THE ASSOCIATION OF *RICKETTSIA* WITH TRENCH FEVER.

By J. A. ARKWRIGHT, M.D., A. BACOT and F. MARTIN DUNCAN.

(*From the Lister Institute of Preventive Medicine.*)

(With Plates II and III.)

The following contains a preliminary account of the work on the Etiology and Pathology of Trench Fever which has been done at the Lister Institute in connection with the War Office Committee on Trench Fever under the Chairmanship of Major-General Sir David Bruce, F.R.S. A first account from the Clinical side has been published in a paper by Major Byam, R.A.M.C., and his colleagues at the New End Military Hospital, Hampstead, and read before the Society for Tropical Medicine and Hygiene in May of last year. We have been mainly dependent for our material on Major Byam and the rest of the Medical Staff of the New End Hospital and are very much indebted to them for their help and courtesy. We are under an especial obligation to Lieut. Ll. Lloyd, R.A.M.C., who has carried out that part of the entomological work done at the Hospital, taking immense pains in supervision and in obtaining the material which we required.

HISTORY AND DESCRIPTION OF *RICKETTSIA* BODIES.

The class of microorganism to which the name of *Rickettsia* has been given by da Rocha-Lima is associated chiefly with two human diseases, Typhus and Trench Fever and the lice which transmit them. The parasites found in Rocky Mountain Spotted Fever are probably very closely related; they are described as occurring in blood films and in very large numbers in the Tick, which transmits this disease, by Ricketts (1909), Wolbach (1916, 1918). A fourth species has been found by Nöller (1917) in the "sheep tick," *Melophagus ovinus*; this species, *R. melophagi*, is not known to be associated with any mammalian disease. Töpfer (i. 1917) further associates war nephritis with another form of *Rickettsia*.

The chief characters distinctive of *Rickettsia* are the following: (1) morphology: they are of very small size, 0·3 to 0·5 × 1·5 to 2·0 microns. Their shape resembles a coccus, diplococcus or a short bacillus. They stain rather feebly by aniline dyes, do not retain Gram's stain, and are not acid fast, but stain well by Giemsa, when they appear as small dots, double cocci, or bipolar staining bacilli with an unstained central part. They are non-motile. (2)

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Occurrence in the blood: they occur in blood films sparsely, and are best seen in dehaemoglobinised thick drops taken during the periods of fever (Rocky Mountain Fever, Ricketts, 1909; Typhus, Ricketts and Wilder, 1910; Trench Fever, Töpfer, x. 1916). It is, however, now generally acknowledged that the recognition of scanty *Rickettsia* in the blood or tissues is very difficult and uncertain. (3) Occurrence in the insect vector: Ricketts and Wilder (1910) found these microorganisms in the louse in Mexican Typhus. Sergent, Foley and Vialatti (1914), da Rocha-Lima (1916), Töpfer and others confirmed this observation and described enormous numbers of the parasite in the midgut of lice that had bitten a patient during the height of the fever a few days previously. (4) Artificial culture: attempts to cultivate *Rickettsia* on artificial media have been usually unsuccessful, but Nöller records that he grew *R. melophagi* on a blood agar medium. The claims made by Töpfer (1917) and Csernel (1916) appear to have been based only on single occurrences and are unconfirmed.

There has been a tendency to regard *Rickettsia* as a Protozoon as first suggested by Prowazek and favoured by da Rocha-Lima. The reasons for this view appear to be largely *a priori*, on account of the fact that it is insect-borne, the relapsing character of the fever in Trench Fever, and in addition the peculiar staining properties attributed to the bodies by da Rocha-Lima. However Ricketts, Wilder, Töpfer and the present writers find that with Giemsa the staining reaction is very like that of other bacteria.

Nevertheless, this class of microorganism and its associated diseases appear to have sufficiently distinct characteristics to justify the retention of the name *Rickettsia* for the present.

THE ASSOCIATION OF TRENCH FEVER AND *RICKETTSIA* BODIES.

