A Laboratory Study of Abyssinian Louse-Borne Relapsing Fever

R. KIRK

ANNALS
Edited by
Prof. W. YORKE
Prof. D. B. BLACKLOCK
Prof. R. M. GORDON
Dr. T. SOUTHWELL
Prof. T. H. DAVEY

REPRINTED FROM THE
ANNALS OF TROPICAL MEDICINE AND PARASITOLOGY
Vol. 82, No. 4. December 21, 1988

ISSUED BY
THE LIVERPOOL SCHOOL OF TROPICAL MEDICINE

THE UNIVERSITY PRESS OF LIVERPOOL
A LABORATORY STUDY OF ABYSSINIAN LOUSE-BORNE RELAPSING FEVER

BY

R. KIRK*

(From the Staff Medical Research Laboratories, Khartoum, Anglo-Egyptian Sudan)

(Received for publication September 17th, 1938)

CONTENTS

I. INTRODUCTION .......................................................... 339
II. MORPHOLOGY AND MOVEMENTS OF THE SPIROCHAETES ....... 340
III. CULTURE ............................................................. 341
IV. ANIMAL INOCULATION .................................................. 343
V. TRANSMISSION ........................................................... 346
VI. IMMUNITY ............................................................. 348
VII. EFFECT OF SPLENECTOMY ............................................ 349
VIII. RESIDUAL INFECTIONS ................................................ 352
IX. SUMMARY ............................................................... 353
X. REFERENCES ............................................................ 353

I. INTRODUCTION

During the past two years, cases of relapsing fever have been recorded in the Anglo-Egyptian Sudan, and the writer has had the opportunity of studying some of these. The complete restriction of the disease to immigrants from Abyssinia and Eritrea leaves no doubt that these countries are the source of the infection. The tendency to extension at the present time is probably related to the recent military upheaval in East Africa, for relapsing fever is essentially one of those diseases which flourish in times of war.

Relapsing fever of Abyssinian origin has not previously been encountered in the Sudan, although the existence of the disease in Abyssinia has been known for many years. The infection was first reported by Doreau (1908), and in the same year Brumpt (1908) reported that he had successfully infected a monkey in Paris by means of Ornithodorus moubata sent to him by a missionary from Harrar, thus establishing the existence of a tick-borne strain. Mesnil (1908), in a review, called the spirochaete Sp. abyssin; and Bergsma (1928) gave an account of some clinical cases, and noted the prevalence of Ornithodorus moubata and Ornithodorus savignyi.

Louse-borne relapsing fever has been reported from Addis Ababa by Sibilia (1937). This author claims to be the first to report the louse-borne disease; but Nägelsbach (1934), three years earlier, had noted that in the highlands of Abyssinia, where ticks were not found, lice and relapsing fever were both common, and concluded that the lice must be the vectors.

*The writer is indebted to the Director of the Sudan Medical Service for permission to publish this article.

339
It is interesting to note that de Paoli (1890), describing an epidemic of relapsing fever in Asmara in 1902, in which ticks were not found but lice were plentiful, states that the disease was imported into Eritrea from Abyssinia.

Corroboration of his views is given by Bruns (1937), who records a very fatal epidemic of louse-borne relapsing fever in the Dajig district of Abyssinia in 1929. According to Cacciatore (1938), the disease is now endemic in many towns and villages in Eritrea.

According to Bruns (1937), relapsing fever is endemic in many districts of Abyssinia, but is apt to break out in epidemic form during the rainy season. The louse, Ornithodorus moubata and Ornithodorus savignyi are all cited as the vectors by this author.

It may be noted that, with the exception of Brumpt (1908), these authors rely largely on epidemiological data for their views on transmission, and do not substantiate them by experimental transmission of the disease by the alleged vectors.

The writer has carried out a laboratory study of the spirochaetes from some of the cases seen in the Sudan. It has been possible to incriminate the louse as the vector, and the results of animal inoculation have been concordant with eleven strains obtained from the blood of infected patients, and one strain isolated from an infected louse.

II. MORPHOLOGY AND MOVEMENTS OF THE SPIROCHAETES

Typical spirochaetes (Borrelia) were found in the blood of the patients during the pyrexial stages of the disease. In stained films and under the dark field, their morphology and movements correspond closely with text-book descriptions and need not be detailed here.

