MEMOIR XXI
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CONTENTS

AN EXPERIMENTAL STUDY OF THE PARASITE OF THE AFRICAN TICK FEVER

(Spirochaeta duttoni)

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AN EXPERIMENTAL STUDY OF THE PARASITE OF THE AFRICAN TICK FEVER

(*SPIROCHAETA DUTTONI*)

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I. Introduction

The work embodied in the following pages was done with the spirochaete found to be the cause of the African tick fever by Dutton and Todd[8] in the Congo Free State. The parasites were brought home in infected ticks, and the strain was recovered from monkeys which had been infected through their bites. In their report,[8] Dutton and Todd state that the parasite is identical with *Spirochaeta obermeieri*, but further study of the animal reactions, and the results obtained by inoculating animals immune to tick-fever or European relapsing fever with the causative agent of the other disease, have shown that the spirochaete of tick fever differs from that of ordinary relapsing fever. We have, therefore, called the parasite of African tick-fever* *Spirochaeta duttoni* in honour of the late Dr. Dutton.[6]

We have been enabled to make a direct study of the disease in four cases contracted in these laboratories during the progress of the work by one of us and three of the laboratory assistants.

The morphology of the parasite will not be discussed fully in this report, but will be reserved for a future publication.

* After a study of two slides sent from these laboratories, and of the few experiments given by Dutton and Todd, Novy and Knapp, in a paper[24] published six weeks earlier than ours, considered that they had sufficient evidence to call this spirochaete a new parasite, which they accordingly named *Spirillum duttoni*.

II. Technique

The routine method of examining the blood for spirochaetes was the ordinary thick-film one. Two or three drops of blood were placed on a perfectly clean slide and then spread out over a surface 2 × 3 cm. After drying in the air, the films were fixed in the flame in the same way as a bacteriological specimen and the haemoglobin was removed by washing the films in distilled water. After being so treated, they became quite colourless and were then stained with Romanowsky's stain for half an hour. The stain was made in accordance with the directions given by Stephens and Christophers.[34]

A.	Medicinal methylene blue	1 part.
	Sodium carbonate	0·5 ,,
	Distilled water	100 ,,
B.	Eosin	1 : 1000 ,,

Before using dilute each solution with 19 parts of distilled water, and then mix in equal parts for staining. This method gave us better results than any other modification of Romanowsky.

In specimens prepared by this method the spirochaetes are well-defined and of a deep purple colour. The leucocytes are well-stained, while the red cells appear as mere shadows. The examination is much facilitated by reason of this.

For more detailed study, very thin films were made on slides heated to 37° C., in order to dry the blood film more rapidly; these were fixed in absolute alcohol and stained with the above modification of Romanowsky and by Giemsa's and Laveran's method. In our hands Marino's method did not yield satisfactory results.

When the presence of precipitates interfered with examination, it was found advisable to place the preparations in oil of cloves for a short time, and then in xylol after the excess of oil had been blotted off.

Carbol-fuchsin stains the spirochaetes very readily and intensely, but is not as valuable a stain as Romanowsky. Heidenhain's iron-haematoxylin was also used, but without any advantage, as it stains the spirochaetes uniformly black.

In order to study the structure of the parasite the wet film method was used. Perfectly clean slides were covered with an exceedingly

DISEASE.

TICK FEVER

Notes of Case

Name A.B.

Age

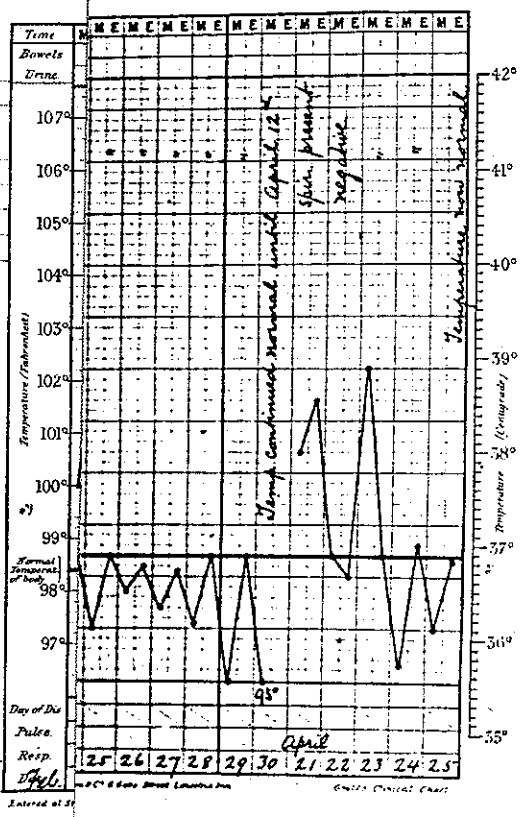
Diet

Case Book N°

Chart 1.

Date of admission

Result



thin layer of Mayer's albumen. A drop of blood was spread out as quickly as possible over the layer of albumen, and while still wet the slide was dropped into Flemming's fixing solution and left for ten minutes. In this the albumen was quickly coagulated and firmly fixed the blood to the slide. From the fixing solution the slide was passed through the different alcohols and stained.

Grawitz's method for cutting sections of the blood was also employed, but we were not successful in seeing spirochaetes in the sections.

The routine examination was made with a Zeiss 1/12 oil immersion and No. 4 ocular. The number of spirochaetes given throughout this publication refers to the number that were seen in the field thus obtained.

III. Description of Cases of African Tick Fever in Whites

CASE I (Chart I)—A.B. European, age 26. Felt sick February 1st, 1906. No prodomal symptoms were noticed other than profuse sweating the previous day with no apparent reason. On arising in the morning the patient felt tired, but was able to work as usual until the afternoon, when he was suddenly seized with aching in the lower limbs, slight headache and a marked degree of fatigue. At 2 o'clock, when first taken, the temperature was 100° F., and in the course of the next eight hours rose to 103° F. Preparations of the peripheral blood were made early in the afternoon, and the characteristic parasites were found. The symptoms became steadily worse as the temperature rose, and the most predominant was an intense pain in the region of the spleen. The slightest pressure there caused paroxysmal pain. A slight diarrhœa had been noticed during the day. In the evening the patient vomited once, and was finally forced to go to bed on account of the diffuse headache and very severe pain in the bones and spleen. An intense feeling of chilliness persisted in spite of all measures to relieve it. The physical examination, in addition to the general symptoms of fever, revealed slight enlargement of the spleen, the free border of which could be palpated one finger's breadth below the costal margin.

During the night the symptoms became more severe, and the patient was very restless and sleepless. On the morning of the 2nd

the temperature fell slightly, but there was no amelioration in the severity of the symptoms. Great thirst was noted and also slight coughing, which was suppressed on account of pain caused by it in the splenic region. In the afternoon the temperature again rose, and at 10 o'clock had reached 104·6° F., the highest reading observed during the disease. With the increase in temperature the pain became worse, and slight delirium set in. The symptoms continued without much amelioration until the night of the 4th when the crisis occurred, accompanied by heavy sweats. The temperature fell below normal and with this the characteristic symptoms disappeared completely, leaving the patient very weak and tired. A noticeable feature was the pseudocrisis on the third day of the disease, which was not followed by a corresponding improvement in the condition of the patient.

The parasites were found in preparations of the blood with the first rise of temperature, 1 to 10-25 fields, and the number increased slightly until, on the second day, from 2-3 per field were counted. At the time of the crisis one parasite to 150 fields was seen, and none were found in the preparations made on the following day.

The only changes found in the blood were a slight decrease in the number of the erthyrocytes, with a fall in the percentage of haemoglobin and a marked leucocytosis just before the crisis. In stained specimens, polychromatophilic degeneration of the red cells and a very decided increase in the number of platelets were noticeable.

After the crisis the patient regained his appetite, and the feeling of sickness slowly passed away.

On February 10th a relapse occurred. With the rise in temperature the original symptoms reappeared; the parasites, which had not been seen in the peripheral circulation during the interval, were again present and did not disappear until twenty-four hours afterwards. As in the original attack, the headache and pain in the long bones and spleen increased steadily in severity until the temperature reached its maximum. A pseudocrisis was observed on the 13th, but the symptoms persisted until the true crisis on the following day. Marked sweating accompanied this fall of temperature.

At intervals of eight days, four more relapses followed. Each time the symptoms came on very suddenly, increased in intensity

with the rise of temperature and disappeared with the crisis. Each succeeding relapse was less severe than the previous one.

Other remarkable features of the disease were the very great thirst experienced during the whole of the attack, and the loathing for food during the febrile period. In the intervals of apyrexia the patient recovered to a great extent, and was able to pursue his occupation.

After the last relapse on the 22nd March, the patient quickly recovered his usual health, and the only abnormality was a subnormal temperature in the morning, at times as low as 95° F. On the 22nd of April, another attack occurred in which the symptoms were of peculiar intensity. With the height of the fever the patient was very restless and delirious. After a pseudocrisis on the previous day, there was a critical fall of the temperature on April 24th. No further relapses have occurred since.

Treatment

As quinine and other forms of treatment of relapsing fever are very unsatisfactory, it was thought advisable to try the effect of Atoxyl, which has such a marked effect in the treatment of experimental trypanosomiasis.[37] It was given hypodermically for a fortnight in daily doses of 0·6 ccm. of a 20 % solution, increasing to 1 ccm. No effect either on the disease or on the parasites was observed.

The mode of infection in this case seems well defined. Seven days previous to the onset of the symptoms the patient was performing an autopsy on a heavily-infected monkey, and in the course of this abraded the skin of his hand.

Subinoculations into eleven rats and one monkey were made.

On February 2nd, two rats were inoculated with 0·6 and 0·8 ccm. of citrated blood and showed parasites in preparations of the peripheral blood on the following day. On the third day after inoculation both died from pneumonia.

One rat was inoculated on the 6th of February with 0·2 ccm. of citrated blood, was infected on the 11th (5 spirochaetes in film) and shewed the parasites for fourteen consecutive days, during most of the time numerously.

On the 11th, two rats were inoculated with 0·8 and 1 ccm. of citrated blood respectively. Parasites were found in blood preparations on the fifth day, and were continuously present for 10 and 12 days.

The monkey was inoculated on the 22nd of February and became infected on the evening of the 23rd. It passed through an attack of five days duration, with three relapses, and showed parasites in preparations of the blood for the last time on March 23rd. Two rats were inoculated at the same time with 2 ccm. of citrated blood, and both had an attack followed by two relapses.

It is of interest to point out here, that one rat, Experiment 1,042, inoculated on the 6th, when the patient's blood was negative to microscopical examination, became infected on the 11th, the day on which the patient had his first relapse.

Another rat, Experiment 1,043, inoculated on the 8th, showed parasites for the first time on the 10th, when the spirochaetes reappeared in the patient's blood.

Experiment 1,049.—Rat, inoculated on the 19th of February, and showed parasites in blood preparations on the afternoon of the 22nd, agreeing perfectly with the onset of a relapse in the patient.

Experiment 1,064.—Rat, inoculated with 3 ccm. of citrated blood on March 22nd and never became infected. Reinoculated on the 5th of April from an infected rabbit and showed spirochaetes in preparations of the peripheral blood after an incubation period of three days.

Experiment 1,110.—Rat, inoculated on the 22nd of April and showed spirochaetes in preparations of the peripheral blood made on the following day. It passed through an attack and two relapses.

A consideration of the experiments outlined above shows that the blood of patients suffering from relapsing fever is infective for susceptible animals during the periods of apyrexia. Rats so inoculated showed the parasites for the first time in blood preparations at a period closely agreeing to the onset of the succeeding relapse in the patient; in one case even at the same hour.

In view of the remarkable fact that the rat which was inoculated from the patient on March 22nd, when a marked rise in temperature occurred, did not become infected, whereas the one inoculated a month later did so, we are inclined to consider the "relapse" on April 22nd as a *re-infection.* The shortness and severity of this attack are also in accord with the facts published by other authors, *e.g.*, Litten,[14] about re-infection.

CASE II (Chart II)—J. Fg. European, age 18. Previously healthy. On the 21st of June he came to work as usual, but complained of headache, diarrhœa and aching in his legs and spleen. Later in the morning he vomited a greenish, slimy fluid, and was sent home to bed, as a temperature of 101° F. was found. Preparations of the blood were examined in the afternoon, and showed spirochaetes in scanty numbers (1 to $\frac{1}{2}$ film). He passed a sleepless night on Thursday, but felt somewhat better on Friday, although he had no appetite and was very thirsty. On Saturday he felt so much better that he thought he would be able to work again, but on Sunday was seized with an exacerbation of all the previous symptoms accompanied by a rise in temperature to 104·8° F. The examination of blood films

DISEASE.

Tick Fever

Notes of Case.

Name J. Fe.

Age ......

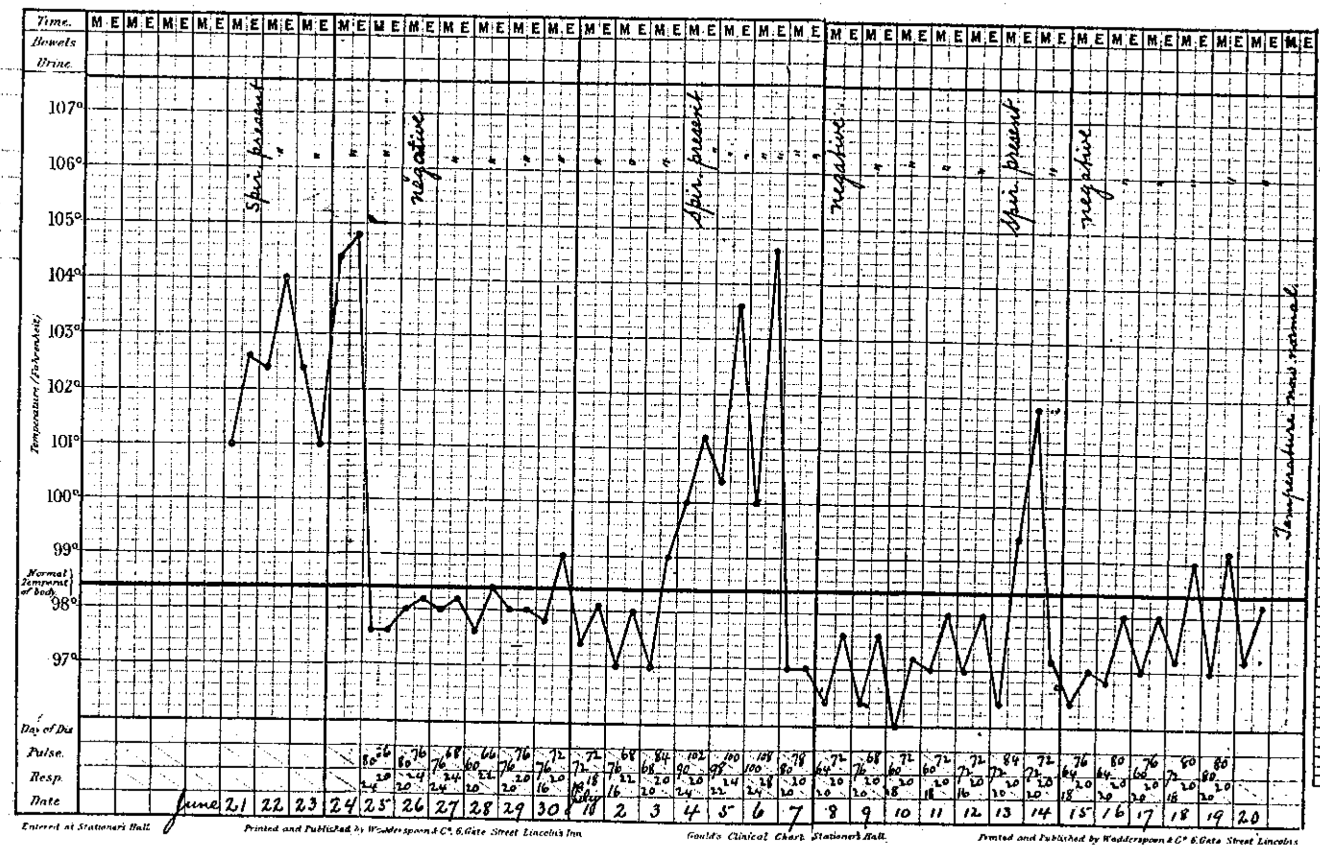
Diet ......

Case Book No ......

Chart 2.

Date of admission ......

Result ......



made at this time revealed the presence of fairly large numbers of parasites (1 to 4-5 fields).

The physical examination showed slight enlargement of the spleen, which could be palpated 2·5 cm. below the costal margin. Other than this, nothing abnormal could be found.

On Sunday evening the crisis occurred, preceded by a pseudo-crisis on Saturday, and accompanied by heavy sweats. The following morning he was much better, but still felt some malaise, and was then removed to the care of Dr. J. Hill Abram.

We are indebted to Dr. Abram for the following notes from the records of the Royal Infirmary, Liverpool:—

June 25th, 1906.

Circulatory system.—Pulse 60, regular, good volume, tension rather low. Artery wall not thickened.