The presence of the virus of Trench Fever in the blood of patients during and just after a febrile attack was shown by McNee, Renshaw and Brunt (12. ii. 1916) by transmitting the disease to man by intravenous or intramuscular injection of blood. This has been confirmed and amplified by the War Office Committee on Trench Fever in England, and the American Red Cross Medical Research Committee in France (1918); the latter also made additional experiments on plasma, filtered material, etc. A few inoculation experiments in Germany (Werner, Benzler and Wiese (ix. 1916)) have also been published. Several observers (Jungmann (iii. 1916), Töpfer (iii. 1916) and others) claim to have seen definite bodies in the blood in wet and dry preparations which resemble diplococci or bipolar staining bacilli and are like those described by Ricketts in Rocky Mountain Spotted Fever, and by Ricketts and Wilder in Typhus.

The suspicion that lice were concerned in the spread of Trench Fever was suggested in McNee's paper, and has been supported by the evidence of Davies and Weldon (3. ii. 1917). Jungmann and Kuczinski (iii. 1917), Werner and Benzler (v. 1917) also make statements on the subject, which, however, do not afford very satisfactory proof of this mode of transmission.

The War Office Committee working in England (Byam (v. 1918)) and the American Medical Research Committee in France have shown conclusively that the disease is readily transmitted by lice from patients to healthy volunteers. The former Committee has shown that the excreta or the body contents of infected lice, rubbed into a scarified area on the skin of a healthy man almost invariably reproduces the disease, usually with relapses, and other characteristic symptoms after an incubation in man of six to 14 days. They also showed that infected lice fed for over 30 days on several healthy men failed to infect them, although the lice were frequently fed on Trench Fever patients during that time. The latter Committee found that when infected lice were allowed to feed on healthy men the majority contracted Trench Fever after a period of about 2–4 weeks had elapsed.

Töpfer (17. x. 1916) described microorganisms in lice from Trench Fever patients which very closely resembled those found in lice from Typhus Fever, but claimed that he could distinguish the two kinds of parasites—those associated with Trench Fever being shorter and thicker than those from Typhus lice, but he did not consider important da Rocha-Lima's distinction between Typhus and other kinds of *Rickettsia*, namely, the invasion of the epithelial cells of the gut wall by the former. He states that some of the lice caught on every Trench Fever patient contained these parasites, and that uninfected lice fed on Trench Fever patients were found subsequently to harbour similar forms. These microorganisms were first found in the midgut of lice on the 5th day after the first infecting feed and were present in very large numbers on and after the 8th day. Not every louse was found to be infected.

Jungmann and Kuczinski (23. iii. 1917) state that they regularly produced a fatal infection in wild mice with the blood of Trench Fever patients injected intraperitoneally and that they found the same bipolar staining parasites in the peripheral blood of the mouse and in the patients' blood. They also, like Töpfer, found that the guts of lice, which had fed on patients, became infected with parasites, but they could not distinguish them from those found in Typhus lice. They say that in both diseases the microorganism develops inside the epithelial cells of the louse's midgut. They state that the gut of an infected louse injected intraperitoneally into a mouse causes its death in the same way as when a patient's blood is inoculated. They infected normal lice by letting them bite Trench Fever patients, and both these and also 80 % of the lice caught on Trench Fever patients were found to be infected on microscopical examination.

Munk and da Rocha-Lima (30. x. 1917) confirm the occurrence and multiplication of *Rickettsia* in lice fed on Trench Fever patients. Da Rocha-Lima claims that parasites found in lice fed on patients suffering from the two diseases can be distinguished by the position in and destruction of the insects' gut-cells by *Rickettsia prowazeki* which is associated with Typhus, and the irregular and only occasional entrance into the cells by *Rickettsia*

quintana and *R. pediculi* which occur in lice from Trench Fever cases and normal lice respectively. He acknowledges that he is unable to distinguish *R. quintana* from *R. pediculi*. He maintains that the inability of other workers to distinguish *R. prowazeki* from the other two species is due to their examination having been made by means of smears, whereas a differentiation can only be made with certainty by examining serial sections 3-5 microns thick, of which he claims to have examined over 25,000. He also describes slight morphological differences.