Variations in length and in the number of turns in the spiral were considerable. It was found that by freezing citrated blood containing spirochaetes, keeping it for a few days in the ice-chest, and thereafter making films, beautiful preparations could be obtained, in which the regular spiral structure of the organisms was preserved (Plate IX, fig. 2). These films were ideal for measurements. The average length of 200 consecutive spirochaetes seen in such a film was found to be 18μ, the extremes being 10μ and 40μ. Wider extremes were observed in other films.

In these films of frozen blood irregular globose swellings were frequently seen in the spirochaetes, sometimes terminal, sometimes situated at various distances along the length of the spirochete. They probably correspond to the swellings seen by Butler (1908) in Sp. duttoni which had been kept for some time outside the human body. In all probability they represent degenerative changes.

In these films of frozen blood, too, various structures could be seen inside the red blood corpuscles. Some of them recalled the appearances figured by Balfour (1911) in the development of the intracorpuscular granules of Sp. anserina

(Spirochaeta granulosa penetrans) (see text-fig.). Indeed, all the appearances seen by Carter (1908) could be readily identified in the corpuscles of these films. Carter regarded them as stages in the life-cycle of the spirochaete. The appearances seen in the frozen blood certainly suggest penetration of the red blood cells by the spirochaetes, but it is more reasonable to regard them as artefacts, because:

1. They were seen only in frozen blood, never in fresh films.
2. The spirochaetes showing these peculiarities were always motionless in the dark field, and therefore presumably dead.
3. Other and obvious artefacts were observed in the same films.

III. CULTURE

Yuan-Po's medium. It was found impossible to grow the spirochaetes in the egg-medium described by Yuan-Po (1933, 1936). At the outset much time was lost by confining one's attempts at culture to this medium, and refusing to accept repeated failures, largely owing to the glowing reports of Yuan-Po,
who claimed to have obtained 100 per cent. successes with \textit{T. recurrens} in this
medium.

Spirochaetae could be found in the cultures for periods up to 10, and even
20, days after inoculation. From the seventh day onwards their movements
under the dark field became very feeble. Finally they stopped, and the
spirochaetae disappeared. It was not possible to determine the manner in which
they disappeared. Short, immobile forms of only two or three turns were
observed, and probably resulted from the disintegration of dead or dying
spirochaetae.

Batches of medium were made up according to the original recipe (Yuan-Po,
1935), as well as according to the modified simple method (Yuan-Po, 1936);
but there was no difference in the result. No evidence of multiplication could
be adduced in either medium. The instructions of the author were followed to the
letter in both cases, and the resulting pH was found to be identical with that
obtained by him, so that there is no reason to believe that the media differed in
any way from those used by Yuan-Po.

After many attempts this medium was finally discarded, and other methods
were tried.

\textit{Citrated human blood}, kept either at room-temperatures or at 37°C, was not
found successful. The spirochaetae died rapidly, as estimated by their move-
ments under dark-ground illumination. They were always completely immobile
in three days, and there was no evidence of multiplication. It was found that,
as the corpuscles sedimented, the spirochaetae tended to settle in the layer just
above the corpuscles, and a false impression of multiplication might be obtained
if fluid from this layer were selected for examination. In some cases a tendency
to auto-agglutination was observed. The blood used was of human origin,
taken from the infected patients and containing spirochaetae at the time of
withdrawal.

\textit{Diluted human serum}, far from being a successful culture fluid, appeared
to have a lethal action on the organisms, which were all motionless in 24 hours.
\textit{Diluted horse serum} (Kligler and Robertson, 1922) was similarly found to be
useless as a culture medium. Although six tubes were inoculated from two
different strains, it should be admitted that the serum was all obtained from one
horse; Holter and Zablotzkaia (1926) have stated that sera from different
horses often vary considerably, one giving a good growth, while another appears
to have an inhibitory effect.