Cardiac impulse felt in Vth space, 6 cm. to the left, localized, regular.

Second pulmonary sound, slightly accentuated.

Abdomen.—Liver not enlarged. Spleen can be felt 2·5 cm. below costal margin. Tongue slightly coated.

Urine.—Alkaline, deposit of phosphates. No albumen.

After admission to the hospital the malaise gradually passed off. The temperature after the crisis remained below normal until the 3rd of July, when the first relapse set in with the usual symptoms—headache and pain in the bones and spleen. The relapse lasted three days and ended by crisis preceded by a pseudocrisis, and the patient was free from any symptoms until a week later, when a second relapse occurred of twenty-four hours' duration. The symptoms were very slight in this relapse, which was the last.

The treatment was wholly symptomatic.

The etiology of this case is not very clear. The disease is highly infectious, and the patient may have become infected through the bites of animals suffering from spirochaetal infection. In this event the parasites would pass directly from abrasions in the gums to the bite, and not from the saliva. We have never been able to see spirochaetes in the saliva of infected animals which resembled those in the blood, and the inoculation of saliva into rats was never followed by the appearance of spirochaetes in the blood.

Subinoculations.—One monkey, Experiment 1,227, was inoculated with 2 ccm.

of citrated blood during the crisis of the first attack and became infected after an incubation period of five days. The attack lasted three days and was followed by a relapse nine days later.

Two rats, Experiment 1,228, inoculated intraperitoneally at the same time with 3 and 2·5 ccm. of citrated blood respectively, became infected after incubation periods of one and three days and passed through the usual course of infection.

On the 26th June two other rats, Experiment 1,230, were inoculated intraperitoneally with 3·5 and 3 ccm. of citrated blood in which no spirochaetes could be seen. They became infected after incubation periods of three and four days.

CASE III (Chart III)—J. Fd., age 14. Felt ill for the first time on the evening of August 5th, when he fainted and afterwards vomited. At this time his temperature was normal, but had risen to 104° F. on the following morning. The attack was accompanied by all the usual symptoms—severe headache, pains in the bones and especially the spleen, complete anorexia and great thirst. Spirochaetes were found in the blood on Sunday (1 to 5 fields). The patient was removed to the Northern Hospital on August 7th, and we are indebted to Dr. W. B. Warrington for placing the records at our disposal. The disease in this case followed the ordinary course; the relapses occurred at the usual intervals, and were accompanied by a recrudescence of the original symptoms. During the first relapse the patient vomited very frequently, but this was the only remarkable feature of the case.

The etiology of this case is obscure. As in Case II, the only possible mode of infection seems to be the bites of infected animals.

IV. A Clinical Comparison of African Tick Fever and European Relapsing Fever

Koch, in his "Ergebnisse einer Forschungsreise nach Ostafrika,"[12] has drawn attention to the shortness of the attacks in tick-fever and to the small number of parasites in the blood in comparison with the European relapsing fever. We have been able to confirm these observations in the few cases which we have seen ourselves, and also by a reference to those of Dutton and Todd. The statistics of European relapsing cases show that the attacks and relapses are of longer duration than those of African tick-fever.

Hödlmoser[10] observed in only 1·1 % of his cases attacks with a duration of three days, in 5 % a duration of four days, and in 60·5 % the duration of the attacks was from six to eight days.

DISEASE.

TICK FEVER.

Notes of Case

Name J. FD.

Age 14

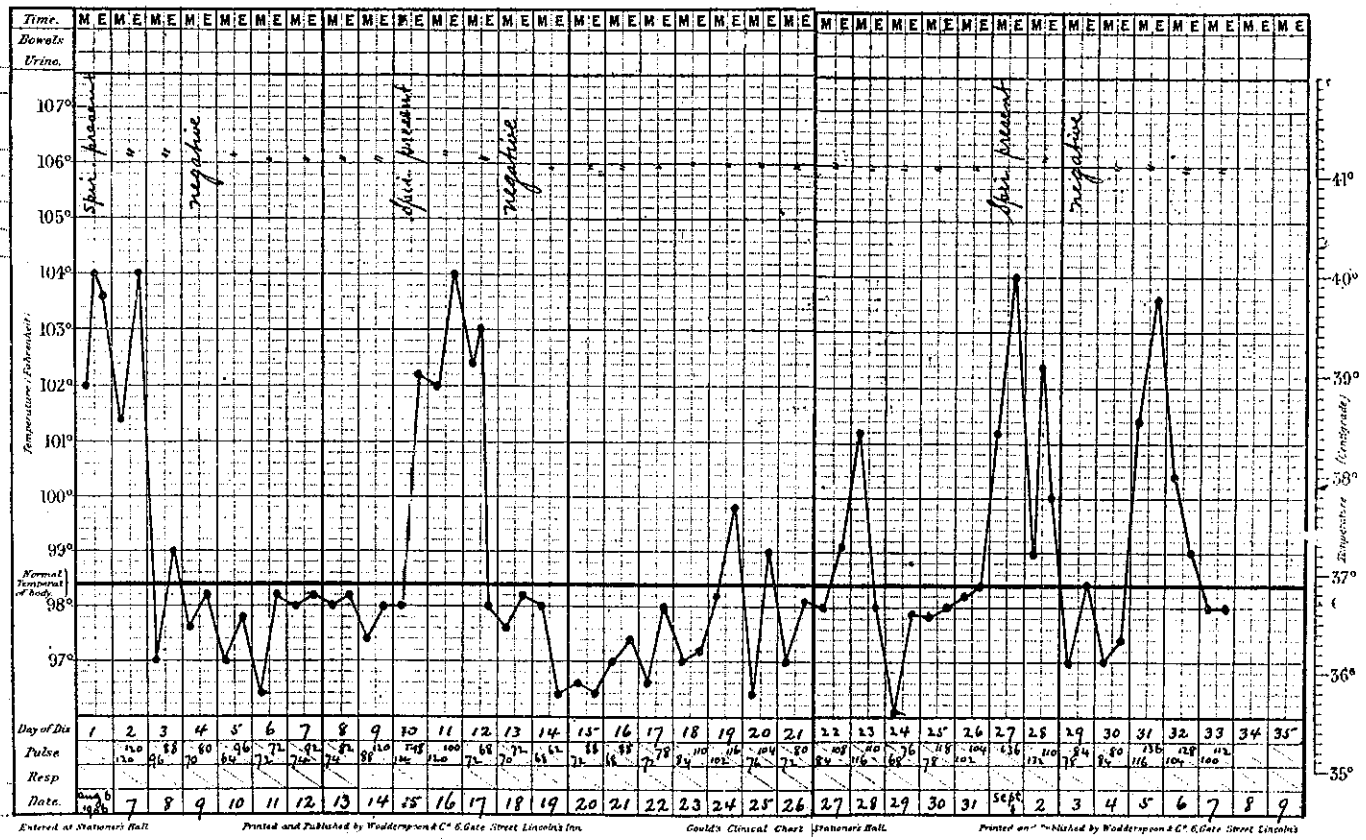
Diet

Case Book No

Chart 3.

Date of admission

Result



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No. of Days	1	2	3	4	5	6	7	8	9	10	11	12	13	14
In I attack per cent. of patients	—	—	1·1	5·0	6·5	21·2	19·1	20·2	9·7	7·9	4·5	2·5	2·2	0·4
II ,, ...	11·3	10·6	16·9	2·5	27·5	7·5	0·6	—	—	0·6	—	—	—	—
III ,, ...	2·5	2·5	2·5	10·7	10·7	—	3·6	—	—	—	—	—	—	—

The following table gives the observations of Moczutkowsky[18]:—

	Duration in Days				
	I	II	III	IV	V
Attacks	$6\frac{3}{4}$	$5\frac{1}{2}$	$3\frac{1}{4}$	$2\frac{1}{8}$	I
Intervals	$5\frac{1}{4}$	6	9	$10\frac{1}{2}$	—

Meschede[15] gives the following averages:—

	Duration in Days				
	I	II	III	IV	V
Attacks ...	7-6	5-4	4-3	3-1	1-0
Intervals ...	7-8	9-10	11-12	—	—

and Oks[27]:—

	Duration in Days		
	I	II	III
Attacks	5·7	3·8	2·0
Intervals	6·6	4·3	—

The length of the first attack was four days in the three cases given in detail above, in a fourth which has not been published as it does not differ from those described, and in Case No. VI of Dutton and Todd (J.E.D.). In the other cases quoted in their report the first attack was not carefully observed from the beginning except in one, J.L.T., in which the attack lasted three days.

No conclusions can be drawn as to the length of the intervals and the number of relapses in tick-fever, as the cases observed have been too irregular in their course and too few in number. Slight prodomal symptoms of malaise may occur, but in our cases the disease set in suddenly with headache, pain in the spleen and bones, and vomiting. Diarrhœa is frequently noticed just before the attack. Although

no distinct chills occur, the patients complain of feeling very cold. Coincidently with the commencement of the infection the spleen becomes enlarged and tender, and the very intense and agonising pain felt in this region was in our cases the most characteristic symptom of the disease. During the attack there is complete loss of appetite and great thirst, and a slight degree of bronchitis is common. The fever falls by crisis, and during this period the patients sweat profusely. In the intervals no morbid symptoms are apparent.

The peripheral blood was examined every day in our cases, and the maximum number of spirochaetes observed was two to three per field at the height of the attack.

V. Animal Reactions of Sp. duttoni

In a preliminary report [4] we have published notes on the reactions of spirochaetes observed in various animals. Since then these have been amplified and completed.

Monkeys

Many monkeys, of different species, were used in the course of the experimental study. The susceptibility to the disease varied greatly with the species and with the age. We have found young Mona (*Cercopithecus mona*) and young Callithrix (*Cercopithecus callitrichus*) monkeys most susceptible to the infection, followed by Rhesus (*Macacus rhesus*), "Sooty" (*Cercocebus fuliginosus*), "Jew" (*Cercopithecus?*) monkeys and baboons (*Papio anubis*) in the order named. The resistance to infection increased with the age. While this is true in general, some animals showed a decided idiosyncracy to the disease, and succumbed very rapidly even when inoculated with small amounts of infected blood. The condition of the monkey at the time of inoculation also had an influence on the course of the disease, which was of a more severe type in the animals which were not in perfect health. All of our monkeys, with a single exception, became infected.

This was a large female Rhesus of 1,800 grms. weight, Experiment 1,272. It was inoculated with 7 ccm. of heavily-infected citrated blood from two rats, and in spite of careful bi-daily examinations no parasites were found during the next eight days. It was then reinoculated with 8 ccm. of citrated blood showing 60-80 parasites to a field, with the same result.

Vandyke Carter,[7] in his classical work, records the same result in

DISEASE

Notes of Case.

Name MONKEY EXP. 1227

Age

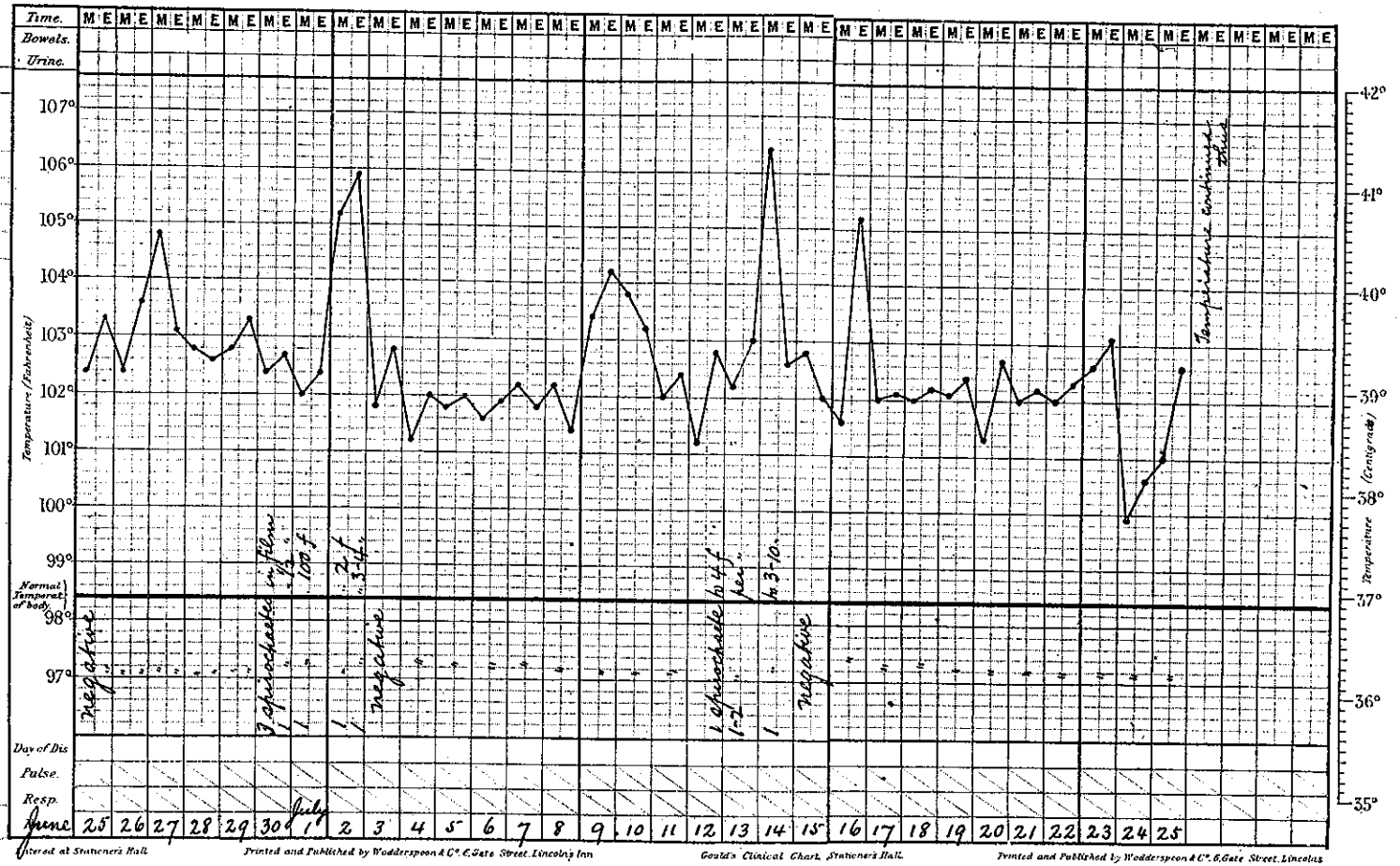
Diet

Case Book N°

Chart 6

Date of admission

Result



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DISEASE.

Notes of Case.

Name MONKEY Exp. 1058

Age

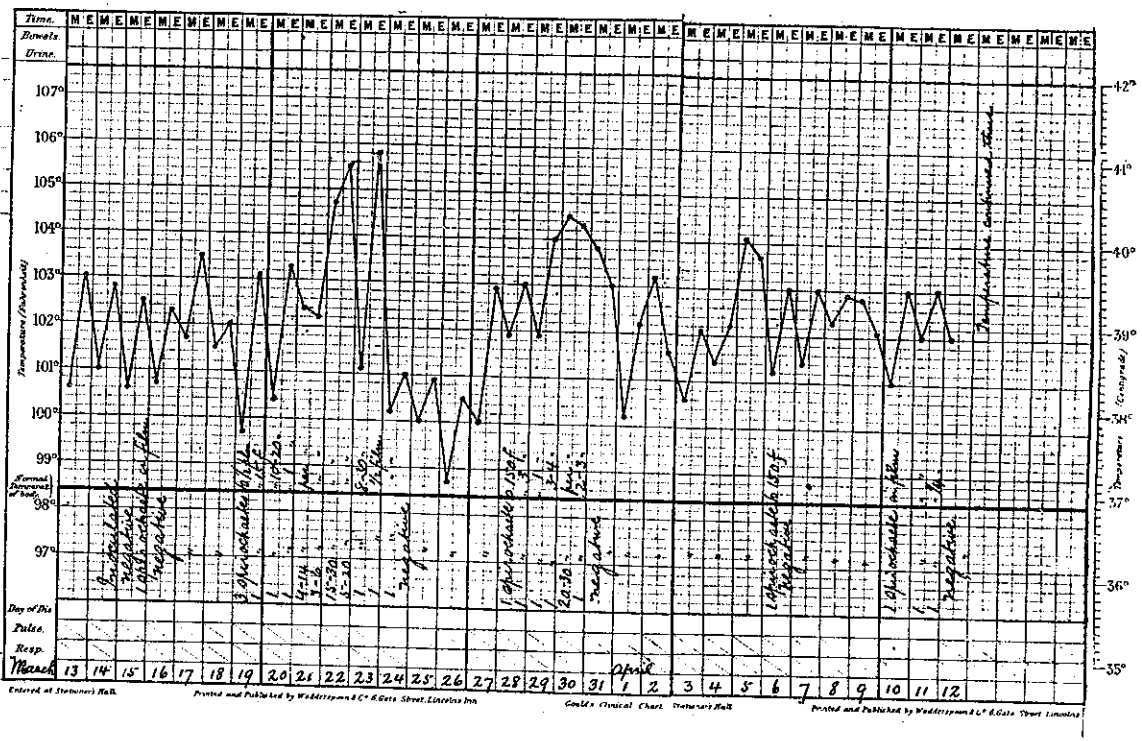
Diet

Case Book No

Chart 5.