ANIMAL EXPERIMENTS.

Da Rocha-Lima states that he infected guinea-pigs and produced a characteristic temperature chart by inoculating various kinds of material (blood, urine, lice) from patients. However, only seven out of 44 animals reacted typically, ten slightly and 27 not at all. He was unable to pass the disease on to other guinea-pigs from those first infected. He could not infect mice. He records 119 experiments made by feeding normal lice on 103 persons suffering from Trench Fever, or other fevers, or who were normal. He used lice from places which were believed to be free from Typhus, or lice bred in captivity. Lice which showed doubtful appearances or only scanty *Rickettsia* were excluded from the results. They were examined partly by smears and partly by serial sections. Of 70 experiments on Trench Fever patients 51 gave a positive result, *i.e.* at least one louse was found to contain *Rickettsia*. In 11 the lice remained negative. In 33 control experiments on men not suffering from Trench Fever, but who were in hospital with other diseases or were healthy, the lice fed on 26 gave negative results and those from six positive results; the lice from one of these latter were heavily infected though the patient had never had any general symptoms.

Of 14 persons examined in Hamburg, where there had been no Typhus fever, in two the majority of the lice were strongly infected. One of these was a man who had been associated with soldiers from the front.

Trench Fever patients were able to infect lice with *Rickettsia* both before, during and after a febrile attack, and after many weeks of convalescence.

TIME OF APPEARANCE OF *RICKETTSIA* IN THE LOUSE.

After the first experimental infecting feed on a patient, *R. quintana* was commonly found in lice on the 3rd to the 6th day, sometimes in large numbers on the 4th day. Lice caught on patients showed the parasites usually on the 6th to the 8th day. *R. prowazeki* was commonly well seen in lice after the 8th day from the first experimental feed on Typhus patients, and only exceptionally as early as the 4th or 5th day.

Trench Fever lice remained healthy, but Typhus lice often died. Lice infected with *R. quintana* remained infected after long continued feeding on a healthy man.

Da Rocha-Lima failed to infect by the bites of lice containing many

Rickettsia of the *R. pediculi* type. Korbsch (1916) reports a failure after two "infected" lice had fed on him for eight days, and also no result following scarification and rubbing in of a single "infected" louse on two occasions.

Strisower (iv. 1918) described three cases of transmission of Trench Fever to men by feeding infected lice on them and also claims to have infected mice and cats in series. Several other writers (*e.g.* His and Stintzing) criticise the work on *Rickettsia* adversely and support the hypothesis of a spirochaete as the infective agent.

DISCUSSION OF THE FURTHER EVIDENCE IN THE LITERATURE AS TO THE CAUSAL RELATION BETWEEN TRENCH FEVER AND *RICKETTSIA*.

(1) The hypothesis that *Rickettsia* causes Trench Fever is largely founded on the analogy of Typhus Fever in which the evidence brought forward is more complete owing to experiments on monkeys and guinea-pigs. The claims to have infected guinea-pigs, cats, mice and rabbits with Trench Fever in a recognisable form are very unconvincing in the published reports.

(2) Observations on forms resembling *Rickettsia* in the blood of Trench Fever patients are very difficult to interpret and unsatisfactory, since these forms are so scanty and their morphology alone when present in small numbers in a film is inconclusive.

(3) The inability to obtain growth of *Rickettsia* on artificial culture media make inoculation and re-infection experiments, after several subcultures, impossible at present.

