirochetes to 100 fields 2/7— 32 spirochetes to 100 fields

PASSIVE IMMUNITY

Two attempts have been made to produce passive immunity by the subcutaneous injection of blood from a convalescent animal a moment after infected blood had been injected intraperitoneally.

Mouse 17, inoculated December 23 from Mouse 13, when the latter had one spirochete in 10 fields. Immediately afterward blood was drawn from the tail of Rat 7, formerly No. 1, 0.5 c.c. of blood being used and injected subcutaneously. Rat 7, formerly No. 1, had been infected with human blood strain A October 11, and was subsequently inoculated with blood from infected Mouse 2. This quantity of blood from an animal which had recovered was not sufficient to prevent a severe infection in Mouse 17 with three paroxysms after an incubation period of four days.

Rat 45, inoculated December 27, from Rat 41, when the latter had 450 spirochetes to 100 fields; inoculation intraperitoneal followed by a subcutaneous injection of 5 drops of heart's blood from Rat 31, whose blood had been free from spirochetes for nine days. This quantity of blood from a convalescent was not sufficient to prevent Rat 45 becoming infected after a period of forty-eight hours.

Blood from convalescents, therefore, in the quantities used is of no value in preventing an infection. The period of incubation, however, as in Rat 45 and Mouse 17, may be prolonged.

In these experiments very large amounts of infected blood were not used in the attempts to infect or reinfect animals. Only that quantity was used which was amply sufficient to cause the disease in a susceptible

non-immune animal. When very large quantities of blood are used the contained spirochetes become disseminated through the blood stream of the inoculated animal; they are merely diluted and their presence does not positively mean that they have multiplied within the blood stream.

HYPERIMMUNITY: PREVENTION

A goat has been gradually hyperimmunized, in addition to its natural immunity, by successive injections of infected white rat's blood, containing strains A and B, and one injection of blood from human source (Case 1). In all, 11 subcutaneous injections of infected blood have been given from three human sources.

TABLE 11.—INOCULATIONS RECEIVED BY GOAT

Inoculation	Blood From—	Inoculation	Blood From—
7/31, 1907	Lindoff, Case 1	1/ 2, 1908	Rat 46, Strain A
12/ 6, 1907	Rat 21, Strain A	1/11, 1908	Rat 49, Strain A
12/12, 1907	Rat 24, Strain A	1/16, 1908	Rat 51, Strain A
12/15, 1907	Rat 26, Strain A	1/24, 1908	Rat B-1, Strain B
12/24, 1907	Rat 38, Strain A	1/28, 1908	Rat 59, Strain A
12/27, 1907	Rat 41, Strain A		

Serum was collected from this goat February 6, 5 p. m., placed in a refrigerator at 51 degrees F. until the following afternoon, when it was used in the two following experiments:

Mouse 68, inoculated February 7, 3 p. m., from Rat 66, which had 90 spirochetes to 100 fields, using citrated peripheral tail-blood, 2 drops intraperitoneally, followed immediately by an intraperitoneal injection of 5 c.c. of hyperimmune goat serum. Spirochetes appeared in Mouse 68 on February 13, 2 spirochetes to 100 fields, or after the prolonged incubation period of six days. Most of the mouse's red blood corpuscles were diminished in size, strongly resembling goat's red blood corpuscles; the larger red blood corpuscles showed considerable polychromatophilia and fine granulations, but no basophilic granulation was noticed.

TABLE 12.—COURSE OF INFECTION IN MOUSE NO. 68

Date of Inoculation. 1908	Notes.
February 7, 3 p. m.	
February 8.	
February 9.	
February 10	No spirochetes in 400 fields; most of the red blood corpuscles are small—goat size, and the larger ones show polychromatophilia and granular degeneration—no basophilia.
February 11	No spirochetes in 300 fields. Red blood corpuscles same as on the 10th.
February 12	No spirochetes in 300 fields. Polychromatophilia increased; 60 per cent. of the r. b. c. are polychromatophilic. R. b. c. increasing in size.
February 13	2 spirochetes to 100 fields.
February 14	24 spirochetes to 100 fields.
February 15	No spirochetes to 300 fields.
February 16	No spirochetes to 300 fields. Polychromatophilia still marked. R. b. c. normal size.
February 17	2 spirochetes to 100 fields.
February 18	16 spirochetes to 100 fields.
February 19	12 spirochetes to 100 fields. Polychromatophilia diminishing. R. b. c. normal size. Died. Spleen enlarged. Autopsy otherwise negative.