Date of admission

Result



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DISEASE.

Notes of Case.

Name MONKEY EXP. 1063

Age

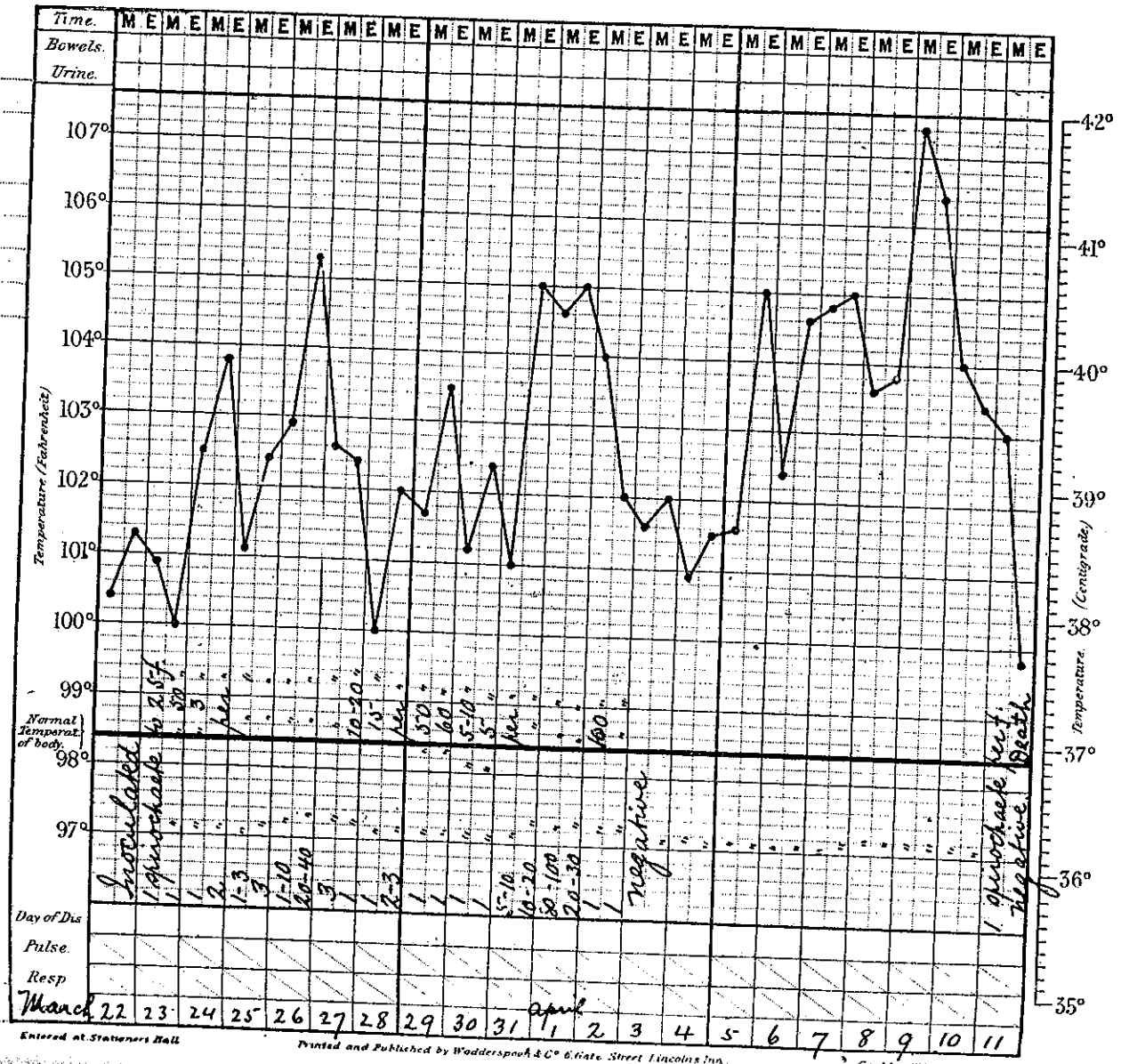
Diet

Case Book N°

Chart 4.

Date of admission

Result



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Gould's Clinical Chart

the case of two monkeys with the spirochaete of Indian relapsing fever.

The incubation period varied, as in rats, with the quantity of spirochaetal blood injected. When from 5-10 ccm. of infected blood was used, the parasites appeared in the peripheral blood within two hours; when smaller doses were employed, at times varying between two hours and three days. The parasites were found, at first, in very scanty numbers, increased slowly, and in two or three days were fairly numerous, so that in some cases they could not be counted. When the increase occurred very quickly the parasites disappeared, as a rule, very rapidly, and within one or two days the peripheral blood did not contain any. When, on the other hand, the increase was very slow, the spirochaetes did not disappear promptly but were present in the blood stream for a much longer period, up to twelve days in one case. Again, when the parasites became innumerable and remained present in the peripheral blood in such a number for two or three days, the death of the monkey was a foregone conclusion.

Coincidently with the appearance of the parasites the temperature rose, and during the course of the attack went as high as 107° F. in some cases (Charts IV, V and VI). It fell with the decrease in the number of parasites, but even when these were completely absent from the peripheral blood the temperature remained of a more or less irregular type. With the commencement of the relapse there was usually a distinct rise in the temperature again.

When the temperature fell markedly, with numerous spirochaetes in the blood, it could be regarded as a sign of approaching death. In these cases the temperature became subnormal, and remained low until the animal died. The height of the temperature did not always correspond to the number of spirochaetes in the blood, as the parasites were sometimes present in monkeys with normal temperatures.

After the disappearance of the spirochaetes, none were observed in the peripheral blood, as a rule, until the next relapse, which occurred at varying intervals after the attack. In typical cases of the disease, the relapse was ushered in by a rise of temperature, and the spirochaetes were found at the same time. When the first attack was of a severe type the relapse was milder in character, and *vice versâ*. One or two relapses were observed in most of the cases,

though frequently three occurred. Abortive relapses were also noted, *i.e.*, the temperature rose for a short time but spirochaetes were not found in the blood preparations.

The disease lasted from three days to eight weeks, counting from the first appearance of the parasites until the last day on which they were found.

When the infection was caused by the bites of ticks, the incubation period varied between five and seven days, five being the commonest. The course of the disease was identical with that observed after the inoculation of blood containing spirochaetes.

The monkeys suffered more or less from the disease. Usually with the appearance of the parasites the animal became quiet and listless, did not eat, and remained crouched up. In some cases, however, the monkeys showed no change in appearance and ate as in health. Loss of weight was observed as a general rule. During the infection a loss of from 200-400 grammes was noted, but on recovery this was quickly regained. A very marked feature was the anæmia which occurred. When the ear was cut for the purpose of making preparations, the blood was very pale and watery and did not coagulate as rapidly as normally. The blood counts revealed a noticeable decrease in the number of red cells, together with a marked fall in the percentage of haemoglobin. The number of leucocytes varied with the stage of the disease. At the commencement of the attack there was a slight increase in the number of white cells, and this continued until the spirochaetes were about to disappear, when a marked leucocytosis was observed, as many as 60,000 white cells being counted in one case.

At the autopsy made on monkeys dying of the disease, the heart muscle was degenerated. A varying quantity of clear transudate was found in the pleural and pericardial cavities. Small subpleural petechiae were commonly seen. The lungs were oedematous, and exhibited haemorrhagic infarcts. The peritoneal cavity frequently contained transudate. The liver was usually enlarged, congested and showed small haemorrhagic areas throughout the substance. When the disease had lasted some time before the monkey died, small necrotic areas were found. Of all the organs the spleen was most markedly changed. It was enlarged, as a rule, to twice or thrice its usual size, deeply congested

and very soft. Sometimes the surface was uneven and coarsely granular. These granules extended into the underlying tissue, and were composed of rounded necrotic areas of whitish colour intermingled with small haemorrhages. In acute cases numerous haemorrhagic infarcts were found scattered through the whole organ, occasionally involving half its extent. As in the liver, necrotic areas of varying size were commonly seen in cases of longer duration. The Malpighian bodies were enlarged and of a greyish-white colour. The other abdominal organs showed only some congestion. The lymph glands were frequently haemorrhagic. The marrow of the long bones was of a dark purple colour and softened to a semi-fluid consistence. Throughout its substance small greyish necrotic areas were often seen. The brain and spinal cord showed some congestion of the superficial vessels. Films were made from all the organs, and were examined in order to see whether the spirochaetes showed a predilection for any one organ. Whilst the number of parasites found in the spleen and bone marrow was far less than that observed in the blood, approximately the same number was found in the other organs. These pathological changes correspond to those found by Ponfick[28] in human cases of European relapsing fever.

Subinoculations from monkeys were made into rats at different stages of the disease. The blood was infective, not only when the spirochaetes were present in the peripheral circulation but also in the interval between the relapses. In the latter case, however, the incubation period in the rats was prolonged and the infection was of a milder type than usual.

Dogs

Experiment 1,018, mongrel, was inoculated intraperitoneally with 15 ccm. citrated blood showing 60-80 parasites per field, and spirochaetes were found in blood preparations two hours later (1 to 10 fields). With the appearance of the spirochaetes the temperature rose and on the second day had reached 103·8° F. and then fell to normal by the fourth day. A marked leucocytosis occurred with the appearance of the parasites. The number of parasites increased very slowly until the next evening when 2-3 per field were counted in fresh specimens of the blood. In the afternoon of the third day one parasite to 20-25 fields, in stained preparations, was found but three hours later the spirochaetes had disappeared and were not seen again.

Experiment 1,054, pup, four months old. Inoculated intraperitoneally with 13 ccm. of almost pure blood from a heavily-infected rat. Spirochaetes were present in the blood for the next two days, but always very scantily (1-2 per film) and then disappeared finally.

Experiment 1,053, pup of same litter. On February 24th, 41 young infected ticks were fed on this dog. As parasites were never seen in preparations up to March 22nd, 49 ticks were then fed again, but without result.

CATS

Four cats were inoculated with large amounts of heavily-infected blood, but spirochaetes were never seen in preparations of the peripheral blood.

HORSES

One pony, Experiment 1,019 (Chart VII), was inoculated intraperitoneally with 27 ccm. of citrated, heavily-infected blood from two rats. The mixture showed 5-20 parasites per field. The blood was examined every two hours after the inoculation, and parasites were found for the first time seven hours afterwards (4 to ¼ film). The number increased up till the evening of the next day, when as many as 2 spirochaetes to a field were observed, and then decreased until, on the third day, the examination proved negative. Although examined twice daily no spirochaetes were seen again in the blood until the ninth day, when in the morning film one spirochaete was found. The preparation made in the evening did not contain any parasites.

Later a rise of temperature occurred, but no parasites were seen in the blood preparations, and subinoculations into rats were not followed by infection, although heavy doses were used (10 ccm. citrated blood).

GOATS

One goat, Experiment 1,058, was inoculated intraperitoneally with 50 ccm. citrated blood from heavily-infected rats. The mixture showed 2-3 spirochaetes per field. A slight rise of temperature from 102·5° F. to 103·6° F. was observed on the evening of the inoculation. Parasites were never found in preparations of the blood, but a rat inoculated with 10 ccm. of citrated blood from the goat on the third day became infected after an incubation period of two days. The attack lasted nine days, and was followed by three relapses. Another rat was inoculated seven days after the inoculation of the goat, but without result.

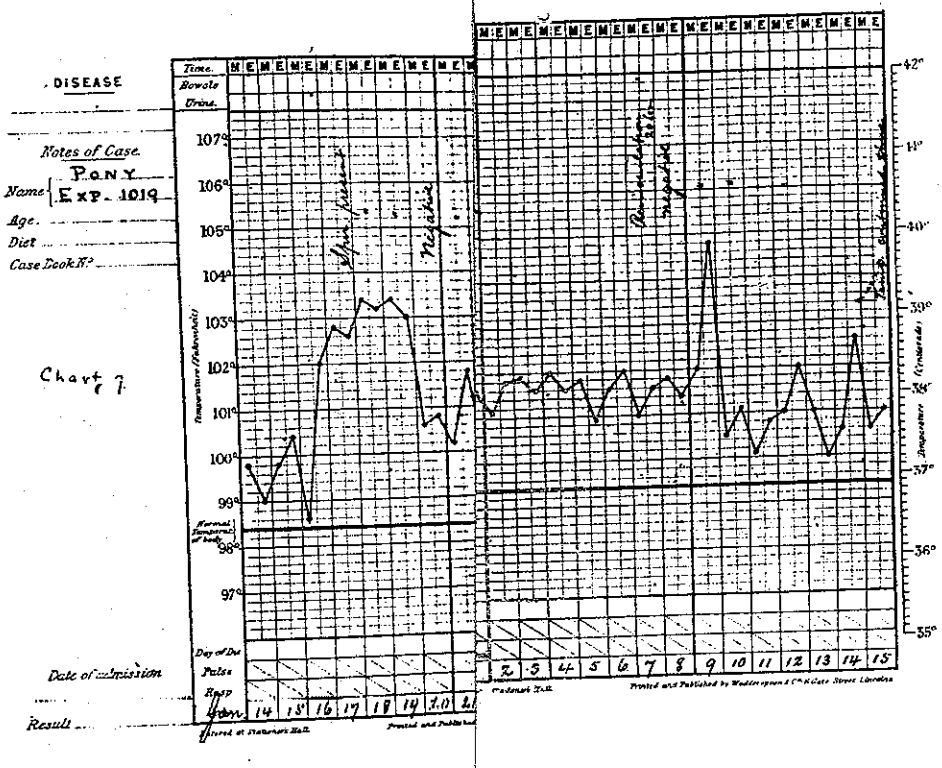
SHEEP

One sheep, Experiment 1,150 (Chart VIII), was inoculated intraperitoneally with 50 ccm. of citrated blood containing 10-20 spirochaetes per field. Parasites were found in preparations of the blood made on the same evening (4 to a film) coincidently with a rise in temperature, and then disappeared. Two days later a rat was inoculated from the sheep, and became infected after an incubation period of eight days.

RABBITS

From our experiments, it seems that the spirochaetes when inoculated in doses of more than 5 ccm. are very pathogenic for rabbits. Small doses do not seem to have any effect.

Many adult rabbits were inoculated, in every case with a fatal issue when a dose of 4 ccm. or more was employed. As a rule, spirochaetes in very small numbers were found in preparations of the


DISEASE
Notes of Case.
Name
P.O.N.Y.
Exp. 1019
Age.
Diet
Case Book N°
Chart 7.
Date of admission
Result
Time.
Bowels
Urine.
Temperature (Fahrenheit)
107°
106°
105°
104°
103°
102°
101°
100°
99°
98°
97°
Normal Temperature of body
Day of Dis
Pulse
Resp
Jan.
14
15
16
17
18
19
20
21
Temperature (Centigrade)
42°
41°
40°
39°
38°
37°
36°
35°
1
2
3
4
5
6
7
8
9
10
11
12
13
14
15

DISEASE.

Notes of Case.