(4) The chief reasons for the failure to obtain acceptance or a better hearing for *Rickettsia* as the probable cause of Typhus and Trench Fever are: (*a*) The not very rare occurrence according to Continental observers of *Rickettsia* in lice from sources where Typhus and Trench Fever have not been suspected; (*b*) connected with this objection, and perhaps largely the cause of it, is the difficulty in distinguishing the forms of *Rickettsia* associated with Typhus and Trench Fever from each other, and from those found in "normal" lice. There is no agreement as to definite means of distinguishing these parasites, but da Rocha-Lima's claim to differentiate them by their position in serial sections is more convincing than Töpfer's very slight description of differences in morphology. (*c*) The confusion has perhaps been enhanced by the failure of the German observers to work with a clean stock of lice. Da Rocha-Lima did use some lice bred in the laboratory, but he makes no statement as to their number or the proportion of such lice used or the results obtained with them, as distinguished from lice caught in surroundings believed to be free from infection. Moreover, the captive lice would require feeding on some human being and the infection of laboratory workers with Trench Fever is common.

The wide distribution and high incidence of Trench Fever on the Continent during the War makes it very difficult to obtain definitely uninfected lice for experiments or uninfected men on whom to feed them, except by breeding

lice, feeding them on a man of unimpeachable freedom from Trench Fever infection, and watching their excreta through several generations.

(5) The counter-claims of other workers to have discovered the cause of Trench Fever in a spirochaete mainly rest on the very insecure basis of occasional observations of a single spirochaete in the blood, supported by *a priori* arguments, except in the case of (1) Riemer (i. 1917) who, in addition, obtained these organisms in culture from one patient, but his observations have been unconfirmed, and (2) Couvy, Dujarric and de la Rivière (12. i. 1918), who experimentally infected guinea-pigs with patients' blood and passed the infection on to other guinea-pigs; these observers recovered the spirochaetes in considerable numbers from the kidneys, etc. Their observations have so far not been corroborated by other workers.

THE AUTHORS' OBSERVATIONS AND EXPERIMENTS.

Early in 1918 it had become clear from the experimental work on volunteers of the Committee (Byam 1918) that the virus of Trench Fever was contained in a very active form in the excreta and bodies of lice, since the body contents of 11 lice or a small pinch of dry excreta was sufficient to infect a man through the excoriated skin.

Much microscopic and cultural work had been done on the blood of patients by previous workers, at the New End Hospital, Hampstead, especially by Captain Dimond, and, since its formation, by the members of the War Office Committee.

It was determined therefore early in May, 1918, to concentrate our attention on the excreta and contents of the intestinal canal of infected lice, while some cultural and other observations on the blood of patients were continued.

Cultures of the excreta and guts of lice, both infected and normal, had sometimes yielded a variety of bacteria and sometimes been sterile, but one form of *Bacillus* was the most constant and had been previously studied by Bacot. In young cultures on agar or blood agar it takes the form of a coccus 1·5 to 2·0 microns in diameter and is often seen dividing. In older cultures, and sometimes even in the first 24 hours, films show irregular threads amongst the round or oval organisms. When first isolated growth takes place best at about 27° C., but later it grows well at 37° C. It is Gram-negative and non-motile; ferments glucose, mannite and lactose very slowly; forms acid and clot in milk in about 14 days, and is not pathogenic for guinea-pigs. Attempts were then made by means of wet and dry films and by cultures to discover other organisms in the excreta of infected lice which were not present in those of normal lice.

One or two films made by the late Professor Plimmer from the excreta of infected lice and fixed wet with formalin and iodine vapour showed an immense number of very small particles which were probably minute organisms, and turned our attention to a search for the *Rickettsia* described by Töpfer and Rocha-Lima. In many films of excreta, whether fixed wet or dry, a large

number of stained granules were seen, which recalled descriptions of *Rickettsia*, but it was very difficult to distinguish the granules of altered blood, etc., from the more definite forms, and one was continually in doubt as to whether one was looking at minute microorganisms or precipitated protein. In order to get rid of the débris of red corpuscles, the films after drying were fixed for one or more hours in absolute alcohol containing 20 drops of strong hydrochloric acid per c.c. as recommended for thick blood drops in Malaria work by W. M. James. By using this technique, there has seldom been difficulty in distinguishing *Rickettsia* when present from granules of débris, though the disintegrating nuclei of leucocytes occasionally have presented a somewhat similar appearance. The chief further source of error lies in the danger of confusing these parasites with other microorganisms, especially larger bacteria only stained in the centre or at the poles, and with the small bacteria occasionally seen, which approach *Rickettsia* in size.