Excellent cultures were finally obtained in a \textit{modified Noguchi’s medium},
prepared as follows:

Under aseptic precautions, half an inch of egg albumen was pipetted into
the bottom of 5 in. × ½ in. test-tubes, and then coagulated by placing the tubes in
hot water at approximately 80°C. Thereafter the tubes were filled to within
an inch and a half from the top with freshly drawn acetic fluid. The tubes
were inoculated by introducing 3–5 drops of blood containing spirochaetae, and
incubated at 37°C. Within 24 hours there was an obvious increase in the ease
with which spirochaetae could be found in the dark field, and the high proportion
of long and dividing forms was evidence of active multiplication. On the third
day after inoculation, each dark field from the fluid lying just above the coagu-
lated egg-white contained 20–30 actively motile spirochaetae, with a high pro-
portion of dividing forms. Enormously long filamentous forms were sometimes
encountered. In one of these the appearance of thinned parts along its length
suggested that it was in the process of dividing transversely into four daughters.

This medium never failed to grow the spirochaetae. The impression
was gained that growth was improved by the addition of 5–10 drops of sterile
50 per cent. glucose, but it was difficult to be certain. The addition of the
glucose was certainly not necessary in order to obtain a good growth.

The addition of a layer of liquid paraffin on the top of the acetic fluid
was found to have no influence on the growth. Culture tubes without the
paraffin on the top were indeed preferable, as the oil tends to adhere to the
pipette with which samples are withdrawn for examination, and refractile oily
globules appear in the dark field. As compared with Yuan-Po’s medium,
which is full of granular debris, the acetic fluid gives beautifully clear dark
fields, against which the spirochaetae stand out distinctly.

Subcultures were readily made by introducing 3–4 drops from a culture
showing plentiful spirochaetae into a fresh culture tube by means of a capillary
pipette, adding at the same time 3–4 drops of citrated or freshly drawn rabbit’s
blood. One strain was passed through 12 successive subcultures at 2–4 day
intervals without any apparent loss of vitality, and the procedure was only given up
because of the writer’s departure on leave. After nine subcultures, the inocula-
tion of 3 c.c.m. of the culture fluid into the peritoneum of a mouse resulted in the
appearance of spirochaetae in the peripheral blood in 24 hours. The course
of this infection was not followed out.

\textbf{IV. ANIMAL INOCULATION}

\textbf{RODENTS}

Of laboratory animals, white rats, rabbits and guinea-pigs proved insus-
ceptible. So also did the ordinary brown and black rats (\textit{Rattus rattus} and
\textit{Rattus norvegicus}) caught locally by the Khartoum Sanitary Service.

This at the outset, suggests a louse-borne infection. Nicolle (1932)
recommends guinea-pig inoculation as the most ready method of differentiating
between louse-borne and tick-borne strains in northern Africa, and suggests
that, if this procedure were resorted to more frequently, many infections in
Egypt, Algeria and Libya at present regarded as louse-borne would prove
by their virulence to guinea-pigs to be due in reality to tick-borne strains.

\textit{White mice}, obtained originally from the Wellcome Bureau of Scientific
Research, London, were found to be susceptible. The infection in these animals
was observed to run a very constant course. As a general rule the intraperitoneal
route was used for inoculating the spirochaetes. Spirochaetes were invariably found in the peripheral blood 12-24 hours after inoculation. They were always present 48 hours after inoculation, but usually disappeared during the following 12 hours. The infection ran an exceedingly mild course in these white mice. It was apparently symptomless, and daily examination of the blood for 20 days after the disappearance of the spirochaetes failed in every case to show any evidence of relapse. Subinoculations from mouse to mouse were not successful, even when a large inoculum of infected material was given. This was effected by bleeding a mouse to death into sterile citrate solution 30 hours after inoculation, when the blood, as shown by films taken immediately before bleeding, contained numerous spirochaetes. About three-quarters of a c.cm. of blood was obtained in this way, and inoculated immediately into the peritoneum of two fresh mice, but no infection resulted. Negative results were likewise obtained when the organisms were inoculated subcutaneously instead of intraperitoneally, and also by the percutaneous method, which Tomioka (1924) had found to give rise to a much more severe infection with the European strain in mice than intraperitoneal inoculation.

According to the tables drawn up by Balfour (1911) and Manson-Bahr (1935), passage through monkeys increases the virulence of the European strain of T. recrurentis for small rodents. This was not found to be the case with the Abyssinian strain. Rats, rabbits and guinea-pigs were still refractory after passage of the spirochaetes through monkeys, and the virulence of the organisms for white mice was, if anything, diminished. Twenty-four hours after inoculation the spirochaetes were present in the peripheral blood of the mouse, but at 48 hours they were exceedingly scanty and difficult to find. After this they disappeared and no relapse took place. Subinoculations from mouse to mouse were still unsuccessful.