TABLE 13.—COURSE OF INFECTION IN MOUSE 69

Date of Inoculation. 1908	Notes.
February 7	(3 p. m.)—From Rat No. 66, when the latter had 90 spirochetes to 100 fields, using 2 drops of citrated tail-blood, followed by a subcutaneous injection 0.7 c.c. hyperimmune goat-serum.
February 8.	
February 9	—No spirochetes in 300 fields.
February 10	—No spirochetes in 300 fields. Blood shows many small goat-size r. b. c. The larger normal size corpuscles are polychromatophilic and finely granular.
February 11	—No spirochetes in 300 fields.
February 12	—No spirochetes in 300 fields.
February 13	—20 spirochetes in 100 fields.
February 14	—No spirochetes in 400 fields.
February 15	—No spirochetes in 300 fields.
February 16	—No spirochetes in 300 fields. Polychromatophilia still marked. R. b. c. normal size.
February 17	— 3 spirochetes to 100 fields.
February 18	—12 spirochetes to 100 fields.
February 19	— 1 spirochete to 300 fields.
February 20	—No spirochetes to 300 fields. Polychromatophilia much diminished. R. b. c. normal size.
February 21	— 1 spirochete to 300 fields.
February 22	—No spirochetes to 300 fields—and none in 5 successive daily examinations.

In this experiment the period of incubation was greatly and equally prolonged in both mice, although Mouse 68 received about seven times as much serum as Mouse 69, and this intraperitoneally. Mouse 68 died during the second paroxysm, while Mouse 69 had a light primary paroxysm and a very mild secondary paroxysm. There was considerable blood destruction in each mouse. The supply of rats became exhausted at this time, so that it was not possible to increase the immunizing substances in the goat serum. A serum of this strength and character, in the quantity used, apparently merely prolongs the period of incubation, for, after the injection of the hyperimmune serum, the immunizing substances are very possibly slowly eliminated or rendered inert; the spirochetes then multiply as in an animal receiving a minimum amount of infected blood without the addition of immune serum.

It was necessary to use white mice in this experiment so as to avoid excessive hemolysis, the goat having been immunized with infected white rat's blood, which had rendered the goat serum hemolytic for the red blood corpuscles of the white rat.

HEREDITARY IMMUNITY

No opportunity arose for making observations on the immunity resulting from infection through the placenta.

PREVENTION BY VACCINATION

The results of experiments on the immunization of rats and mice with strains A and B show conclusively that curative or preventive serums, if derived at all, must be obtained by the inoculation of several strains. Polyvalent serums must be used and the question arises as to

whether it will be necessary to hyperimmunize an animal to each of the strains or whether, perhaps, single inoculations of a large number of strains may be sufficient to hyperimmunize.

A method of treatment not yet investigated is that of vaccination. This is suggested on account of the fact that phagocytosis plays an important part in the protective mechanism against the infection. In the investigation of this question it will be necessary to devise a practicable method for the cultivation of the spirochetes.

INFLUENCE ON THE INFECTION OF DRUGS ADMINISTERED HYPODERMICALLY

A few experiments were carried out to determine the influence, if any, of certain aniline dyes and other substances on the course of the disease in rats. The following substances were used:

Neutral red.
Congo red.
Orcein.
Saffranin.
Bordeaux red.
Sulphanilic acid.
Quinin and urea hydrochlorate.
Methylene blue.

Saturated solutions of these substances in normal saline solution were injected subcutaneously immediately after a peritoneal injection of infected blood had been given. A control rat was always inoculated at the same time. The result of such injection in almost every instance was an earlier and more severe infection than in the control rat which had not been treated with the dyes.

Methylene blue, when given in very large doses, prevented an infection, but the animals were profoundly poisoned by the drug and died on the second and third day. Dilute solutions of methylene blue were of no value in preventing an infection. The minimal protective dose of methylene blue could not be worked out at this time, but the use of methylene blue and allied substances seems to afford a favorable line for investigation.