The criteria which we have adopted in deciding on the presence of *Rickettsia* have been (1) its minute size, smaller than *M. melitensis* or *B. influenzae*, usually about $0{\cdot}3 \times 0{\cdot}3$, or $0{\cdot}3 \times 0{\cdot}5\mu$; (2) its irregularity in shape, round, oval, diplococcal or bacillary with stained poles; (3) its occurrence in very large numbers, or even in masses, especially on flakes of solid material in the excreta; (4) its well-stained appearance when coloured by Giemsa, the colour being purple like that of the nucleus of a leucocyte.

When small granules, somewhat resembling *Rickettsia* in size and shape, but stained pink or lilac, have been met with, or if the bodies have been very few and scattered about the film, the result of microscopical examination has been recorded as negative or doubtful. The number of "doubtful" specimens has very much diminished as our experience has increased, and now it is very seldom necessary to return an uncertain result, though *Rickettsia* no doubt are sometimes present but unrecognized on account of their rarity in the film.

METHOD OF CONDUCTING EXPERIMENTS WITH LICE.

A large, healthy stock of *Pediculus corporis*, maintained by Bacot for over three years, was used. The lice were confined in boxes, covered with fine gauze, and were fed twice daily by the method described by him (Bacot, 1917). When not in use the lice were kept in an incubator or in an inside pocket at 27° to 30° C. except in those experiments in which another temperature is recorded. The lice in each box usually numbered 50 to 100. They were fed once or twice for 20 to 30 minutes on Trench Fever patients during an attack of fever, also in some instances during non-febrile periods; thereafter they were fed on a healthy man or in some experiments on the same infected man throughout.

Excreta were examined by shaking them as a dry powder out of the boxes through the gauze, and making an emulsion on a glass slide with a drop of salt solution. The contents of the gut were examined by dissecting it out

and emulsifying it on a slide with needles in a small drop of salt solution. The film was dried and fixed in acid alcohol and stained with Giemsa. Dead lice were also examined by soaking the body in a drop of salt solution and teasing it up on a slide. It was found that films made from dead lice showed *Rickettsia* very clearly when these were present.

Some infected and uninfected lice were also examined by serial sections.

INCUBATION OF *RICKETTSIA* IN THE LOUSE.

It was found that if a boxful of lice were given an infecting feed the excreta obtained from the box did not show *Rickettsia* for some days.

Table I.

Showing the results of examining microscopically the excreta of lice on a series of days after the first infecting feed, and also the results of inoculation of excreta from two of the boxes, 150 and 134.

Days from first infecting feed	Box No. 175	142	A 15	A 18	A 25	A 34	193	A 33*	150 Microsc. exam.	150 Result of inoculation	134 Microsc. exam.	134 Result of inoculation
1st	–	–	...	–	–	–	...	–	–	–	–	–
2nd	...	+ ?	...	–	–	–	...	–	...	...	...	...
3rd	...	–	...	–	–	–	–	–	...	...	– ?	–
4th	+	–	...	...	–	–	–	–	...	...	...	...
5th	+ ?	–	–	–	–	...	–	...	+	+	– ?	–
6th	–	–	...	+ ?	–	–	–	–	–	–	...	...
7th	...	+	...	–	–	–	–	–	+ +	+	...	...
8th	...	+	+ + +	+ + +	–	–	–	–	–	–	+	–
9th	+	+	+ +	...	–	+	–	–	+	+	...	...
10th	+ +	+	...	+ + +	+ + +	+	–	–	+	+	...	...
11th	...	+	+ + +	+ + +	+ + +	+	+	–	+	+	...	...
12th	+ +	+	...	+ + +	+ + +	+	+	–	...	...	...	+
13th	...	+ + +	+ + +	+ + +	+ + +	+ + +	+ +	–	...	...	+	...
14th	...	+ + +	...	+ + +	...	+ +	+ + +	...	...	...	...	...
15th	...	...	...	+ + +	...	+ +	+ +	–	...	...	...	...
16th	+ +	+ + +	+ + +	+ + +	...	...	+ + +	–	...	...	...	...
17th	...	+ +	...	+ + +	+ + +	+ + +	+ +	–	...	...	...	...
18th	...	+ +	...	+ + +	...	...	...	...	...	...	...	...
19th	...	+	...	+ + +	+ + +	...	...	...	...	...	...	...
20th	+ + +	+	...	+ + +	+ + +	...	+ + +	...	...	...	...	...
21st	...	...	...	+ + +	+ + +	...	...	...	...	...	...	...
22nd	...	...	...	+ + +	...	...	...	...	...	...	...	...
23rd	...	...	...	...	...	...	...	...	...	...	...	+
24th	...	...	...	...	...	...	...	...	...	...	+	...