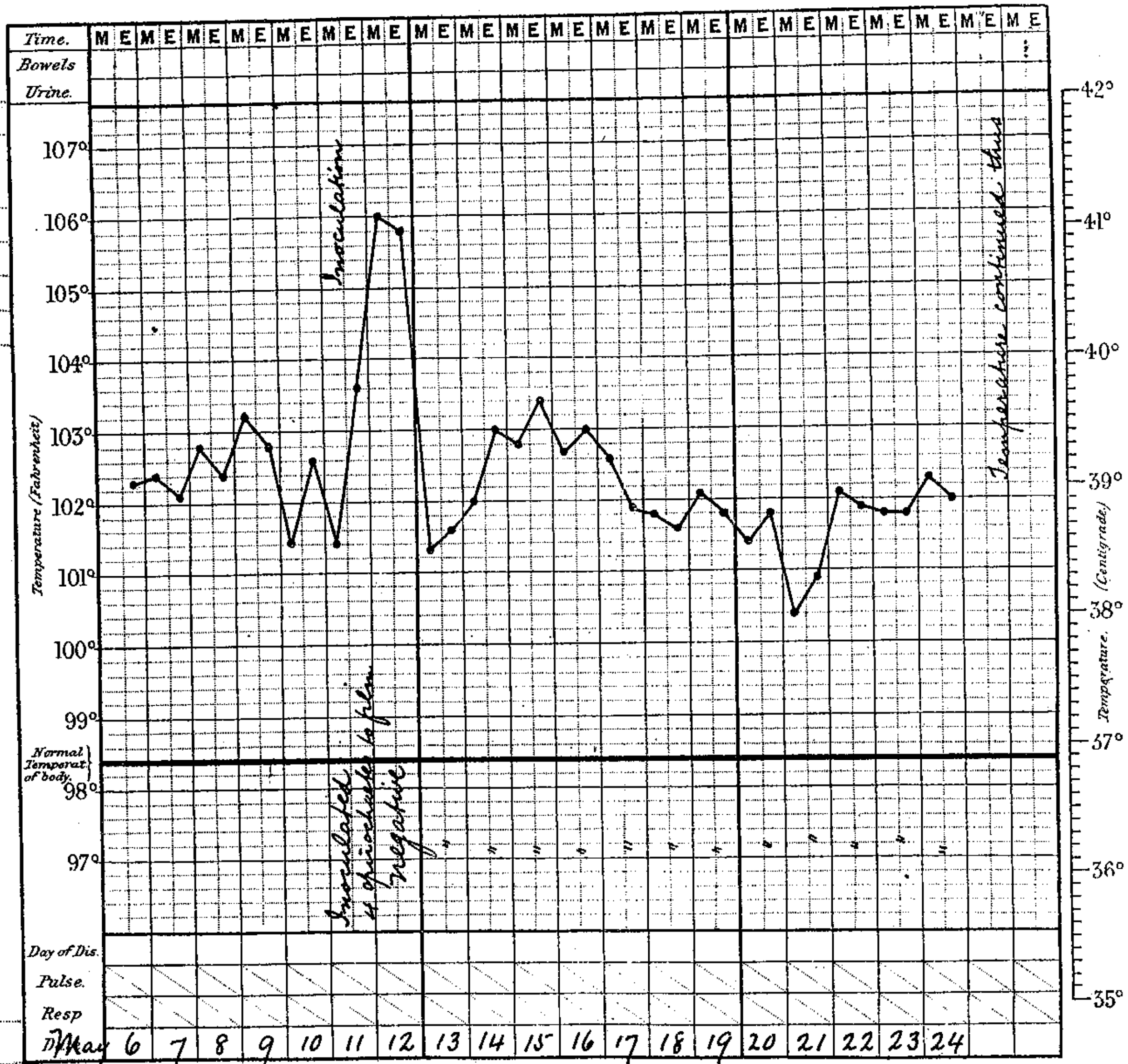
Name SHEEP EXP. 1150

Age

Diet

Case Book No.

-Chart 8.



Date of admission.

Result

Entered at Stationer's Hall. Printed and Published by Wodderspoon & Co 6, Gate Street, Lincolns Inn. Gould's Clinical Chart

DISEASE.

Notes of Case.

Name RABBIT Exp. 995A

Age

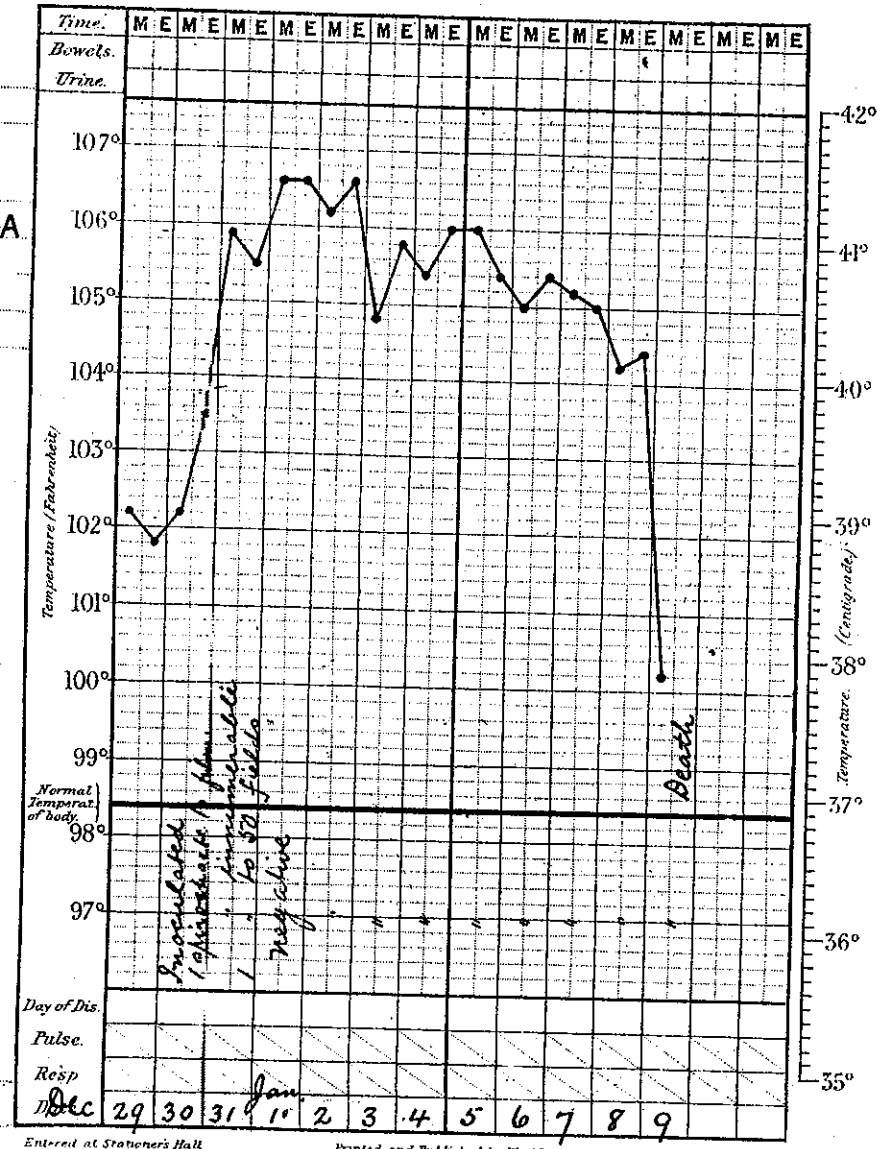
Diet

Case Book N°

Chart 9.

Date of admission

Result



Entered at Stationer's Hall

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peripheral blood two or three hours after the inoculation. At the same time a marked rise of temperature occurred (see Chart No. IX). The parasites usually multiplied during the next few hours and then gradually decreased. In preparations of the peripheral blood made at this time degenerating forms were seen. A second increase in the number of parasites then followed until, after seven or eight hours, from one to three per field could be counted in fresh blood preparations. In a few cases the blood was simply swarming with spirochaetes. They were present in the peripheral blood for from 1-3 days and then disappeared finally. A leucocytosis of the polymorphonuclear type set in with the appearance of the parasites. Spirochaetes were found in the peritoneal fluid withdrawn twelve and fourteen hours after inoculation, but no leucocytosis or phagocytosis was seen. The temperature usually remained high, on occasions even rising to 107° F. The animals appeared to be very sick, remained constantly in one corner of the cage, did not eat at all and emaciated rapidly. At the same time mucous discharges from the rectum and intractable diarrhœa were noticed. During the course of the disease the rabbits suffered from a progressive anæmia. The blood became very watery, and the coagulability was greatly lessened. In from three to ten days death supervened.

In very young rabbits (eight weeks old) the disease was of much shorter duration than in adult ones. The parasites appeared in the blood shortly after the inoculation, and were present for from two to three days. The animals usually died on the fourth day without any previous symptoms.

At the post-mortem examination few changes were found in the thoracic viscera. The pleural sacs occasionally contained a small quantity of exudate poor in cellular elements, the lungs showed small subpleural petechiae and the heart was soft and flabby. Very rarely exudate was found in the peritoneal cavity. The liver was slightly enlarged, showed small superficial haemorrhages which sometimes involved the underlying tissue, and in two cases small necrotic infarcts. The spleen was more or less enlarged—in one case 10 × 2·5 × 1·5 cm.—deeply congested, very soft and of a dark purplish-red colour. The Malpighian bodies were very prominent and greyish-white in colour. In some cases haemorrhagic infarcts from 1 to 5 mm. in diameter were found, while in others numerous small, whitish,

necrotic areas were scattered throughout the spleen substance. The bone marrow was very soft, and scattered through the reddish marrow were numerous greyish areas.

Subinoculations were made in rats with various amounts of blood, up to 6 ccm. Usually, the rats became infected only when spirochaetes were seen in the rabbits' peripheral blood. In one case subinoculations were made on the day of the rabbit's death, four days after inoculation, and the rat became infected after an incubation period of eight days.

GUINEA-PIGS

In guinea-pigs the course of the disease was much the same as observed in rabbits, but a fatal termination was less common. The incubation period varied between two hours and three days, depending on the amount of blood used for inoculation, and the parasites were found in the peripheral blood preparations for two to three days before they finally disappeared. In the small percentage of guinea-pigs which died, the only changes observed at the autopsy were sub-pleural haemorrhages and enlargement of the spleen.

Very young animals were more susceptible than adults.

Subinoculations in rats were followed by infection only if made when the guinea-pigs showed spirochaetes in preparations of the blood.

RATS

Rats are very susceptible to the disease, young ones more so than adults. Of the total number used in the work, only two displayed a remarkable resistance against infection.

One of these never became infected, although inoculated twice with large amounts of spirochaetal blood. The other remained negative after an injection of 8 ccm. of infected blood, but showed the parasites for two days when inoculated five days later with a second dose of 7 ccm.

The incubation period varied directly with the amount of infected blood injected and with the mode of inoculation. When small quantities were used—up to 2 ccm.—spirochaetes were found in the peripheral blood in small numbers within from twelve to twenty-four hours after the inoculation, while when larger doses were injected—up to 5-10 ccm.—the spirochaetes were found two hours later. Very occasionally the incubation period was much prolonged—up to twenty-one days in one case. Rats inoculated subcutaneously became

infected a few hours later than those receiving the injection intraperitoneally. The parasites multiplied more or less rapidly, depending on the dosage, and on the second day from 20-30 per field were seen in the blood preparations. On the following day they were numerous and on the fourth day innumerable, as a rule, *i.e.*, the whole field was densely covered with the spirochaetes. In the majority of the animals a marked leucocytosis was observed later on the fourth day, and with this the spirochaetes commenced to decrease in numbers. Their diminution at this period was followed out by hourly examinations of rats, and one of these experiments may be quoted.

When the examination was begun at 2 p.m., from 60-70 parasites, and 20-25 leucocytes per field were counted. The leucocytes were chiefly of the polymorphonuclear type, and many of them contained large granules, and occasionally erythrocytes. The parasites were very active and appeared perfectly normal. This condition continued up till 9 p.m. with hardly any change except that the spirochaetes, in freshly drawn blood, did not appear to be quite so active. At 9 o'clock the leucocytes had increased markedly (30-35 per field), and many of them contained dark, irregular inclusions and large granules. The spirochaetes for the most part were normal in appearance, but a few were fastened to the slide at either end, and in these the spirals moved very slowly and jerkily from side to side. At the same time a slight change in the form of a few parasites was seen. A small, rounded or oval, translucent body was observed at the side and towards the middle of the spirochaete, and moved longitudinally and laterally in unison with the parasite. The rapidity with which many of the spirochaetes ceased moving in freshly drawn blood was very marked. Whereas at the commencement of the examination most of the spirochaetes were active, half an hour later a very large number were absolutely motionless. This was all the more noticeable as the parasites in preparations of blood made from an animal at the height of an attack usually retained their activity unchanged for hours.

At midnight the number of leucocytes was smaller. From the first, many of the spirochaetes were motionless, and very many more ceased moving within the next fifteen minutes. From 10-15 parasites per field were counted. In preparations kept in the incubator (37° C.) hardly any phagocytosis was observed—only two or three leucocytes were seen engulfing the spirochaetes during the whole period of observation.

Until two o'clock the parasites had decreased in number very slowly, but after this time there was a decided and sudden fall in number observed. At 3 a.m. only 3-4 per field were counted, at 5-30 only one spirochaete to about 150 fields was seen, and in the thick stained specimen of blood made at 9 a.m. only three spirochaetes were found. During the period in which the rapid diminution occurred, viz., from 2 to 5-30 a.m., a greater number of the spirochaetes than observed earlier had the central rounded bodies mentioned above, while many of the parasites were much more irregular in outline than normal, presenting at intervals along their bodies slight thickenings. In others, which were normal in shape, small, well-defined, translucent dots were seen.

As a rule, the attack lasted five days, but spirochaetes have been seen in the peripheral blood for fifteen consecutive days. When the

spirochaetes were about to disappear, after the height of the attack was reached, a peculiar phenomenon was seen frequently in fresh blood —irregular clumps composed of large numbers of entangled parasites and red blood cells.

After the attack the spirochaetes disappeared from the peripheral circulation, and even to the most careful examination (four preparations in one day) the blood was negative. In from three to eight days a relapse occurred, but was of shorter duration than the original attack, and during it the examination at no time revealed the presence of the spirochaetes in so great numbers as seen during the attack itself. A second, third and even a fourth relapse, at varying intervals, was observed, each less severe than the previous one and with fewer parasites in the peripheral blood until, in the fourth, only one in a film was seen. The total duration of the disease varied between three and forty-five days.

While this was the typical course of the disease, variations were observed. Fairly often after the number of parasites had increased to the maximum in the manner indicated they did not diminish, but remained constantly present in very large numbers until the rat died in the course of two or three days. In other cases, in which the amount of blood inoculated was small or was from an animal in whose peripheral blood no parasites could be seen, the spirochaetes increased in number very slowly and were never very plentiful. In these rats the first relapse was more severe than the attack, and during its course the spirochaetes were much more numerous than in the attack.

After the inoculation the symptoms observed were usually very slight. The rats did not show any change, but when the attack was at its height some became drowsy and remained huddled up in one position. Frequently, however, the rats appeared absolutely normal, being very lively and eating freely. Loss of weight during the infection was common and there was also an anæmia corresponding in degree to the severity of the infection. After the final disappearance of the parasites, rapid recovery ensued. In the cases followed by a fatal termination the rats passed from a drowsy to a semi-comatose condition. To the touch they were quite cold, and the thermometer did not register. A very common feature was diarrhœa, and rarely haematuria was observed, even when very

small doses of rat blood were used for inoculation. Just before death clonic contraction of the muscles of the legs was observed, and sometimes paraplegia.

Attempts to get the temperature curve of the disease in rats were without result, as it was very irregular and bore no relation to the presence of spirochaetes in the blood.

While we have been unable to infect guinea-pigs and rabbits through the bites of infected ticks, this method succeeded perfectly in the case of rats. The incubation period was from 5-7 days, and after this time the spirochaetes appeared in the peripheral blood in scanty numbers, but were never seen in nearly so great a number as was observed after inoculation. The first attack was usually of prolonged duration, and was followed by two to three relapses. On the whole, the course of the disease in rats infected by tick-bites was milder than in those infected by direct inoculation.

The post-mortem appearances in the rats dying of the infection were much the same as in other animals which died from the disease. The mediastinal connective tissue was gelatinous. In the pleural and pericardial cavities a varying quantity of clear fluid was often found. The lungs were oedematous, and sometimes contained small haemorrhagic infarcts. Small subpleural petechiae were seen. The heart was pale and soft. The liver was enlarged slightly, contained small haemorrhagic infarcts, and in those cases in which the disease had lasted for some length of time, anæmic infarcts of different sizes. The spleen, in the acute cases, was usually very much enlarged (sometimes measuring 6 × 2 × 1·5 cm.), of very dark colour, and contained haemorrhagic infarcts of varying size. In cases of longer standing anæmic infarcts were observed. The Malpighian bodies were enlarged and grey in colour. The lymph glands were unchanged macroscopically. The bone marrow was very soft and congested.

Mice

The course of the disease in these animals was identical with that observed in rats, but mice succumbed more readily to the infection. The changes in the organs, at the post-mortem, were also similar in character to those observed in the rats.

All attempts to infect chickens, pigeons, and goldfish with *Spirochaeta duttoni* have proved negative.

The above experiments show that we have been able to infect nearly all the usual laboratory animals with *Sp. duttoni*, i.e., monkeys, dogs, horses, goats, sheep, rabbits, guinea-pigs, rats, and mice. In some the parasites were found only in the subinoculations. Cats have shown themselves to be entirely refractory to the infection. The most susceptible animals, in our experience, are white rats and then monkeys.