It is interesting to note that the course of the infection in these mice inoculated from monkeys did not vary with the severity of the disease in the monkeys. Nor did it seem to signify whether the original monkey was splenectomized or not, or whether it was originally infected by the inoculation of blood from a case, or by experimental transmission from an infected house. These differences made a considerable difference in the severity of the infection in the monkeys.

The gerbille, or desert rat of the northern Sudan, was also susceptible, and in this animal the course of the infection was similar to that in mice, but the parasites sometimes persisted in the blood for three or even four days. Subinoculations from gerbille to gerbille sometimes resulted in a transitory appearance of scanty spirochaetes about 48 hours after inoculation. The gerbille seemed undoubtedly to be the most susceptible of the available small rodents.

An interesting observation made in these rodent infections was that in a small percentage of white mice the spirochaetal infection was accompanied by, or more commonly followed by, a low-grade septicaemia in which a mixture of organisms were concerned, principally diphtheroids, cocci and bacilli. This always persisted for some time after the spirochaetes had disappeared from the blood, and generally seemed not to upset the health of the animals, although in two mice the number of organisms observed in the blood-films steadily increased, and the animals became ill, and finally succumbed. Post-mortem examination revealed no peritonitis or abscess-formation, and clinical examination of other affected mice showed no evidence of peritonitis or local reaction at the site of inoculation.

At the time, not much attention was paid to these organisms which were seen in the blood-films, except to exclude the possibility of their being due to faulty technique at the time of taking the blood-films. It is difficult to understand why the intraperitoneal inoculation of spirochaetes withdrawn directly from a human vein should cause a septicaemia without local reaction, unless the organisms were of extreme virulence, in which case the septicaemia might reasonably be expected to be rapidly fatal.

It is possible that the condition may have been due to an increase in the invasive powers of commensal organisms, brought about by the spirochaetal infection. Sanarelli (1927) maintains that the spirochaetal infection is harmless in itself, but in some way is able to increase the invasive powers of other organisms, and that these are responsible for the disturbance which takes place. Kulesha and Titowa (1923) stated that many of the relapsing fever fatalities in the Russian epidemic of 1920-1921 were due to secondary infection, and pointed out that from the literature it is apparent that many of the past European epidemics have been complicated by a pyaemia in the same way.

Monkeys

The common grey monkey of the Sudan (Cercocebus aethiops) was found to be susceptible, and in these animals the infection is similar to the human disease. The spirochaetes commonly appear in the peripheral blood after an incubation period of 2-3 days, persist for 2-5 days, and then, after a lapse of some 10 days, reappear for 1-3 days, after which there is no further relapse. Some variation between individual monkeys was encountered. Thus, one monkey had no relapse, and another was found which appeared to be quite immune: attempts to infect it with two different strains were quite unsuccessful.

It was found that one monkey could readily be infected by the inoculation of blood containing spirochaetes from another monkey. A strain obtained from infected human blood was passed through two monkeys in this way, and one obtained directly from a house was passed through three successive monkeys.

No attempt was made to ascertain how many passages could be effected in this way without the strain losing its virulence. It may be recalled that Sergent and Foley (1910) were unable to pass the Algerian strain even to a second monkey, and that Gambier (1923) in the French Sudan had the greatest difficulty in infecting even one monkey.
Enlargement of the liver and spleen was readily detected in all the infected monkeys, but no attempt was made to follow the clinical course of the disease in these animals. Except for very obvious illness, attention was confined to the examination of the blood for organisms.

In general terms the virulence of the strain for laboratory animals may be summarized as follows. Rodents, with the exception of white mice and gerbilles, were insusceptible. In these two latter animals the infection runs an exceedingly mild course, ending in recovery. There is no relapse, and the strain cannot be maintained in these animals. In monkeys the infection runs a mild clinical course with one relapse, and tends to recovery. Passage through at least three monkeys is possible.

These results conform in the main with those which have been found fairly universally with the louse-borne strains of relapsing fever, and especially with the north African group.