\+ = *Rickettsia* seen in a few microscope fields.
\+ + = ,, ,, several ,, ,,
\+ + + = ,, ,, enormous numbers.
– = No *Rickettsia* seen.
... = No examination.

* The lice in boxes A 33 and A 34 were fed at the same time, but A 33 was kept at about 20° and A 34 at 27° C.

When *Rickettsia* have once been found in a box in large numbers they usually continue to be found in daily examinations till all the lice of the infected generation are dead.

Table I shows the results of a daily microscopical examination of the excreta from ten of the boxes of experimental lice which had previously fed on a Trench Fever patient. The results of a series of experimental inoculations into volunteers of the excreta from two of the same boxes, 150 and 134, are also shown. It is seen, (1) that *Rickettsia* appear in the excreta after a series of negative examinations following the infecting feed, and (2) that the numbers of these bodies can often be seen to be smaller when they first appear than on later days, (3) also that when the infection is thoroughly established a positive result is obtained every day. The period elapsing between the first infecting feed and the recognition of *Rickettsia* varies from about 4 to 10 days in this series. Examinations of boxes kept at about 20° C. remained consistently negative; though the lice were apparently healthy.

The results shown in this table, *i.e.* an incubation period, the appearance of *Rickettsia* after about a week or ten days, and its persistence for three or four weeks, have been a constant phenomenon when we have been able to examine a series of specimens.

Table II relates to experiments with 20 boxes of lice which were fed on ten different patients with Trench Fever. The boxes were examined at frequent intervals, in most cases daily, either by making films from the excreta, or from lice. It is seen that *Rickettsia* was first seen on the 5th to the 12th day, most commonly on the 7th to the 10th day from the first infecting feed, when the lice were kept at about 27° C. between the feeds. Boxes A 20 *b*, A 28, A 30, and A 33 were kept at about 20° C. and *Rickettsia* did not appear, though examinations were continued till the 17th, 19th and 22nd day from the infecting feed in these experiments. It was also shown that if a box was kept at 20° C. for two or three days and then at 27° C. the parasites were found after a week at the higher temperature.

Lice in eight other boxes fed on seven infected patients (six of whom were different men from those mentioned in Table II) have been found to be infected with *Rickettsia* after a variable number of days, but the examinations have not been sufficiently numerous to determine the date when these first appeared. In two of them, however, the parasites were present in the excreta on the 6th day and in one on the 7th day from the first infecting feed.

Seven other boxes of lice, which have been fed on patients believed to be suffering from Trench Fever, have only been examined on two or three occasions, and *Rickettsia* has not been definitely found.

Table II also shows the number of days which elapsed after an infecting feed before the excreta were proved to be infective, *i.e.* capable of producing Trench Fever when inoculated into volunteers in two series of experiments.