RAT, EXPERIMENT 994
Inoculated December 30, 1905

Date	Day of Disease	No. of Parasites	Leucocytes
Dec. 30	1	ev. 1 to 150 f.	
„ 31	2	1 to 3 f.	
Jan. 1	3	m. 1 to 1 f. e. 30 to f.	
„ 2	4	Negative	Very numerous
„ 3	5	„	Decreasing
„ 4	6	3 to ¼ film	Normal
„ 5	7	Very numerous	Numerous
„ 6	8	„	Decreasing
„ 7	9	1 to 100 f.	
„ 8	10	5 to film	
„ 9	11	1 to 80-100 f.	
„ 10	12	2 to film	
„ 11	13	1 to 100 f.	
„ 12	14	2 to film	
„ 13	15	Negative	
„ 14	16	„	
„ 15	17	„	
„ 16	18	„	
„ 17	19	„	
„ 18	20	„	
„ 19	21	„	
„ 20	22	„	
„ 21	23	„	
„ 22	24	„	
„ 23	25	1 to 3-8 f.	
„ 24	26	3-7 to f.	
„ 25	27	1 to 180 f.	
„ 26	28	1 to film	
„ 27	29	Negative	
„ 28	30	1 to 6-22 f.	
„ 29	31	Negative	
„ 30	32	„	
„ 31	33	1 to 10-70 f.	
Feb. 1	34	1 to 20-25 f.	
„ 2	35	1-2 per f.	
„ 3	36	Negative	
„ 4	37	„	
„ 5	38	„	
„ 6	39	„	
„ 7	40	1 to 150 f.	
„ 8	41	1 to 5-35 f.	
„ 9	42	2 in film	
„ 10	43	Negative	
„ 11	44	„	

RAT, EXPERIMENT 990B
Inoculated December 26, 1905

Date	Day of Disease	No. of Parasites
Dec. 27	2	Numerous
„ 28	3	Innumerable
„ 29	4	„
„ 30	5	„
		Death

RAT, EXPERIMENT 1022
Inoculated January 17, 1906

Date	Day of Disease	No. of Parasites
Jan. 17	1	Negative
„ 18	2	„
„ 19	3	1 to 30-40 f.
„ 20	4	6-7 per f.
„ 21	5	5-6 per f.
„ 22	6	1 per f.
„ 23	7	1 per 60 f.
„ 24	8	1 per 5 f., 2 to 1 f.
„ 25	9	3 per 1 f., 1 to 3 f.
„ 26	10	Negative
„ 27	11	„
„ 28	12	1 to 10-15 f.
„ 29	13	1 to 150 f.
„ 30	14	Negative
„ 31	15	1 in film
Feb. 1	16	1 to 7-9 f.
„ 2	17	2 to 1, 1 to 3 f.
„ 3	18	Negative
„ 4	19	„
„ 5	20	„
„ 6	21	„
„ 7	22	„
„ 8	23	„
„ 9	24	„
„ 10	25	„
„ 11	26	1 in film
„ 12	27	1 to 300 f.
„ 13	28	1 to ½ film
„ 14	29	Negative
„ 15	30	„
„ 16	31	„
„ 17	32	„
„ 18	33	„
„ 19	34	„

RAT, EXPERIMENT 1045
Inoculated February 13, 1906

Date	Day of Disease	No. of Parasites
Feb. 13	1	Negative
„ 14	2	„
„ 15	3	„
„ 16	4	1 to 50 f.
„ 17	5	5 to 1, 1 to 6 f.
„ 18	6	20 per f.
„ 19	7	Innumerable
„ 20	8	3 to 1 f.
„ 21	9	1-5 per f.
„ 22	10	1 per f.
„ 23	11	1-5 per f.
„ 24	12	10-20 per f.
„ 25	13	20-30 per f.
„ 26	14	20-30 per f.
„ 27	15	5-10 per f.
„ 28	16	Death

VI. Chronicity of the Disease

The disease, once contracted, ran an acute or chronic course. In the latter case the infection was occasionally of long duration; for example, Case I quoted above. In some of the monkeys, *e.g.*, Experiment 199/I infected through tick bites, spirochaetes were seen first on October 13th, 1905, and the monkey died on December 28th, death being preceded by a rise of temperature, during which period parasites were found in preparations of the peripheral blood; none had been seen for the previous six weeks. In another case, Experiment 184/I, the spirochaetes were seen first on February 19th, and were found last on the 13th of May. In rats also the infection was occasionally of long duration. One rat, Experiment 994 (see table), was inoculated on December 30th, 1905, and spirochaetes were seen last on February 9th, 1906. A point which may be emphasised is that a rise in temperature occasionally occurred in monkeys some time after the last evident relapse. Although spirochaetes could not be found in the peripheral circulation at this period, subinoculations into rats were followed by infection after a prolonged incubation period, but the subinoculated rats passed through a very slight attack.

VII. Virulence of the Spirochaetes

In susceptible animals of a given species the course of the disease was usually of a similar type. Some rats died two or three days after the spirochaetes had become extremely numerous in the blood, and it was thought that the strain derived from these might be more virulent, but the rats subinoculated from them did not suffer from a more severe infection than those inoculated with a seemingly less virulent strain. Efforts were made to increase the virulence by passing the spirochaetes quickly through a succession of thirty or forty rats, but this was also ineffectual. The same method practised on young animals, which are distinctly more susceptible to the disease, was without effect. A more or less close relation existed between the number of spirochaetes inoculated and the number appearing in the blood of the subinoculated animal. When blood containing few parasites was used the animal inoculated showed few in its blood, and when heavily-infected blood was injected a correspondingly greater number was observed. When blood was used in which no parasites were seen, obtained from an animal in the interval between

two relapses, the inoculated animal had a slight attack and only few parasites were found in its blood. No difference was noticed in the virulence of strains which had passed through a long series of animals and that derived from animals directly infected through tick bites. The passage of the spirochaetes through man did not lessen the virulence.

VIII. Immunity

A complete list of the literature on immunity in spirochaetal infections was given, first by Wladimiroff in the Handbuch der pathogenen Mikro-organismen,[38] and brought up to date in detail by Novy and Knapp,[24] who believe that they have established "a sound basis for the prevention and cure of relapsing fever and the related tick-fever."[24]

It is a peculiar phenomenon that the spirochaetes disappear from the blood so that they cannot be seen by microscopical examination and then reappear, and the explanations offered are not satisfactory. It is a question whether the disappearance is caused by the formation of a germicidal substance or whether it is characteristic of a life-history of the spirochaete, or is the result of a combination of these two. When fresh preparations were made from an animal during the onset of an attack the spirochaetes lived for some time at the room temperature, and at 37° C.; in both cases a distinct increase in their numbers occurred. The behaviour of the spirochaetes in preparations made during the decline of an attack differed with the temperature at which they were kept. At room temperature the spirochaetes behaved as in the preparations made during the onset, but at blood heat the parasites died in a very short time.

Blood containing numerous spirochaetes, taken on the second or third day of the disease, was mixed with serum obtained from rats which had recovered from the disease in the proportion of 1 : 1 and kept at room and incubator temperatures. Controls were made with normal serum in the same dilution. At room temperature (20° C.) no difference was noted in the appearance of the spirochaetes in the two cases. At incubator temperature the changes in the immune serum specimen were well marked. After a lapse of ten minutes the spirochaetes moved more slowly than usual, and tended to conglomerate with the red blood cells. After half an hour the first dead parasites were seen. Some showed a small oval thickening towards the middle, or occasionally at the extremity, and moved very sluggishly; after an hour and a quarter many of the spirochaetes were still motile, while many others had become entangled in irregularly-shaped masses similar to the appearance observed in the case of *Sp. gallinarum* just before the death of the fowl. Some of these masses were absolutely motionless, but in others the projecting spirochaetes still moved. Lying between the spirochaetes, small,

rounded, refractile granules were seen. At this time the first true agglutination rosettes were noticed. After two and a quarter hours still more rosettes were seen, and only a few actively motile parasites were still present. Five hours afterwards not a single rosette was to be seen, and only very few sluggishly motile spirochaetes; nearly all were clumped together. After seven hours all the spirochaetes were dead. A good number of the spirochaetes preserved their form, even after the lapse of twenty-four hours.

In the control no change in the spirochaetes was seen for the first four hours. After this time the spirochaetes displayed a tendency to clump together, but showed no change in form whatever. Some of them were dead, but living spirochaetes were seen after thirty hours.

When spirochaetal blood was mixed with serum from a hyperimmunised animal in equal parts and kept at incubator temperature (37° C.), many rosettes composed of several hundred spirochaetes were seen after fifteen minutes. At the centre the parasites were stuck together, but the free extremities were moving actively. In three-quarters of an hour many of the spirochaetes were dead and clumped together, while the number of rosettes was lessened. Along the course of a fair number, one, or sometimes two, of the oval thickenings described above could be seen. Other spirochaetes showed on one side and towards the centre small, rounded, or oval bodies, which appeared to be joined to them by delicate pedicles. After three hours most of the parasites were dead, and appeared more refractile than when alive. After five hours all the rosettes were broken up, and most of the spirochaetes clumped together. Even after ten hours motile spirochaetes were still seen.

In the control, made with normal serum, the spirochaetes acted in the same way as described in the controls mentioned above.

When blood containing spirochaetes was inoculated intraperitoneally in normal rats, the parasites shortly afterwards increased markedly in number, and this multiplication continued until the parasites were found in the peripheral circulation. Granular leucocytes appeared very slowly in the peritoneal fluid, and became numerous only after the lapse of three or four hours. The spirochaetes continued to increase, but the fluid was slowly absorbed until in the course of ten hours none could be withdrawn.

In rats which had passed through the infection the results were somewhat different. In the fluid drawn from the peritoneal cavity 15 minutes after inoculation a distinct increase in the number of parasites was apparent. The leucocytes appeared more rapidly than in the normal rat, and in the course of two hours from 15 to 20 per field were counted. In fresh preparations made two hours after the inoculation very few spirochaetes were seen, most of them deformed, swollen up, and stuck to the leucocytes. After a lapse of from three to four hours no parasites and only a very few leucocytes were seen in the fresh and stained specimens. The stained specimens showed the same appearances.

In rats which had been repeatedly inoculated with infected blood the results were still more different. Living but very sluggish spirochaetes were seen in blood withdrawn two minutes after the inoculation. In the fresh specimen made ten minutes after, no living spirochaetes were seen, while in the stained the parasites were collected together in small clumps. The leucocytes were very scanty and some were filled with broken-up spirochaetes.

The same method of examination was used on rats previously treated with immune and hyperimmune serum, but the phenomena observed were practically the same as those seen in normal rats. Phagocytosis was slightly more pronounced.

1. ACTIVE IMMUNITY

A short preliminary note on the active immunity in experimental animals has been published already from these laboratories.[6]

Many monkeys which had recovered from the disease were reinoculated with the same strain of spirochaetes at varying intervals after the attack.

One *Macacus rhesus* was reinoculated ten days after spirochaetes had been seen last in the blood preparations, and after subinoculation into rats had failed to infect. The monkey received 10 ccm. of heavily-infected blood intraperitoneally, and on the following evening, simultaneously with a rise in temperature, scanty parasites were present in the peripheral circulation (1 in 40-90 fields). On the following morning only one parasite in half a film was found, and no spirochaetes were seen afterwards.

After a lapse of several days it was reinoculated again, but without result.

Another monkey (*Cercocebus fuliginosus*), Experiment 1,015, which had passed through an attack with two relapses was re-inoculated seven weeks after the last relapse. On the third day a rise in the temperature from 102° to 105° F. was noted, but in spite of most careful examination no spirochaetes were found. A rat which was subinoculated at this time did not become infected. Thirty-three days later the monkey was again reinoculated, but not even a rise of temperature followed.

A second monkey of the same species reacted in a similar way.

A *Macacus rhesus*, which had been infected through the bites of ticks and had recovered from an attack followed by one relapse, was reinoculated five weeks later with 8 ccm. of heavily-infected rat blood. A subinoculation, made four weeks after the last relapse, had proved negative. On the second evening after the monkey had been reinoculated two spirochaetes were found in a preparation, but they then disappeared and were never seen afterwards.

Analogous observations were made on a large *Cercopithecus callitrichus*. The reinoculation was followed by a marked rise in temperature to 105·2° F., and although no spirochaetes could be seen in preparations of the peripheral blood, a rat inoculated with 5 ccm. of the monkey's blood, became infected after a prolonged incubation period of seven days.

The experiment given below is of especial interest, in that it suggests the duration of the active immunity.

This monkey (*Cercopithecus sp.?*) was inoculated for the first time on February 22nd, 1906, and the parasites were last seen in the preparations on March 22nd. On July 30th it was reinoculated with 7 ccm. of heavily-infected blood, and on the morning of the fourth day one spirochaete was seen in the blood film, but the parasites had disappeared by evening and were never seen again. On August 2nd a rat was subinoculated with 4 ccm. of nearly pure blood and was infected coincidently with the monkey. This was the only occasion on which parasites were found in the rat's blood.

In rats the results obtained were much the same as in monkeys. A large number of rats were reinoculated at various intervals after recovery from the disease, but in only three cases were the parasites seen in preparations of the peripheral blood, and then, usually, only

within 6-8 hours after the inoculation. Subinoculations, which were made within two or three days of the reinoculation of the rats, were followed by infection in the majority of the cases, but the attacks were always slight in the subinoculated rats.

Active immunity still persisted after a period of seven months, as shown by two rats which were reinoculated with 2·5 ccm. of infected blood each after this time and did not become infected.

The above experiments show that **there is a relatively active immunity against re-infection** as animals reinoculated at various intervals after recovery up to seven and a half months did not become infected at all or only had a very slight attack. Only the first reinoculation of monkeys was followed by a rise of temperature; succeeding ones did not even cause this.

2. TREATMENT

A. Immune Sera

The immune sera used in these experiments were derived from a horse, Experiment 1,019; and from three monkeys and from fifteen rats which had recovered from the disease. The horse serum was obtained seven weeks after the first inoculation.

1. Horse Serum

(*a*) *Preventive*

Monkeys

Experiment 1,073, monkey (*Macacus rhesus*), weighing 1,538 grm., was inoculated on March 23rd, with 10 ccm. of horse serum. On March 25th and 27th the same amount of serum was given subcutaneously. On the 30th the monkey was inoculated intraperitoneally with 5 ccm. of citrated blood showing 3-5 parasites per field. Although examined carefully twice daily spirochaetes were not found in the blood preparations until seven days later. At first they were very scanty (one to half a film), but increased for the next two days, when 1-5 to a field were seen. Two days later no parasites were found. Only one relapse occurred.

The control monkey of the same species, Experiment 1,074, 1,748 grms., was infected on the third morning (2 to 1 film); the spirochaetes increased in number and were continuously present in the peripheral blood in varying numbers for the next ten days. No relapse occurred.

Experiment 1,104.—*Macacus rhesus*, 2,165 grms., was inoculated on April 6th, 9th, 11th and 14th with 10 ccm. of horse serum on each occasion. Four days later 49 infected ticks were fed on the monkey, but as no parasites had been seen in the peripheral blood, 53 ticks were fed again a week later. The following day a few spirochaetes were found in the preparation (3 to a film), were present for the next five days and then disappeared. A slight relapse of two days' duration occurred five days later. No spirochaetes were found afterwards.

RATS

Experiments 1,112 ((*a*) 160 grm.; (*b*) 145 grm). These rats were inoculated seven times with 7 ccm. of horse serum at intervals of two days. Two days after the last serum injection they were inoculated intraperitoneally with 2 ccm. of citrated blood showing 1-3 spirochaetes per field. In both rats parasites were found in the peripheral blood three days later, and were continuously present for the next eight days. After an interval of 5 days, during which no spirochaetes were found, rat 1,112(*a*) had a relapse lasting one day, while (*b*) had no relapse. The control rat was infected three hours after the inoculation, and spirochaetes were found in the blood for five days, but always in greater number than in the rats treated with the serum. After five days' interval a relapse occurred which lasted for five days.

Other experiments carried out in the same manner with horse serum were followed by similar results.

(*b*) *Curative*

Two monkeys in different stages of the infection were treated with horse serum, but without the slightest effect. The attack continued as in untreated animals, and the relapses occurred as usual. Ten rats, seven mice, and one rabbit treated in the same way showed no difference from the controls.

2. RAT SERUM

(*a*) *Preventive*

MONKEYS

Experiment 1,159, *Cercopithecus mona*, weighing 945 grms., received 15 ccm. of rat immune serum in the course of five days and was inoculated two days later with 2 ccm. of citrated spirochaetal blood. The monkey was infected after an incubation period of 48 hours, passed through an attack of four days' duration, and had one relapse lasting three days.

The control monkey passed through a similar course of the disease.

RATS

All efforts to prevent the disease in rats by means of rat immune serum were unsuccessful. The only result noticed was a slight prolongation of the incubation period, but the rats always became infected, and then passed through the ordinary course of the disease. One experiment only will be quoted in detail, as it presents some remarkable features.

Experiment 1,123, rat weighing 189 grms. It received 9 ccm. of rat immune serum in three doses and was inoculated two days after the last injection with 2 ccm. of citrated heavily-infected blood. Spirochaetes were found in the peripheral blood nine hours afterwards (1 to 30 fields), but disappeared in the course of the next few hours and were not seen again until twelve days later. The rat then had an attack lasting fourteen days, and during part of this time the spirachaetes were very numerous. No relapse followed.

(*b*) *Curative*

Those animals treated with rat immune serum at various stages of the disease displayed no marked difference from the controls. The attacks were not shortened, nor were the relapses prevented.

3. Monkey Serum

The results obtained by the use of monkey immune serum were similar in every respect to those following the use of horse and rat immune serum.

The above experiments lead to the conclusion that immune serum, whether derived from horses, monkeys or rats, has no appreciable value either in preventing the occurrence of the attacks in susceptible animals or in curing the disease once contracted. The incubation period may be prolonged to a greater or less extent, but the inoculation of infective blood is always followed by infection.

B. Hyperimmune Sera

As the use of immune serum conferred no marked passive immunity, serum derived from animals after a varying number of inoculations with spirochaetal blood was employed.