V. TRANSMISSION

Spirochaetes found in lice. It has been possible to demonstrate that the louse is the intermediate host of the relapsing fever under study. Metacyclic forms of the spirochaete were found in the haemocoele fluid of lice from infected persons. Compared with the organisms found in the blood, these forms are smaller, finer, and more regularly spiral in structure. Stained by Leishman's method they do not show the same tendency to twist themselves into figures-of-eight and other coiled or irregular forms. They were frequently found in enormous numbers, forming large tangled masses in the haemocoele of the louse (Plate IX, fig. 4).

The best method of demonstrating the spirochaetes in the louse was found to be that described by Riding and Macdowell (1927). The louse was seized with fine forceps, and laid on its back in a small drop of distilled water on a clean slide. The abdomen was transfixed by a needle, lateral to the gut, and the haemocoele fluid was allowed to escape from the puncture, the movements of the legs of the louse assisting the mixing of the haemocoele fluid and the water on the slide. The fluid could then be examined by dark-ground illumination or allowed to dry in the air and stained by Leishman. This method was infinitely superior to making smears of crushed lice, and gave beautiful preparations, free from debris. The lice were kept in a test-tube for 24 hours between removal from the patient and examination for spirochaetes.

The percentage of infected lice varied in different cases, presumably according to the duration of the disease, although it was not possible to verify this, as histories were unreliable, and steps were taken to cut short the disease with treatment as soon as the diagnosis was verified under the microscope. Most commonly 15–20 per cent. of the lice were found infected. In 30 per cent. of cases no infected lice were found. In one case the infection rate was as high as 60 per cent. (6 infected out of a total of 10 lice obtained from the patient's clothing).

No infected nits found. Both male and female lice were found to be infected. No evidence of hereditary transmission in the louse was found, although large numbers of nits were examined, including a number actually deposited in captivity by infected lice. Microscopical examination alone was made: the nits were crushed on a slide and examined under the high power stained by Leishman. No animal inoculation experiments were carried out, so that the possibility of hereditary infection by an invisible granule stage was not investigated.

Transmission of the disease to a monkey from a louse. The spirochaetes in the lice were mostile under dark-ground illumination, and they were able to reproduce the disease in monkeys. Only one successful transmission experiment was carried out, but this took place under somewhat dramatic circumstances. A blood-slide from a patient admitted to the Church Missionary Hospital in Omdurman on February 24th, 1938, was found to be positive for relapsing fever. The hospital was informed, and it was requested that the lice (if any) from this patient be collected and transmitted to the writer. Unfortunately, it transpired that the patient had already been washed and his clothes removed for disinfection.

On the following day, however—thanks to the Medical Officer of Health, Khartoum—a single louse was found on a pair of pants, which must somehow have escaped the delousing process. This louse was given a feed from the writer's arm, and kept for another day at 37° C.

On February 28th, 1938, this louse was transfixed on a slide and its haemocoele fluid was allowed to mix with distilled water in the manner described above. Some of this fluid was rubbed into a small scarified area on the shaved abdomen of a splenectomized monkey, and allowed to dry, as in a vaccination. The residue on the slide was stained by Leishman's method, and examined microscopically. It was found to contain spirochaetes.

After an incubation period of four days spirochaetes appeared in the monkey's blood. During the next eight days the spirochaetes became progressively more numerous, until there appeared to be as many organisms as red blood cells. The monkey became obviously ill on March 8th, 1938, and died on March 10th, eight days after the first appearance of the spirochaetes in the blood.

According to this observation, the disease acquired from the louse is more virulent than that which results from the inoculation of blood containing spirochaetes. In no other experimental animals were the spirochaetes observed in the peripheral blood in such enormous numbers as in this monkey, which was, moreover, the only animal to die in the first paroxysm of experimental relapsing fever. Although this is in accordance with the experimental findings in tick-borne relapsing fever, Lipstein (1938), working with *S. noyai*, found that the infections derived from lice were always benign, while those which followed the inoculation of infected blood were always fatal.
VI. IMMUNITY

Evidence of immunity. On January 24th, 1938, blood containing numerous spirochaetes was inoculated from a human case in Khartoum North directly into two mice which had previously been infected on two occasions, and at the same time into two fresh mice. In 24 hours the latter developed a typical 2-days' infection, while spirochaetes failed to appear in the blood of the two previously inoculated mice. A similar result was obtained on February 28th, when blood was inoculated from an infected monkey into two fresh mice and two previously inoculated mice.