In two experiments with orcein such heavy infections followed that it was thought that orcein might break down an actively acquired immunity; accordingly Rat 27, having passed through an infection between December 13 and 15, was inoculated from infected Rat 38 and treated hypodermically with 10 drops of saturated solution of orcein in normal saline solution; the animal failed to become infected. Orcein,

therefore, in the quantity used can not destroy an actively acquired immunity.

PARALLEL INFECTION WITH SPIROCHETES AND TRYPANOSOMA LEWISI, IN MUS RATTUS

There is no striking modification of a trypanosomal infection in rats by the parallel infection with spirochetes, nor, on the other hand, are the spirochetes affected by the presence of trypanosomes. In one instance (Rat 56) the trypanosomes increased at equal pace with the spirochetes, but the trypanosomes remained in the blood continuously for weeks after the spirochetes had disappeared. (See Fig. 9, chart of parallel infection of trypanosomes and spirochetes.)

TRANSMISSION OF RELAPSING FEVER

The transmission of African relapsing fever by means of infected ticks, *Ornithodoros moubata,* from monkey to monkey was accomplished by the late T. Everett Dutton and John S. Todd of the Liverpool School of Tropical Medicine in 1904. During the next year infected ticks were sent to Liverpool, and from these ticks monkeys were infected. Dutton and Todd were also successful in transmitting the spirochetes by the bites of young ticks newly hatched in the laboratory from eggs laid by infected parents.

Breinl, Kinghorn and Todd were unsuccessful in their attempts to transmit *Sp. duttoni* or *Sp. obermeieri* from monkey to monkey by means of bedbugs. The analogous disease in fowls, as determined by Marchoux and Salimbeni,[24] is transmitted by a tick, *Argus miniatus.*

In an interesting note concerning the earliest mentioned epidemics in Great Britain Begbie quotes Welsh, who wrote from Edinburgh in 1819, as follows:

> When acting as a clerk in the Royal Infirmary, in the course of four months my three colleagues, two of the young men in the apothecary shop, two housemaids and thirteen or fourteen nurses caught the disease and the matron and one of the dressers died of it. Since I left the Infirmary three more of the gentlemen acting as clerks, one of the young men in the shop and many more of the nurses have caught the infection. When it begins in a family we always expect more than one of them to be affected. I could mention instances of 4, 5, 6 and 7 being sent to the hospital out of one family; 8, 9 and 10 out of one room; 20 and 30 out of one stair and 30 and 40 out of one close, and this all in the course of a few months. Hardly any of the nurses, laundry-women or others coming in contact, either with the patients or their clothes, have escaped. At one time there were 18 nurses off duty from the fever. It appears sufficiently remarkable that, as specially noted by Dr. Connant in 1843 and 1844, laundry-women engaged in washing the clothes of the sick, though never brought into direct communication with the patients themselves, suffered frequently from the disease.

24. Marchoux, E., and Salimbeni, A.: Am. de l'Inst. Pasteur, 1903, xvii, 569.

After reading the accounts of epidemics of relapsing fever, together with our knowledge of the mode of transmission of tick fever and spirillosis in fowl and Mackie's observations in India, one can hardly escape the belief that the spirochetes of our local relapsing fever are conveyed mechanically or by an intermediary host in the person of some suctorial insect or acarid—fleas, bedbugs, ticks, lice or mosquitoes. The disappearance of relapsing fever for long periods after extensive epidemics from some localities, such as Scotland and Ireland; the failure of the disease to assume any extensive character in the United States and certain other places after introduction, and the limitation of the disease frequently to recently arrived immigrants or sailors and to seaport or neighboring towns, point strongly to a suctorial insect as the agent of transmission, and render it highly probable that the conditions for the existence of this host are not favorable in the United States and certain other places, either due to the fact that the suctorial host does not survive, or that it does not possess a suitable alternate host to subsist on when man is not available.