1. Horse Serum

The pony, Experiment 1,019, was reinoculated on April 12th, April 30th, June 7th, July 17th, 19th, 23rd, 26th and 28th with a total of 250 ccm. of heavily-infected blood. The inoculations were partially intravenous and partially intraperitoneal. The pony was bled on the 30th of July and the serum obtained was used in the following experiments.

Unfortunately, large doses of this serum could not be used in rats, as the blood with which the pony was inoculated was derived from these animals. When it was used, haemolysis was set up, and the rats died very quickly from the effects.

(*a*) *Preventive*

Monkeys

A *Macacus rhesus* monkey, weighing 1,513 grms., Experiment 1,290, received in the course of a week 49 ccm. of horse hyperimmune serum. Two days after the last injection sixty infected ticks were fed on it, but as spirochaetes were never found in the peripheral blood, in spite of bi-daily examination, forty more ticks were fed eleven days after the first feeding. Two days afterwards scanty parasites were found in the blood and slowly increased in numbers for the next two days, but never became very plentiful. The monkey had one relapse lasting five days after an interval of one day.

As the incubation period of the infection after tick-feeding is never shorter than five days,[8, 4] the infecting feed, in this case, was the first one. The previous treatment with the hyperimmune serum prolonged the incubation to thirteen days, but the animal then became infected and passed through a mitigated infection.

Experiment 1,345, *Macacus rhesus*, of 1,845 grms. weight. Received 15 ccm. of horse hyperimmune serum in the course of four days, and three days later was inoculated with 4 ccm. of infected blood. The parasites appeared in the blood after an incubation period of seven days, and were present for four and a half days in scanty number (maximum 1 to 40-50 f.). Eight days later a relapse of two days' duration occurred, and was followed after five days by a second relapse lasting three days.

In this experiment the hyperimmune serum had the effect of prolonging the incubation period very markedly and of moderating the severity of the attack.

(*b*) *Curative*

MONKEYS

A large callithrix (*Cercopithecus callitrichus*), Experiment 1,287, weighing 2,200 grms., was inoculated with 5 ccm. of citrated rat blood, showing 1-2 spirochaetes per field. After the usual incubation period the parasites appeared in the peripheral circulation and increased in numbers. On the third day, when there were from 20-25 spirochaetes per field in the blood film, the monkey was injected subcutaneously with 9 ccm. of the horse serum, but without result. The spirochaetes continued to increase and on the second day after the serum was administered were innumerable. On the third day the monkey died from the disease.

Another monkey (*Macacus rhesus*), Experiment 1,326, weighing 1,370 grms., was inoculated with 3 ccm. of citrated infected blood from a rat (mixture showed 1 parasite to a field). On the third day when there were 1-2 parasites per field in the preparation, it received subcutaneously 15 ccm. of horse serum, but, nevertheless, the number of the spirochaetes increased. Two hours later from 2-4 per field were seen; on the following day 6-20 per field, and on the next day 5-7 per field. On the following day no spirochaetes were seen in the peripheral blood. A relapse occurred after an interval of three days.

These two experiments demonstrate that **horse hyperimmune serum is of no pronounced value in the treatment of the disease in monkeys.** The one monkey died from the disease, while the other passed through an attack almost identical with that observed in the controls.

RATS

It was scarcely possible to make valid experiments to judge of the efficacy of the hyperimmune horse serum in preventing the occurrence of the disease in rats, as small doses had no effect and larger ones

caused the death of the animals through haemolysis. An attempt was made, however. The serum was used in the treatment of the disease in rats.

A series of four were injected subcutaneously with 0·5, 1, 1·5 and 2 ccm. respectively, on the day after they had been inoculated with infected blood. The rat which received 2 ccm. died four days later with all the signs of haemolysis, and with very many spirochaetes in its blood. The other three became infected at the same time as the control and passed through an attack followed by relapses in the ordinary manner.

In another experiment three rats were inoculated simultaneously with hyperimmune serum and infected blood in the proportions of 3 : 1, 1 : 1, and 1 : 2. In each case the dose used was 1 ccm. The first rat was infected on the sixth, the second on the fifth, and the third on the third day. The control rat was infected on the day after inoculation. The treated rats then passed through a slight attack followed by one relapse. The number of the parasites was always small (maximum 2-3 per field) as compared with the control (60-80 per f.).

2. Monkey Serum

Serum from three hyperimmunised monkeys was also used in efforts to prevent and cure the disease.

The monkeys were allowed to pass through an attack and after they had recovered, *i.e.*, in about four weeks, were reinoculated with large doses of infected blood. Two weeks later they were again reinocculated, and after another week received from five to seven injections of infected blood at intervals of two days. In the course of this treatment large amounts of blood containing numerous spirochaetes were used, up to 80 ccm. in one case. The animals were then bled and the serum obtained.

(*a*) *Preventive*

Rats

A rat weighing 84 grms. which had received subcutaneously 4·7 ccm. of serum was inoculated the next day with spirochaetal blood, and became infected on the fourth day afterwards. The control was infected on the day following inoculation. The course of the disease ran a similar course in both rats but the spirochaetes were never as numerous in the serum animal as in the control.

In another experiment, five rats received injections of 2, 3, 4, 5 and 6 ccm. of the serum respectively, and were inoculated after an interval of two days with infected blood. They were all infected on the third day, and then passed through the ordinary course of the disease. Spirochaetes were found in the peripheral blood of the control rat on the day after inoculation, and were always present in greater numbers than in the serum rats.

In another series, three rats were inoculated respectively with 1 ccm. of mixtures of monkey hyperimmune serum and spirochaetal blood in the proportions of 3 : 2, 2 : 3, and 3 : 1. The only noticeable feature in this experiment was that these rats did not become infected until the third day afterwards, while the control was infected on the following day. The severity of the attack differed in the three rats. In the one receiving the serum and blood in the proportion of 3 : 1 the attack and relapse were very slight, and the parasites were always scanty. In the other two the infection was more typical, but the number of spirochaetes was always smaller than in the case of the control.

(*b*) *Curative*

MONKEYS

A monkey (*Cercopithecus callitrichus*), Experiment 1,299, weighing 1,950 grms., was inoculated with a small dose of heavily-infected rat blood, and on the third day of the disease, when there were two or three spirochaetes per field in the blood films, received subcutaneously 5·5 ccm. of monkey hyperimmune serum. Four hours later the parasites had disappeared from the peripheral blood, but reappeared after thirty-six hours. The monkey then had the usual attack, and on the fifth day, when the blood preparations showed 1-2 spirochaetes in a field, received a further injection of 5 ccm. of the serum. Spirochaetes were seen in the preparation made on the same evening, but were very scanty (2 to ½ film). None were found for the next seven days, but on the eighth day the spirochaetes were again present in the peripheral circulation, and the monkey then had a relapse lasting five days.

RATS.

Five rats which had been inoculated with blood containing numerous spirochaetes were treated on the second day of the disease when showing many parasites, with monkey hyperimmune serum (0·5, 1, 2, 3 and 5·5 ccm. respectively). There was no effect. The parasites increased rapidly as in the control and the rats passed through typical attacks and relapses.

3. RAT SERUM

The hyperimmune serum was obtained from twenty-five rats which had recovered from the disease and were then inoculated interperitoneally at intervals which shortened from two weeks to every second day. Some rats received as many as sixteen doses of from 2·5-4 ccm. of heavily-infected blood. The rats stood the many inoculations perfectly.

(*a*) *Preventive*

MONKEYS

No preventive experiments were made on monkeys.

RATS

Three rats were inoculated respectively with 3, 4 and 5 ccm. of the serum, and on the following day with 3 ccm. of heavily-infected blood. All of them became infected after prolonged incubation periods (3, 3 and 5 days), and passed through a slight infection.

(*b*) *Curative*

MONKEYS

A small *Cercopithecus callitrichus*, Experiment 1,319, weighing 1,215 grms., was inoculated with spirochaetal blood and became infected as usual. On the third day there were 10-15 parasites per field in preparations of the peripheral blood, and the monkey then received 10 ccm. of rat hyperimmune serum subcutaneously. Four hours later the number of spirochaetes was the same as before the injection of serum, but in six hours had diminished to 6-8 per field, and by the following evening only one to two hundred fields was seen. The monkey had no relapse. Six weeks later the monkey was reinoculated with 7 ccm. of heavily-infected blood and spirochaetes were found in the peripheral blood on the following morning. This attack lasted three days.

RATS

Seven rats, weighing from 120-150 grms. were treated at different stages of the initial attack with hyperimmune serum. The serum was administered subcutaneously in doses of from 1-3 ccm. In every case the results were very discouraging. In only one rat the number of parasites did not reach the same level as in the control.

The above experiments show that hyperimmune serum, whether derived from horses, monkeys or rats, does not prevent the infection, although it lengthens the incubation period very markedly when given in sufficiently large doses, and mitigates the course of the disease to a marked extent. No cure has been effected by the use of this serum; in some cases the disappearance of the spirochaetes was hastened, but they reappeared in the course of a few days. The relapses were prevented only in one case, Experiment 1,319, but occurred in all the others.

Animals which have been treated with immune and hyperimmune sera and have then been inoculated with spirochaetal blood and have recovered from the resulting infection, usually exhibit no greater degree of active immunity than do those animals which have recovered from the disease but have not been treated with immune sera. In the one case in which we were able to cut short the attack and prevent the occurrence of relapses the immunity was not very pronounced, and corresponded in degree to the immunity observed in animals which had originally only a slight attack.

3. INBORN IMMUNITY

As spirochaetes were present in the fœtuses of infected rats, the question as to whether the young, born from such mothers, possessed any immunity against the disease naturally suggested itself. Experiments to throw light on this question were made.

Two rats, three weeks old, from a mother which had littered just after an attack lasting eleven days, were inoculated with 0·5 ccm. of infected blood. A control of the same age was used. The rats were infected on the following day and had an attack of three days' duration after which the spirochaetes disappeared from the peripheral blood and were never seen again. The control had a typical attack with one relapse.

Two of the same litter were inoculated when six weeks old with 0·5 ccm. of spirochaetal blood and had attacks lasting seven days, but no relapses were observed. The control rat of the same age passed through the ordinary course of the disease with one relapse.

Another young rat of another litter, two months old, borne by a heavily-infected mother in the first attack, was inoculated and passed through an attack identical with that of the control.

Infected ticks were fed on two rats nine weeks old, born while the mothers were heavily-infected in the first attack, but they became infected as readily and passed through as typical attacks as did normal rats of the same age.

These experiments show that **there is a slight degree of inborn immunity which disappears in a very short time.**

The results of all the work on immunity may be summed up in the following conclusions:—

(1) In animals which have recovered from the infection there is a relatively active immunity of comparatively long duration.

(2) We have been unable to produce passive immunity through the use of immune serum.

(3) Serum from hyperimmunised animals, whether horses, monkeys or rats, does not protect a susceptible animal against the disease, but does prolong the incubation period and mitigates the severity of the infection.

(4) Immune serum has no curative action whatever; hyper-immune serum occasionally cuts short an attack but does not prevent the occurrence of relapses.

(5) There is a slight inborn immunity of short duration.

IX. The Specific Nature of Sp. duttoni

The question of the active immunity observed after infection by *Spirochaeta duttoni*, and also after infection by *Spirochaeta obermeieri*, has been discussed elsewhere in this report. As no morphological peculiarities sufficient for exact differentiation existed, the only method by which the specific nature of the spirochaete of the African tick-fever could be demonstrated was by inoculating animals which have recovered from that disease with the spirochaete which caused European relapsing fever, and *vice versâ*. With this end in view, many experiments have been made, and the results of these show conclusively that the spirochaete of the African tick-fever is a distinct species.

A *Macacus rhesus* was inoculated on March 22nd, with *Spirochaeta obermeieri*, and after recovering from the disease was reinoculated with the same strain. No parasites were seen after this inoculation, but a subinoculated rat had a slight attack. Ten days later the monkey was inoculated with the African strain, and became infected on the following day. The attack lasted five days and was followed by one relapse. The disease was of the ordinary type observed in monkeys suffering from a first attack of African tick fever.

Another *Macacus rhesus* which was inoculated with *Sp. obermeieri* had an attack of four days' duration followed, after an interval of four days, by a relapse lasting five days. When it had recovered from the disease subinoculations were

made, but the rats did not become infected. The monkey was then reinoculated with the African strain, became infected after an incubation period of one and a half days, and then passed through the typical course of the disease seen in monkeys inoculated with that strain. The attack was followed by two relapses, the last of which was of two days' duration.

The same results were obtained in rats when the strains were crossed.

In the blood preparations of a rat inoculated with *Spirochaeta obermeieri*, which had been kept in defibrinated blood for twelve days, no spirochaetes could be seen, most probably on account of insufficient observations. At intervals of four and seven weeks after the original inoculation this rat was reinoculated, but spirochaetes were never seen in the peripheral blood, although it was very carefully examined. Eight days after the last reinoculation, it was inoculated with the African strain and became infected after a period of nine hours. The attack was of the usual type and was followed by two relapses.

Another rat which was inoculated with *Sp. obermeieri* had an attack lasting three days, and one relapse. Five weeks later it was reinoculated with the same strain, but the parasites were never seen in the preparations. When inoculated, seven days later, with the African strain the rat passed through a course of infection similar to that of the control.

In another case two rats which had recovered from the infection with *Spirochaeta obermeieri* were reinoculated four and five times respectively with the same strain. No parasites were found after these inoculations. The rats were inoculated with the African strain three days after they had received the last injection of *Sp. obermeieri*. The parasites appeared in the peripheral circulation after the ordinary incubation period, 24 hours, and the two rats then had a severe attack.

When animals were inoculated, first with the African strain and then with the *Spirochaeta obeimeieri* the same results were obtained.

One monkey, Experiment 1,227, after inoculation with the African strain, had a typical attack with one relapse, and was reinoculated without result two weeks after the parasites had been seen last in the blood. Six weeks later it was inoculated with *Sp. obermeieri* and had an attack of four days' duration. Similar observations were made in the case of rats.

Two rats which had recovered from infection with *Sp. duttoni* were reinoculated four and a half weeks later with the same strain but did not become infected. Four and a half weeks afterwards they were inoculated with *Sp. obermeieri* and then became infected in the same manner as the controls.

Two other rats were treated in the same way and with exactly the same results.

From the above it will be apparent that **the spirochaete of the African tick-fever is of a species differing from** *Spirochaeta obermeieri*, **since each confers a relatively active immunity against itself, but not against the other.** Therefore, for the spirochaete of the African tick-fever the name *Spirochaeta duttoni* was suggested.[6]

X. Passage of Spirochaetes through the Placenta

In a publication on *Spirochaeta obermeieri*, Albrecht states that he found the parasites in three fœtuses, seven months old, from

mothers who were suffering from European relapsing fever.[1] Spitz made a similar observation in the case of a five-months' fœtus, finding the parasites in an intracranial haemorrhage.[32]

During the course of our experimental work on *Spirochaeta duttoni*, we have been able to demonstrate the passage of the parasites from mother to fœtus in the case of four rats and one guinea-pig.[5]

Experiment 1,082C.—This rat was inoculated on April 3rd with heavily-infected blood, and after it had shown parasites for sixteen consecutive days, it was killed for subinoculation. In preparations of the heart blood from 20-30 spirochaetes per field were seen. Three half-grown fœtuses were found in the rat, and the heart blood of these together with that of the placentas was examined with the following results:—

Placenta I.—1-2 spirochaetes per field: Fœtus I.—1 spirochaete to film.
„ II.—6-8 „ „ „ II.—4 „ „
„ III.—1-3 „ „ „ III.—1 „ „

Film preparations of the brain, bone marrow and spleen of one fœtus were examined; parasites were found only in the preparations from the spleen.

Experiment 1,084.—Rat, inoculated on April 6th. Three days later, when the blood contained innumerable parasites, it was killed. The fœtuses (seven) were nearly mature. The placentas were darker in colour than usual, but otherwise presented no macroscopical changes. In blood from the uterine vein, the spirochaetes were present in very large numbers, and in the placental blood they were in such numbers that in the preparations nothing but large bundles of spirochaetes could be seen. In preparations from the umbilical vein, one spirochaete was seen in from 5-30 fields. The preparations of the fœtal heart blood showed varying numbers, on an average one to three fields, though in some fields as many as ten spirochaetes could be counted.

Experiment 1,178C.—Rat inoculated on May 26th and killed three days later when the heart blood showed from 70-100 parasites in a field. The seven fœtuses were nearly mature. The blood in the uterine vein showed about the same number of parasites as the heart blood. The results of the examination of the fœtal blood is tabulated below.

	Placenta		Umbilical Vein		Fœtus
I.	80–100 per field	...	1–5, 1–9, 1–30 fields	...	Negative
II.	30–40 „	...	—	...	„
III.	10–40 „	...	1–2, 1–4, 1–48 fields	...	1 in film
IV.	3–30 „	...	2–1, 1–6 „	...	1 „
V.	30–100 „	...	1–7, 1–78, 1–90 „	...	2 „
VI.	30–100 „	...	—	...	2 „
VII.	10–70 „	...	1–57, 1–70, 1–100 fields	...	3 „

Subinoculations were made from rat 1,178C and the fœtuses to ascertain whether the spirochaetes in the fœtal circulation were still infective and as virulent as those in the maternal blood.