A monkey (no. R1) proved resistant the second time an attempt was made to infect it. Another monkey (no. R2), which had been twice infected previously, was unsusceptible when a third attempt was made on March 5th. On both these occasions it was possible to infect other monkeys by inoculation of the same blood which failed to infect monkeys no. R1 and R2 respectively, showing that the absence of infection was not due to loss of virulence of the spirochaetes.

Evidence exists, therefore, that a state of immunity to further infection can be brought about by a previous infection. But this is inconstant and erratic. Other and more numerous experiments can be cited in which previous infection failed to protect against further infection. Thus, two mice infected on November 13th, 1937, were found to be susceptible again on November 16th, and two mice infected on November 16th were as readily susceptible again on November 25th. In both cases the second infection was similar to the first in its intensity and duration. The same is true with monkeys: monkey R1 was susceptible on one occasion and thereafter resistant, while monkeys R2 and R3 were readily infected a second time.

Presence of several antigenic variants. These discrepancies are probably due to antigenic differences in the various strains studied. Baltazard (1906) and other workers have found that no two strains of relapsing fever are the same, and that infection with one strain results in the development of immunity against that strain only and no other.

Cunningham (1925) has postulated the alternation of two serological variants with successive relapses. Some of our experimental results at least suggest that the 'relapse' strain is different from that of the first paroxysm.

On January 24th, 1938, two monkeys and two mice, all of which had previously been infected and had recovered, were inoculated with blood containing numerous spirochaetes from a case in Khartoum North. Of those four animals, one of the monkeys (no. R2) was the only one afterwards to show spirochaetes in its blood, although they had all been previously infected with one strain. On this previous occasion, however, one of the monkeys (no. R1) relapsed, while the other did not (no. R2). The mice were both inoculated from monkey no. R1 during the first paroxysm and again during the relapse. On both these occasions they were susceptible. The fact that both mice were resistant on the third inoculation, as well as monkey no. R1, suggests that the difference between the two monkeys on this occasion was due to other factors than individual variations in natural resistance.

It is reasonable to assume that the resistance of the one monkey and the two mice was due to acquired immunity brought about by previous infection with an antigenically similar strain. That the immunity was not due to the first paroxysm is shown by the successful infection of the other monkey (no. R2), which also had a first paroxysm in the previous infection. Presumably, therefore, the immunity in monkey no. R1 and the two mice was due to the relapse, which never appeared in monkey no. R2. If this be so, then the original and relapse strains were probably different from each other.

Another monkey (no. R3) had a relapse during its first infection, and thus probably experienced two different variants. This did not prevent its being successfully infected two months later by the inoculation of infected blood from another monkey. It is probable, therefore, that more than two antigenic variants were present among the strains.

There is possibly also a factor in the immunity which is not specific to the strains. Two infections seemed always to make our animals immune to further infection. It may be noted, too, that the relapse is usually of much shorter duration than the original attack. It may further be observed that, although the number of our experiments on this point is small, when previously infected monkeys are splenectomized, and then inoculated with a strain to which they are susceptible, the effect of the splenectomy on the infection is different from that which results when splenectomy is done before the first infection.

VII. EFFECT OF SPLENECTOMY

According to Melency (1927), Kritschewski and Rubinstein (1927) and Kalajew (1931), the increased susceptibility of experimental animals to relapsing fever which results from splenectomy is evidenced by a much heavier infection of the blood with spirochaetes and an increase in the death-rate of inoculated animals. On the other hand, Breinl and Kinghorn (1906) found that splenectomy had no effect whatever on the course of African relapsing fever, and Velu, Balozet and Zottner (1931) have reached a similar conclusion with regard to
T. hispanicum infections. Russell (1931) found with the West African strain that splenectomy might result in a slightly higher death-rate, but that otherwise it had no effect on the infection.

In experimental work with organisms of relatively low virulence, such as this Abyssinian strain, anything which will increase the susceptibility of the hosts, and make the infections a little more definite, is a practical advantage. The few experiments carried out by the writer under this heading were undertaken primarily to determine whether any advantage would be gained by the use of splenectomized rather than normal animals for transmission experiments, although, in point of fact, the experiments had already been undertaken with splenectomized animals.

Mice. Two mice, splenectomized five days previously, were inoculated intraperitoneally with approximately 0.2 c.c. of infected blood withdrawn from a monkey on January 26th, 1938. Two normal mice were inoculated at the same time, as controls. In all four animals the disease pursued a similar course, being found in the blood during the first two days following inoculation, after which they disappeared, and did not return.