There is no evidence to prove the necessity of contact as in syphilis or in dourine or *mal de coit,* the trypanosomal disease of horses transmitted during copulation. Inoculation is accomplished with ease, for in two laboratories where animal inoculations have been made with *Sp. duttoni* and *Sp. obermeieri* two investigators and three assistants have been infected through an abraded skin during an autopsy on a severely infected monkey and by the bites of infected monkeys. Recently Mackie, of Bombay, has made an important contribution to our knowledge of the transmission of *Sp. carteri* by *Pediculus corporis.* Mackie studied an outbreak of Bombay relapsing fever at the Nasik Mission Settlement. He found that 14 per cent. of lice taken from the heavily infected boys' ward, 2 per cent. taken from the scantily infected girls' ward and 13 per cent. of artificially fed lice showed multiplication of spirilla in internal organs.

Attempts have been made at the laboratory here to infect animals by means of ticks, but all efforts have been unsuccessful. At the suggestion of Mr. C. L. Marlatt, of the Department of Agriculture, several specimens of *Amblyomma*[25] were tried with rats. These ticks, *Amblyomma dissimile,* taken from iguanas, and *Amblyomma cajennense,* taken from dogs, have long biting parts and are dislodged from their cus-

25. During excursions out into the bush and jungle, I find that specimens of *Amblyomma* (species undetermined) larval and adults, readily attach themselves and feed voraciously.

tomary host with difficulty, but they are probably too large to be used in the transmission experiments with rats.

The ecto-parasites of rats are generally quite small, fleas, mites and small ticks. The common dog tick of Panama, *Rhipicephalus texanus,* has shorter biting parts and is more easily dislodged. This tick will attach itself to rats, but unless the rat is immobilized the ticks are rapidly killed and eaten.

CONCLUSIONS

The relapsing fever of Panama is distinct from the analogous fever of Africa, Europe and Asia, although belonging to the same general class.

The micro-organism causing the local relapsing fever belongs to the group containing *Sp. obermeieri, Sp. duttoni* and *Sp carteri.*

This spirochete causes a recurring infection in man, monkeys (genus *Macacus*) and white mice, and single paroxysms in white and wild rats.

The animal reactions are similar to those obtained by Norris, Pappenheimer, Fleurnoy, Novy and Knapp, with the organism erroneously identified by the latter two as *Spirillum obermeieri.*

The blood of animals very recently recovered from an infection and that between paroxysms, where spirochetes are apparently absent from the peripheral blood, is infectious, and by analogy this affords a valuable means of diagnosis of the fever in man during the afebrile period by the inoculation of susceptible animals, mice and rats with patient's blood.

There is considerable variation in the morphology of the spirochete in the same strain and sometimes in the same smear.

Identification of spirochetes can not be made with certainty on morphologic grounds.

The mechanism of defense is largely that of phagocytosis by hepatic endothelium.

Infected animals sacrificed at different stages of the infection show, as the disease advances, an increasing number of fragmented spirochetes, engulfed by endothelial cells of the liver.

In animals which had recently recovered from an infection a liver emulsion is more infectious than heart's blood. This suggests the probable vitality and unity of fragments.

Infection by one strain of spirochetes is followed by a considerable degree of active immunity for that strain, but such immunity is not potent against another strain from a different source, although of the same species and from the same locality but from a different human host.

For the production of preventive and curative serums polyvalent sera derived from all the strains will probably be necessary.

The blood, in moderate amounts, of subjects which have recovered, is of no value in preventing infections in white mice and white rats.

Relapses may be explained by the multiplication of spirochetes in out-of-the-way places where they do not enter the portal circulation and can not be engulfed by liver endothelium.

Agglutination of spirochetes occurs at least twenty-four hours before the crisis in rats *in vitro* and *in vivo*.

This spirochete is probably a spiral ribbon and not a spiral cylinder.

The group of spiral-shaped micro-organisms needs reclassification on a basis of morphology, pathogenity and habitat.

This spirochete is more closely related to bacteria than to protozoa.

The rôle of the spleen is similar to that observed in anemia.

With suitable emulsions of liver substance and immune serums it should be possible to demonstrate specific opsonins.

The natural mode of infection is probably by means of an intermediary host—some suctorial insect or acarid, either directly or by means of an alternate host, such as a wild rat or other susceptible animal.

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