Experiment 1,182.—Two rats were inoculated from the mother. Both showed many parasites in the peripheral circulation after an incubation period of four hours, and passed through the usual course of the infection. Another rat

inoculated with three cubic centimetres of citrated heart blood from three of the fœtuses showed two spirochaetes in a preparation of the peripheral blood after an incubation period of six hours. No parasites were seen for the next two days, but on the fourth day they were again present, and the rat then passed through the ordinary course of the disease.

Experiment 1,238.—This rat was inoculated on July 3rd, and was killed three days later. From one to two spirochaetes per field were seen in preparations made from the heart blood. In the blood of eight of the ten half-grown fœtuses, parasites were seen in very scanty numbers (never more than two to a thick film). In the placental blood there were, on an average, from one to two spirochaetes per field.

Experiment 1,152.—Pregnant guinea-pig. It was inoculated on May 13th, and became infected after an incubation period of six hours. It died on May 18th, and although no spirochaetes were seen in the blood preparations made after death, a subinoculated rat was infected on the following day. The fœtuses were about three-quarters mature. Although preparations of their blood were examined carefully no parasites were found, but the inoculation of a rat proved positive after a prolonged incubation period of six days, and the infection ran its usual course.

The above experiments lead to the following conclusions:—

I. *Spirochaeta duttoni* passes through the placenta from the circulation of the mother to that of the fœtus.

II. The majority of the fœtuses carried by an infected mother are themselves infected.

III. The parasites are found in the placenta in approximately the same numbers as in the maternal heart blood, but on the contrary, occur in very scanty numbers in the fœtal circulation.

IV. (*a*) From our observations we can add, the spirochaetes in the fœtal circulation show no morphological changes.

(*b*) Infected pregnant rats show no tendency towards abortion, but few of their young, in comparison with those from healthy mothers, reach maturity.

XI. Rôle of the Spleen in Spirochaetal Infection

The rôle of the spleen in spirochaetal infections has always been a question of great interest. Metchnikoff,[16] in 1887, found that spirochaetes were present in the peripheral blood of a monkey 59 hours after it had been inoculated, but that none were present in the spleen. A piece of the spleen was removed by means of the thermocautery from another monkey, when the peripheral blood was filled with spirochaetes, but the examination revealed the presence of only a very few parasites, some free and others engulfed in leucocytes. In another case, when the temperature had reached 41° C. and the

spirochaetes had disappeared completely from the peripheral blood, the spleen was taken out and was found to be filled with parasites, part of them free, part engulfed in spleen cells. The inoculation of a piece of this spleen in another monkey was followed by infection. It was thought at this time that the peripheral blood was non-infective during the interval between attack and relapse. Soudakewitch[31] experimented on two monkeys, which he splenectomised. The first died on the eighth day of the infection with very numerous spirochaetes in the peripheral blood and in that from the Inferior Vena Cava. At the autopsy it was found that a small accessory spleen had been overlooked. The second monkey died on the ninth day of the disease, also very heavily infected. From these experiments, Soudakewitch concluded that the spleen is the only organ in which the spirochaetes are destroyed, and that spleenless animals cannot recover from the infection. Tictin[35] splenectomised a monkey, and then inoculated spirochaetal blood. On the seventh day of the disease it died from tuberculosis, and parasites were present only in the blood. No phagocytosis was observed in the lymph glands or bone marrow. A second splenectomised monkey did not become infected when inoculated, probably because it was immune. A third was splenectomised after the attack, but severe relapses occurred and the monkey finally recovered. The inoculation of a fourth splenectomised monkey with spirochaetal blood was followed by a rather severe infection and recovery.

Lamb[13] splenectomised five monkeys after they had recovered from the first attack and then reinoculated them, in only one case with a resulting infection. Two uninfected monkeys were inoculated with spirochaetal blood after the spleen had been removed, and both recovered from the disease which followed.

In order to determine the rôle of the spleen in spirochaetal infection, a large number of animals were splenectomised at different stages of the disease. The strictest asepsis was observed in the operations. After the animal was anæsthetised, a longitudinal incision, starting just below the costal margin, 2·5 to 5 cm. long in monkeys and 1·5 to 2 cm. in rats, was made, and the peritoneum opened. As a rule, the spleen could be brought out at once and tied off. When a little experience had been gained, it was possible to do this through a very small opening without introducing the fingers into

the peritoneal cavity. The wound was closed by suturing the various tissues separately, and dressed with a cotton and collodion dressing covered over with a layer of thick celloidin. By pouring a few drops of chloroform over the celloidin, it became hard at once and then protected the wound very efficiently. We found the combination of collodion and celloidin much more effective than either alone. The flexible collodion kept the skin from wrinkling up while the hard casing of celloidin prevented the animals from picking off the dressing.

The results obtained were most gratifying, as in every case the wound closed by first intention. All the animals used bore the anæsthetic very well, and usually recovered completely from its effects within a very short time.

The animals stood the operation well, and no after results were noticed. The only change observed in the blood was an absolute, as well as relative, increase in the number of lymphocytes. The animals lived for a comparatively long period afterwards.

The experiments may be divided into the following groups:—

I. Splenectomy, and subsequent inoculation.

II. Inoculation, and splenectomy during the first interval.

III. Tick feeding, and splenectomy during the incubation period.

IV. Splenectomy after recovery, reinoculation.

1. Splenectomy of Normal Animals followed by Inoculation

Experiment 1,147.—This monkey (*Macacus rhesus*) was splenectomised on April 30th and after it had recovered completely was inoculated on the 10th of May with 5 ccm. of infected blood. Two hours later spirochaetes were present in the peripheral circulation and increased in number until the death of the monkey, which occurred on the third day after inoculation. The monkey's death was accelerated by a bad attack of diarrhœa which was present at the time of inoculation.

The temperature did not rise above 103° F. during the whole course of the disease and on the evening before the animal died fell to 98·6° F., and remained subnormal until death.

Post mortem the thoracic viscera did not show any changes. The liver was markedly enlarged and showed subcapsular haemorrhages of varying extent. The bone marrow was very soft, congested and of a dark purple colour. Throughout its substance small, whitish areas were seen. The inguinal and axillary lymph glands showed haemorrhagic infiltration. The gut was deeply infected and the mesenteric lymph glands were enlarged, but of normal colour. Films were made of all the organs and of blood taken from various sources and stained by Giemsa's method. In the films of the heart and peripheral blood very numerous free spirochaetes were seen, but only a very few inside the leucocytes. The number of parasites in the blood from various sources, *e.g.*, heart, inferior vena cava, renal vessels, &c., did not differ.

Experiment 1,205.—*Macacus rhesus*, weighing 1,723 grms. It was splenectomised on May 22nd and inoculated on June 11th with 5 ccm. of infected blood. Spirochaetes were found in the preparations seven hours later. They increased in number for the next three days, on the fourth day only a very few parasites were found and none on the following day. Films were made from the blood when the spirochaetes were diminishing in number, and examined for phagocytosis but very little was found. A relapse occurred seven days later and lasted five days. No spirochaetes were seen after this. A month after the relapse the monkey was reinoculated, and while it did not become infected, a rat subinoculated from it became infected and passed through a slight attack.

The course of the disease in the control was similar to that outlined above.

Four rats were splenectomised and afterwards inoculated. In two of these the disease was of the usual type. They became infected as promptly as the controls and had an attack followed by two relapses. After the attacks the spirochaetes disappeared from the peripheral circulation as in normal rats, to reappear at the time of the relapses. Spirochaetes were present in the peripheral circulation of the other two rats on the day after the inoculation, but had disappeared by the succeeding day. After an interval of three days they reappeared and were continuously present in the peripheral blood for eleven days in both cases. One rat then remained negative but the other had a second relapse of short duration. The control passed through the usual course of the infection.

These experiments show that **the course of the disease in spleenless animals does not differ in any way from that noted in normal animals; the spirochaetes appear in the peripheral circulation, increase in numbers to the maximum, then decrease until finally they are absent from the blood and after an interval repeat the same cycle in the ordinary manner.** The death of the first monkey was due not to the absence of the spleen but largely to the severe attack of diarrhœa.

One puppy, two rabbits and two guinea-pigs were also splenectomised and afterwards inoculated with spirochaetal blood, and they reacted in the same way as normal animals.

2. Inoculation followed by Splenectomy after the first Attack

In order to observe whether the spirochaetes rest solely in the spleen during the intervals, animals were splenectomised immediately after recovering from the first attack, and were watched carefully for the appearance of relapses.

Experiment 1,206.—*Macacus rhesus*, weighing 2,280 grms. This monkey was inoculated and had an attack of five days' duration. Four days after the spirochaetes had disappeared from the peripheral circulation the spleen was removed. A relapse occurred eighteen days later or twenty-three days after the end of the original attack, whereas the control monkey had the relapse ten days after the attack.

At the time of the operation the spleen was ground up in salt solution and injected into two rats (4 ccm. each) and a third was subinoculated from the blood. One of the rats which were inoculated with the spleen *became infected seven days*

later, at the same time as the one which had been inoculated with the blood. It passed through a slight attack followed by a relapse. The other did not become infected.

Films made from the monkey's spleen did not show anything remarkable. Spirochaetes were not seen in the five films examined.

Two rats, Experiment 1,198, which had recovered from an attack were splenectomised, and in both of them the relapse occurred at the same time as in the control. The spirochaetes became numerous in the peripheral circulation and then disappeared finally.

Inoculations into two rats were made with the ground-up spleen from one of the rats, Experiment No. 1,198, when operated on. Another rat was inoculated at the same time with blood from the same rat. In this case all the rats became infected and the two which had received the spleen passed through prolonged attacks lasting seven and nine days respectively. No relapses occurred.

These experiments tend to show that **the spirochaetes when disappearing from the blood do not rest solely in the spleen.** From the fact that rats which were inoculated from the blood during the intervals, and from the ground-up spleen, became infected, we must conclude that the spirochaetes are present in an infective stage in both. Of course, spirochaetes as such may be present during the interval between the attack and relapses, but if so they must be very scanty, since an examination of numerous thick films of the peripheral blood during this period has always proven negative.

3. Tick Feeding and Splenectomy during the Incubation Period

After ticks have been fed on a susceptible animal the spirochaetes are not present in a sufficiently great number to be seen in preparations of the peripheral circulation until after a period of five days has elapsed.[4, 8] In order to solve the question whether the multiplication of the parasites during this incubation period occurs only in the spleen which, of all the organs, is the one most markedly changed, or proceeds in the peripheral circulation as well, we fed infected ticks on a monkey and removed the spleen a few days later. In addition, a rat was subinoculated from the monkey each day during the period of incubation.

Experiment 1,175, Chart X, *Macacus rhesus*, of 2,253 grms. weight. On May 25th, 108 infected ticks were fed and three days later the monkey was splenectomised. On the seventh day after the ticks had been fed the monkey became infected and had an attack lasting seven days. A relapse of four days' duration occurred six days after the attack. Spirochaetes were never seen afterwards.

The rat which was subinoculated on the day following the tick feeding (4 ccm. of citrated blood) became infected on the eleventh day and had a slight attack which lasted two days. No relapse occurred.

The rat subinoculated on the second day (3 ccm. of citrated blood) was infected on the ninth day and the attack lasted seven days. The parasites were present in fair numbers in the peripheral blood.

DISEASE.

Notes of Case.

Name MONKEY Exp. 1175

Age

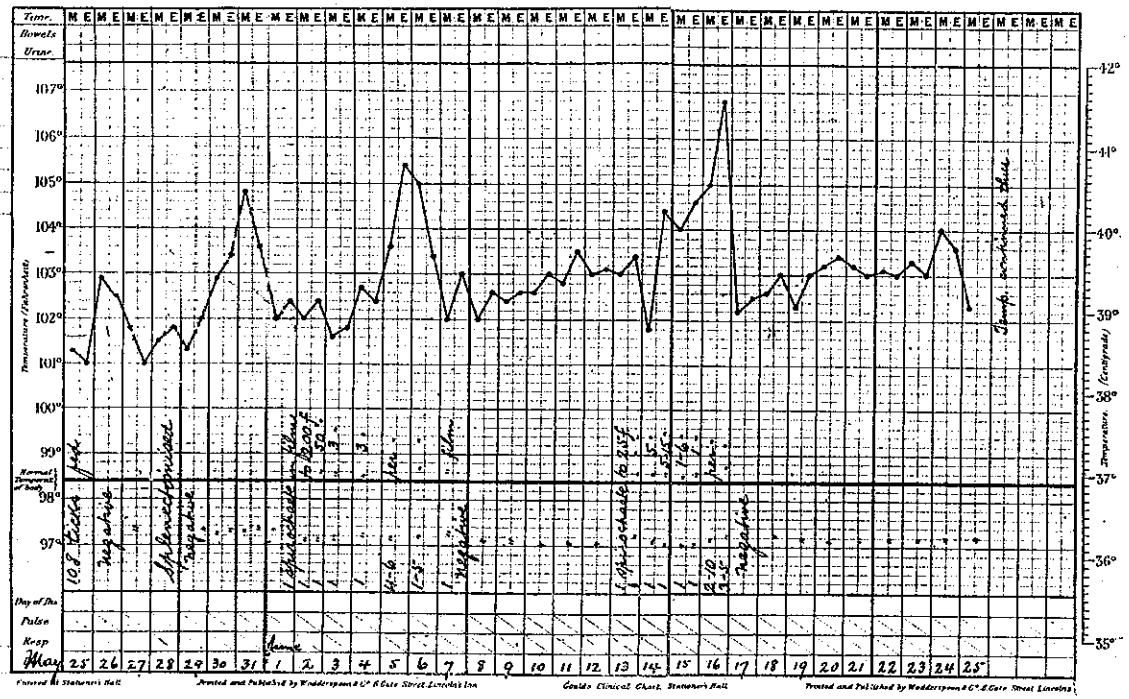
Diet

Case Book N°

Chart 10.

Date of admission

Result



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After an incubation period of seven days the rat inoculated on the third day after ticks were fed (3 ccm. of citrated blood) became infected and had an attack lasting nine days, during which the spirochaetes were found in a fair number in the blood preparations. At the same time two rats were inoculated with the monkey's spleen which was first ground up in normal salt solution. Both these rats became infected after an incubation period of twelve days, and passed through slight attacks of two and three days' duration respectively. The spirochaetes were present in small numbers. Only one of the rats had a relapse.

The rat subinoculated from the monkey on the fourth day after the ticks were fed became infected after an incubation period of four days, and had an attack lasting ten days, with one relapse.

The rat inoculated on the fifth day was infected three days later, and had an attack lasting two days, followed by two relapses.

Spirochaetes were found in the peripheral blood of the rat subinoculated on the sixth day, after an incubation period of one day. The attack lasted two days and the rat had no relapses.

Day after Feeding	Incubation Period	Duration	Relapse
1st Day ...	10 Days ...	2 Days ...	—
2nd „ ...	8 „ ...	7 „ ...	—
3rd „ ...	7 „ ...	9 „ ...	1 Relapse
4th „ ...	4 „ ...	10 „ ...	1 „
5th „ ...	3 „ ...	2 „ ...	2 Relapses
6th „ ...	1 „ ...	2 „ ...	2 „
Spleen Juice Rat A ...	12 „ ...	2 „ ...	—
„ „ „ B ...	12 „ ...	3 „ ...	1 Relapse

This experiment shows that **spirochaetes are present in the peripheral circulation in an infective stage on the first day after ticks are fed on a susceptible animal.** As will be seen from the table given above, the incubation period in the subinoculated rats became shorter, the nearer we drew to the day on which spirochaetes appeared in the peripheral circulation of the monkey, while the severity of the attack increased. From the fact that the two rats inoculated with the spleen pulp became infected five days after the rat inoculated at the same time with blood, it will be evident that the rôle played by the spleen during the onset of the disease is very slight.

As a confirmatory experiment, a white rat was splenectomised on the third day after forty ticks had been fed. At the time of operation a rat was inoculated with blood from this animal and two others were inoculated with the spleen pulp. On the sixth day after the ticks were fed the rat became infected and had an attack of four days' duration. After a negative interval of one day the spirochaetes reappeared and increased in number until the rat died in ten days. Of the rats inoculated at the time of operation, the one injected with blood became infected in four days and only one of the two inoculated with the spleen pulp, after an incubation period of five days. Both the attacks were very slight and lasted one and three days respectively with no relapses.

4. Splenectomy after Recovery from the Disease followed by Reinoculation

In order to determine whether the presence of the spleen has any bearing on the active immunity, a monkey and two rats which had completely recovered from the disease were splenectomised. These animals, however, reacted in the same manner to reinoculation as the controls.

(1) In splenectomised animals, the spirochaetes disappear from peripheral circulation after the attack as promptly as in normal animals and relapses occur in the ordinary way.

(2) When the spleen is removed shortly after the spirochaetes have disappeared from the peripheral circulation after the first attack, the relapses occur as in the controls.