Six days after the disappearance of the spirochaetes, the two control mice were splenectomized, but this did not result in the reappearance of the spirochaetes.

Two of these four mice were infected a second time on March 5th, and the infection ran a typical course, exactly similar to that in two fresh mice inoculated at the same time. A third attempt to infect them 15 days later was not successful.

The effect of splenectomy on the course of the infection in mice appears to be negligible, although it is readily admitted that the number of these experiments is too small to allow of anything more than tentative conclusions being made.

Monkeys. In monkeys the results were different. The number of observations which we were able to make was restricted, but even these few indicated that splenectomy does modify the course of the disease in these animals, and that splenectomy does increase their susceptibility to the infection.

On March 5th, 1938, two splenectomized monkeys and one normal one were inoculated with blood containing abundant spirochaetes. In the normal monkey no. 88 the infection ran a fairly typical course. In the splenectomized monkey no. 88 the infection ran a fairly typical course. In the splenectomized monkey no. 87 and no. 88 no striking departure from the normal was noticed in the first paroxysm, but the relapses appeared earlier, lasted longer, and showed in the first paroxysm, but the relapses appeared earlier, lasted longer, and showed in the first paroxysm, but the relapses appeared earlier, lasted longer, and showed a much heavier infection of the blood with spirochaetes—almost comparable to the fatal infection of monkey no. 84, which was infected directly from the louse. In one of the splenectomized monkeys a second relapse was observed. The other subsequently died. Owing to the writer's departure on leave, it was not possible to follow out these infections fully, so that there may have been further relapses which were not observed.

It is perhaps hardly permissible to include in this comparison the monkey which was found immune, nor one which was infected directly from a louse, but their inclusion in the series gives a very striking picture of the effect of splenectomy, thus:

<table>
<thead>
<tr>
<th>Character of disease</th>
<th>Five normal monkeys</th>
<th>Four splenectomized monkeys</th>
</tr>
</thead>
<tbody>
<tr>
<td>Immune</td>
<td>...</td>
<td>1</td>
</tr>
<tr>
<td>No relapse</td>
<td>...</td>
<td>1</td>
</tr>
<tr>
<td>1 relapse</td>
<td>...</td>
<td>3</td>
</tr>
<tr>
<td>2 relapses</td>
<td>...</td>
<td>1</td>
</tr>
<tr>
<td>Death-rate</td>
<td>...</td>
<td>Nil</td>
</tr>
</tbody>
</table>

50 per cent.

One splenectomized monkey died during the first paroxysm, and another, which ultimately died, probably had more than one relapse.

Splenectomy had no effect on acquired immunity, whether undertaken before or during the second infection. When one normal and two splenectomized monkeys, all of which had been previously infected and had recovered, were inoculated a second time, the course of the second infection was no more severe in the splenectomized monkeys (nos. R1 and R2) than in the normal one (no. R3). Monkey no. R1 was, indeed, immune, and nos. R2 and R3 were susceptible. This was even more clearly demonstrated on March 5th, 1938, when a splenectomized monkey which had been infected twice previously was inoculated with the same blood as a normal monkey which had been infected once. The latter was susceptible, and the former was immune. Kritschewski and Rubinstein (1931) found that, while splenectomy had no effect on acquired immunity, it might cause the reappearance of the spirochaetes in the blood. This was not found in any of our animals.

A possible explanation why the effects of splenectomy were more apparent in monkeys than in mice is suggested by an experiment of Rubinstein (1938). This author found that with the ordinary doses of the infective material the death-rate was the same in splenectomized as in control rabbits. But when a much larger dose was used the death-rate of the splenectomized as compared with the normal animals was strikingly increased. The effect of the splenectomy was apparent only in an infection which was likely to run a severe course in the normal animal; with very mild infections the reserves of the reticulo-endothelial system are sufficient to mask any effects of the splenectomy. In the case of our mice the course of the infection was very mild. But the more severe infection in the monkey entailed presumably a greater tax on the reticulo-endothelial system, which was then less able to withstand removal of a large part of its substance.
Rubinstein and Golubewa (1930) have shown that in *Trypanosoma equiperdum* infections the effect of splenectomy is inversely proportional to the length of time which elapses between the splenectomy and subsequent infection. In this connection it is interesting to observe that the only one of our monkeys in which the inoculation of infected blood resulted in a fatal issue was one which had been recently splenectomized. The others had undergone the operation several months previously, and in the meantime had received numerous inoculations of foreign material (tick contents), a process which might reasonably be expected to induce some degree of compensatory hypertrophy in the remainder of the reticulo-endothelial system.

VIII. RESIDUAL INFECTIONS

Wenyon (1926) and others have recorded that, when mice, previously inoculated with spirochaetes, were infected with trypanosomes several months after the apparent disappearance of the spirochaetes from the blood, this resulted in the reappearance of the spirochaetes in the blood-stream. Buschke and Kroé (1923) and other workers have shown that the spirochaetes, after their disappearance from the blood, may settle in the tissues of the central nervous system, where they may remain viable for long periods.

The importance of these observations from the point of view of the human infection is that they suggest the possibility of spirochaetes, lying dormant in the tissues of recovered patients, again invading the blood-stream as a result of some debilitating infection, e.g., malaria. This might lead to infection of mice, and start a new outbreak.

On January 13th, 1938, three mice, which had been infected with spirochaetes at different periods during the previous two months, were inoculated with *Trypanosoma evansi*. For this I am indebted to Dr. S. C. J. Bennett, Senior Veterinary Research Officer of the Sudan Government. After an incubation period of six days the trypanosomes appeared in the blood-stream. They persisted for four days only. In examinations of the blood carried out for the following 20 days, neither trypanosomes nor spirochaetes were seen.

On another occasion 12 white mice, which had been infected on one or more occasions at periods varying from 14 days to eight weeks previously, were killed. Emulsions were made of their brains, and inoculated subcutaneously into six gerbilles, each gerbille receiving material from two mouse brains. During the course of the following 12 days none of the gerbilles developed an infection, after which their blood was not examined.

Gerbilles were chosen because they had shown themselves to be the most susceptible of the small rodents to the infection, but the experiment was not hopefully undertaken. If passages were not successful when blood rich in spirochaetes was inoculated, they were hardly likely to succeed with brain emulsions containing possibly a few organisms. Negative results in this instance can have no significance.

Probably the only successful way to investigate this aspect of the Abyssinian strain would have been to use monkeys only. As the important point is not so much the proof of latent infection as to estimate the tendency of the strain to cause this, as shown by the proportion of ordinary infections which are followed by latent infection, any satisfactory work along these lines would have required far more monkeys than we had at our disposal.

IX. SUMMARY

1. An Abyssinian strain of louse-borne relapsing fever has been studied in the laboratory.

2. The organism has been successfully cultured in an egg-albumen ascitic fluid medium, but not in Yuan-Po's egg-medium or in other media.

3. Gerbilles and white mice were the only rodents found to be susceptible. In these animals the disease runs a very mild course. There is no relapse, and the strain cannot be maintained in these animals, even after passage through monkeys.

4. Monkeys (*Cercopithecus aethiops*) were susceptible, and in them the infection runs a course similar to the human disease. It appears possible to maintain the strain in these animals for at least three passages.

5. Evidence of immunity was found in infected animals, but several antigenic variants of the spirochaete are apparently present.

6. In monkeys, splenectomy was found to influence the course of the first infection, but had no effect on acquired immunity. In mice, splenectomy had no effect at all. A possible explanation of these results is discussed.

7. No evidence of latent or residual infections was found in rodents after recovery.

8. The intermediate host is the louse. Virulent spirochaetes were found in lice from infected cases, which were able to reproduce the disease in a monkey.

9. In the intermediate host the louse is more severe than that derived from the inoculation of infected blood.

10. This Abyssinian strain of relapsing fever resembles in its general features the other members of the north African group of louse-borne strains.

X. REFERENCES


EXPLANATION OF PLATE IX

Fig. 1. Polymorphous appearance of the spirochaetes in stained blood-films. \(\times 1,900\).  
Fig. 2. Appearance of the spirochaetes in films of frozen blood. \(\times 1,900\).  
Fig. 3. Appearance of the spirochaetes in the haemocoel fluid of the louse. \(\times 1,900\).  
Fig. 4. Large tangled mass of spirochaetes in the haemocoel of the louse. \(\times 1,900\).