(3) During the incubation period, after ticks have been fed on a susceptible animal, the spirochaetes do not develop in the spleen as the site of election.

(4) Active immunity against reinfection is not influenced by the spleen.

XII. Filtration Experiments

Novy and Knapp were the first to demonstrate that spirochaetes are able to pass through a Berkefeld filter.[24] We have repeated the experiments with *Spirochaeta duttoni*, and the importance of the fact necessitates a detailed description. Berkefeld filter cylinders No. 9 were employed. The blood for filtration was diluted in the proportions of 1 : 10 and 1 : 20, with a 2·5 % solution of sodium citrate in physiological saline. The filtration was carried out in from thirty to sixty minutes, and only the vacuum process was employed The manometer during the filtration registered 65 cm. of mercury. As control a laboratory culture of *Bacillus prodigiosus* was used, and subcultures were usually made with 2 ccm. of the filtrate on agar and potatoes, and kept in the incubator at 22° C. At the commencement the blood passed through the filter very quickly, but later the process became much slower, until finally the fluid passed through only in drops. The filtrate was of a ruby colour, and microscopically did not contain any foreign particles. The filtrate was always injected intraperitoneally in rats. The blood used for filtration was obtained from rats at various stages of the disease :—

(*a*) When the parasites were increasing in number.
(*b*) When the parasites started to decrease in number.
(*c*) When the peripheral blood contained no spirochaetes.

The chief points in the following experiments are tabulated below.

Experiment 1,312.—Blood was taken from a rat in which the parasites had just disappeared from the peripheral circulation and was diluted in the proportion of 1 : 10. The filtration lasted an hour. Six ccm. of the filtrate was inoculated in a rat. The subcultures remained sterile. Although examined carefully for the next sixteen days no spirochaetes were found in preparations of the peripheral blood. The rat was then inoculated with spirochaetal blood and passed through an attack identical with that of the control.

Experiment 1,328.—The blood used for filtration was derived from a rat which had numerous parasites in the peripheral circulation on the second day of the attack. It was diluted in the proportion of 1 : 20, and filtered during a period of thirty minutes. Two rats were inoculated, one with 7 ccm. and the other with 5 ccm. of the filtrate. The subcultures did not show any growth of *B. prodigiosus*. The first rat became infected after a period of seven days, and in the preparations only one spirochaete to a film was found. The parasites were never seen again. The other rat remained negative throughout. Both were reinoculated afterwards with spirochaetal blood, were infected at the same time as the control, and passed through a typical infection.

Experiment 1,329.—The blood in this case was also from a rat in the second day of the infection, when numerous spirochaetes were present. The dilution was 1 : 10 and the filtration lasted thirty minutes. The subcultures remained sterile. Three rats were inoculated. (*a*) with 7, (*b*) with 4, and (*c*) with 3 ccm. of the filtrate. (*b*) remained negative. (*a*) became infected on the third day, the spirochaetes on an average were 1 to 10 fields. Two days later this rat had a relapse lasting three days, during which the spirochaetes were always scanty, never more than one to five fields. Rat (*c*) was also infected on the third day in the same way as (*a*), and had a relapse three days afterwards of three days' duration.

Experiment 1,332.—The rat from which the blood was obtained for filtration was in the sixth day of the disease when the parasites had first started to decrease in numbers. A dilution of 1 : 20 was used and the blood filtered during forty-five minutes. Subcultures of the filtrate remained negative. Two rats were inoculated, one with 7 the other with 5 ccm. of the filtrate. Neither rat became infected. They were inoculated with infected blood after eleven days and showed spirochaetes the following day.

Experiment 1,333.—Blood from a rat in the same stage of the disease as the above was filtered. Dilution 1 : 10; time of filtration forty-five minutes. Subcultures remained negative. Two rats were inoculated, one with 7 and the other with 4 ccm. of the filtrate, and both were infected after an interval of two days. The parasites were found in very scanty numbers on one day only.

Experiment 1,340.—The rat was entering on a relapse on the seventh day after inoculation and only one spirochaete in from 1 to 40 fields was seen. The blood was diluted in the proportions 1 : 10 and filtered in thirty-five minutes. Subcultures remained negative. Two rats were inoculated with 7 and 2 ccm. of the filtrate, but did not become infected. When reinoculated later with spirochaetal blood they became infected promptly.

Experiment 1,341.—Blood was taken from a rat in the same stage of the disease as in 1,340. The dilution was 1 : 20 and time of filtration thirty-five minutes. Subcultures remained negative. Two rats were inoculated with 8 and 4·5 ccm. of the filtrate, but did not become infected. They became infected, however, when inoculated with blood containing spirochaetes.

Experiment 1,346.—The blood was from a rat which had one spirochaete in a field in preparations of the peripheral blood. On the previous day numerous spirochaetes had been found. It was diluted in the proportion of 1 : 20 and filtered for twenty minutes. Subcultures were negative.

Two rats were inoculated, (*a*) with 15, (*b*) with 7 ccm. of the filtrate. (*a*) became infected after an incubation period of three days, and in the peripheral blood the spirochaetes were present only in very scanty numbers, 1 to 150 fields. Two days later the blood became negative and remained so. Rat (*b*) never became infected.

Experiment 1,347.—Blood was obtained from a rat in the same stage of the disease as rat 1,346. Dilution 1:10; time of filtration, forty minutes. *The subcultures shewed colonies of B. prodigiosus on the second day.* Both rats which had been inoculated with the filtrate were infected on the second day afterwards, and passed through severe attacks during which numerous spirochaetes were found in the preparations. Nothing can be said from this experiment as a leak probably existed in the filter which allowed the bacilli to pass through.

Experiment Number	Stage of Disease	Dilution	Duration of Filtration	Amount of Filtrate Injected	Result of Inoculation	Subcultures
1,312	Interval	1 : 10	60 minutes	6 ccm.	—	Negative
1,328	Height of Attack	1 : 20	30 ,,	A 7 ,, B 5 ,,	+ After 7 days —	,,
1,329	,, ,,	1 : 10	30 ,,	A 7 ,, B 4 ,, C 3 ,,	+ After 2 days, 1 relapse — + After 2 days, 1 relapse	,,
1,332	Decline	1 : 20	45 ,,	A 7 ,, B 5 ,,	— —	,,
1,333	,,	1 : 10	45 ,,	A 7 ,, B 4 ,,	+ After 2 days + ,, ,,	,,
1,340	Onset of Relapse	1 : 10	35 ,,	A 7 ,, B 2 ,,	— —	,,
1,341	,, ,,	1 : 20	35 ,,	A 8 ,, B 4·5 ,,	— —	,,
1,346	Decline	1 : 20	20 ,,	A 15 ,, B 7 ,,	+ After 3 days + ,, 5 ,,	,,
1,347	,,	1 : 10	40 ,,	A 7 ,, B 4 ,,	+ After 2 days + ,, ,,	Positive on 2nd day

The above experiments show that an infective stage of *Spirochaeta duttoni* is capable of passing through a Berkefeld filter, which does not allow the passage of Bacillus prodigiosus.

XIII. The Morphology of Sp. duttoni

The studies on the morphology of *Spirochaeta duttoni* are not yet completed, so that no definite conclusions on this head can be drawn at present.

FRESH SPECIMENS

The spirochaetes possess from six to ten spiral turns, and are very actively motile. The movement can be analysed into a rotary movement round an imaginary longitudinal axis, a movement of the individual spirals, lateral movement of the spirochaete and progressive and retrogressive movement. As the parasite passes between the blood cells it displaces them in quite a characteristic manner, which is unlike the movement imparted by trypanosomes. From time to time a spirochaete joins end to end to form a ring, and then spins rapidly round, but in the course of a few minutes straightens out and moves in the ordinary way. Others remain motionless for a comparatively long time, and then suddenly start to move again.

The body of the spirochaete in section would look more like a flattened band than like a cylinder. On looking at the parasite an alternation of broader and thinner portions is often seen, the broader parts with ill-defined edges. This appearance is apparently due to a difference in the refraction of the various parts of the spirochaete. They are thinner than the greatest depth of a red blood cell, and consequently the spirals can move up and down in a space equal to this. At a given moment, therefore, the spiral turns of a parasite will be in different planes and thus not all in focus. Those which are out of focus accordingly appear to be broader than those which are in focus.

Along the course of the spirochaetes from six to eight dots which are much more refractile than the general protoplasm can be seen occasionally; most of these are in the uppermost part of the spirals. The width of the single spiral turns of a spirochaete as seen in stained specimens varies with the method of preparation. When the films are exceedingly thin, or are made on warmed slides, the turns are much broader and are fewer in number than when unheated slides are used, three or four as compared with ten to twelve in slowly drying specimens, *e.g.*, in organ films especially. When observed under very high magnifications with monochromatic light, a shadow

was seen passing along the edge of the spirochaetes, but we hesitate to say that this was caused by an undulating membrane, as we have never been able to see such a structure in stained specimens.

Zettnow [41] had described in *Spirochaeta duttoni* and Borrel [3] in *Spirochaeta gallinarum* peritrichal flagella. Although many specimens were examined particularly to this end no evidence of such a condition was ever found. The erythrocytes are extremely plastic and change their shape under the slightest influence, but this was never observed to occur even when the parasites were in very close contact with the red blood cells. Spirochaetes were often seen closely coiled round erythrocytes, but caused no change in their shape such as would be expected to follow were lateral flagella present.

It was impossible to make out any definite structure in fresh specimens. Different reagents which bring out the nuclei of cells were employed, but no changes were noticed in the spirochaetes.

STAINED SPECIMENS

The spirochaetes stain readily with all the modifications of Romanowsky's stain and with some of the basic stains. The individual parasite is then found to measure from 14 to 16μ in length, but chains of three or four are often seen measuring up to 45μ. In specimens which have been stained for a fairly long time the spirochaete is seen to consist of a darkly-stained central core surrounded by a very thin layer of faintly-staining periplast. This periplast extends beyond the termination of the more deeply-stained central portion, and is gradually drawn out to a pointed extremity at one end of the parasite. This forms, in our opinion, what other observers have described as the terminal flagellum.

In a short note on the structure of *Spirochaeta duttoni* Stephens[33] has described "eviscerated forms" produced by mechanical or chemical action on the parasite. Probably these are produced by a separation of the periplast from the central part of the spirochaete.

The chromatic core does not stain evenly; in very many spirochaetes darker portions alternate with lighter ones. Frequently a small unstained area can be seen to completely interrupt the core of chromatin, and is usually situated in about the middle third of the body.

Peculiar forms are seen most often in the "decline" blood. The chromatic core appears to be broken up into from six to eight small portions which stain deeply by Giemsa's method.

Particular reference may be made to a form which was found occasionally in films made from the liver and spleen. In this the spirochaete is coiled up into a small compass, stains a deep red with Giemsa's stain and is surrounded by a well-stained membrane. The whole structure is about three-quarters the size of a red blood cell. The space between the membrane and the spirochaete is filled with a faintly-stained pink substance. We are unable to give any explanation of the origin of this body, but suggest it may be an encysted form (Plate VIII, Fig. 21, page 96).

XIV. Protozoal Nature of Spirochaetes

Novy and Knapp [24] believe that they have brought the final proof of the bacterial nature of *Spirochaeta obermeieri*, and Borrel[3] places all the spirochaetes in the group of spirilla or spirillo-bacteria. Blanchard [2] places these organisms among the protozoa. In his latest publication Prowazek[29] states that he has seen in *Spirochaeta gallinarum* longitudinal division, the presence of an undulating membrane, and the penetration of the parasites into young and old red blood cells which are more oval than normal, and contain granulations in addition to the spirochaetes. He calls the parasite real "Zellparasiten," and states that this speaks against the bacterial nature of the organisms.

The course of the disease in experimental animals is quite different from any bacterial infection known at present. No bacterium causes such a regular recurrence of relapses as do the spirochaetes, and it is a remarkable fact that several rats inoculated from a patient during the interval, when no parasites could be seen in the peripheral blood, became infected *coincidently with the onset of the succeeding relapse*. This points distinctly to a life-history of the spirochaete in the host.

The occurrence of active immunity is not a certain indication of the bacterial nature of an organism, as it is well known that protozoa are capable of conferring this condition, *e.g.*, rats which have passed through an infection with *Trypanosoma lewisi* are immune afterwards, and malaria confers a relatively active immunity. We

have not been able to protect a single susceptible animal against spirochaetal infection by the use of immune and hyperimmune serum.

As the result of their investigations, Dutton and Todd[8] state that the transmission of the spirochaetes by ticks is not merely mechanical and that some developmental process takes place in the tick. The passage of the spirochaetes from the alimentary canal of the ticks to the ovary and eggs is a very interesting and suggestive fact.[12] This has not been shown to occur in the case of any bacterium up to the present, but is known to occur with protozoa.

XV. Animal Reactions of Sp. obermeieri

The experiments given below were carried out with a strain of spirochaetes derived from a case of relapsing fever in the Bellevue Hospital, New York, and is the same as that on which Novy and Knapp based their observations.[23, 24] These observers have identified it as *Spirochaeta obermeieri*. We have used only monkeys and rats as Norris, Pappenheimer and Flournoy,[19] and Novy and Knapp[24] have dealt fully with the animal reactions of this spirochaete.

Monkeys

The incubation period in monkeys varied between one and four days, depending upon the amount of infected blood with which they were inoculated. The attacks varied in length from two to six days. As a rule only one relapse occurred, but occasionally two were noted. The infection was very similar to that with *Spirochaeta duttoni*, but the first relapse usually occurred after a longer negative interval than seen in the case of the African strain. As many as twenty-two days elapsed between attack and relapse.

Rats.

Rats became infected in from twelve to twenty-four hours in the majority of cases, but prolongations of the incubation period up to eight days, in one case, have been observed. The attack lasted from one to three days, as a rule, occasionally four, and the spirochaetes were usually found in the preparations in small numbers. At the height of the attack the maximum number observed was twenty to a field, and this was seen in only a very few cases; in most of the rats one spirochaete to 2-10 fields was found. Relapses were observed in

61 % of 60 rats watched specially for this purpose. The relapses were of very short duration, usually one day only, and very few spirochaetes were present in the peripheral circulation, only one or two in a preparation. In two cases the rats had two relapses each. In the one rat the first relapse occurred on the second day after the attack ended, and the second sixteen days after the first. In the other a negative interval of two days elapsed between the attack and the first relapse, and ten days between the two relapses.

The peripheral blood remains infective after the spirochaetes have disappeared from the circulation, as will be evident from the following:—

A rat which had an attack lasting three days was killed on the fourth day on which no parasites had been found in peripheral blood. Another rat was inoculated from it and became infected after an incubation period of three days. This observation has been repeated in other cases.

The blood was always infective on the day after the spirochaetes had disappeared—at later periods only if larger doses were used. The disease has never been followed by a fatal termination in any animal.

MICE

A few mice were inoculated with this strain and proved to be more susceptible than rats. The disease was more severe than in rats, and two relapses were observed very frequently.

The above experiments show **that the animal reactions of** *Spirochaeta obermeieri* **are quite different from those of** *Spirochaeta duttoni*. The objection may be raised that this difference was due only to a difference in virulence of two strains of the same parasite, brought about by very numerous passages through susceptible animals. Every effort was made to increase the virulence of *Spirochaeta obermeieri* both by slow and quick passage through rats, but always without result. In one rat inoculated with infected blood which had been kept *in vitro* for fifteen days, the attack was of longer duration and was more severe than previously seen, but the rats subinoculated from this suffered the usual slight and passing infection. Moreover, as has been stated already, the strains of African spirochaetes derived from monkeys infected directly by the bites of ticks were just as virulent as others which had been passed through long series of rats.

Novy and Knapp state that they have never seen a relapse in rats. Our experiments show that if the examination is done carefully enough and is continued for a sufficiently long period, relapses are found to occur in the majority of the cases.

XVI. Active Immunity in Sp. obermeieri

The monkeys which had recovered from the disease were reinoculated with the same strain, and this resulted in much the same manner as noted in the similar infections with the African spirochaete.

In one case the subinoculation of a white rat on the first day after the reinoculation of the monkey was followed by infection. Two parasites were seen in preparations of the monkey's blood. Subinoculations were made some days later, but the rats did not become infected. Five rats were reinoculated after complete recovery, and of these two became infected.

A rat which had recovered from an attack with one relapse was reinoculated six days after the first inoculation with 5 ccm. of infected blood. Two days later scanty spirochaetes were found in the peripheral circulation, and then disappeared finally.

Another rat which had passed through a very slight attack lasting one day was reinoculated nine weeks afterwards, and was infected for two days after an incubation period of one day. The parasites were very few in number.

Three other rats did not become infected when reinoculated at periods varying between five and seven weeks after the original attack.

Animals which have recovered from infection by *Spirochaeta obermeieri* acquire a certain amount of active immunity against reinfection, the efficiency of which corresponds directly to the severity of the attack.

*Gabritschewsky 9 was able to infect rats, white mice and a guinea-pig with *Spirochaeta obermeieri* derived from a case of relapsing fever occurring in Russia. The disease was very mild in the rats.

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NOTE