In the case of Box 134 the excreta from the 1st to the 8th day did not infect, but those collected on the 12th day reproduced the disease, whereas

Rickettsia were first demonstrated on the 8th day. The excreta from Box 150 showed *Rickettsia* on the 5th day and the same specimen of excreta infected a volunteer. Excreta from Box A 18 showed the parasite microscopically on the 8th day, and a mixed sample of excreta collected on the 8th to the 22nd day proved virulent for man, but they were not tested earlier for virulence.

Table II.

Showing the day after the first infecting feed on which *Rickettsia* was first found in the guts or excreta of lice.
In the case of Boxes 134 and 150 the day when infective excreta were first obtained is also shown.

No. of boxes of lice	Source of infection of lice: Ref. No. of patient	Source of infection of lice: Day of disease	Source of infection of lice: Febrile or non-febrile	Material examined	Day from infecting feed on which: Microscopic result first positive	Day from infecting feed on which: Successful inoculation obtained
162	Ex. 46 / Ex. 21	2nd / 11th	Febrile / Non-febrile	Midgut	10th	...
175	Ex. 60	1st	Febrile	,,	9th	...
134	G.	79th	,,	Excreta	8th	12th*
150	Ex. 33	2nd	,,	,,	5th	5th*
142	Ex. 27	2nd	,,	,,	7th	...
A 15	A.	2nd	,,	,,	8th	...
A 16	A.	5th	Non-febrile	,,	5th	...
A 18	A.	10th	Febrile	,,	8th	...
A 20 *a*†	A.	23rd	Non-febrile	,,	10th	...
A 24	Ex. 73	25th	Slightly febrile	,,	9th	...
A 25	C.	43rd	Non-febrile	,,	10th	...
A 27	A.	29th	,,	,,	12th	...
A 29‡	Ex. 81	19th	Febrile	,,	9th	...
A 31§	Ex. 81	30th	Slightly febrile	,,	7th	...
A 34‖	A.	49th	Non-febrile	,,	9th	...
A 35‖	A.	49th	,,	,,	7th	...
A 20 *b*†	A.	23rd	,,	,,	Negative 19th	...
A 28‡	Ex. 81	19th	Febrile	,,	,, 17th	...
A 33‖	A.	49th	Non-febrile	,,	,, 17th	...
A 30§	Ex. 81	30th	Slightly febrile	,,	,, 22nd	...

* See also Table I.

† Boxes A 20 *a* and A 20 *b* were alike and treated in the same way except that A 20 *a* was kept at 27° C. and A 20 *b* at 20° C.

‡ Boxes A 28 and A 29 were alike and treated in the same way except that A 28 was kept at 20° C. and A 29 at 30° C.

§ Boxes A 30 and A 31 were alike and treated in the same way except that A 30 was kept at 20° C. and A 31 at 27° C.

‖ Boxes A 33, A 34, A 35 were alike and treated in the same way except that A 33 was kept at 20° C., A 34 at 27° C. and A 35 at 32° C.

These facts show a general agreement in that the virus of Trench Fever and *Rickettsia* both require an incubation period of 4 to 12 or more days in the louse before they are demonstrable—the former by inoculation, the latter microscopically. This point will be dealt with in more detail further on.

The lice in Boxes 162, 175, 134, 150 and 142 were fed on the infected man

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for one day only, and afterwards on a healthy man. Those in the remaining boxes in Table II were fed twice daily on the same or another infected man. The day of the disease in the patient on whom the lice were fed, and whether he was febrile or non-febrile at the time of the first infecting feed, are also shown.

Lice may be infected on a Trench Fever patient when he is febrile or between the attacks, or even some weeks after the last fever has been noticed, as in the case of Boxes 163 and A 16, which were fed in the intervals between attacks, and Boxes A 20 *a*, A 27, A 34 and A 33, which were fed on the 22nd, 28th, 48th and 48th day of the disease, and the 13th, 19th, 39th and 39th days respectively since any fever had been observed, or any symptoms complained of.

RICKETTSIA NOT TRANSMITTED BY HEREDITY IN LICE.

The offspring of infective lice fed on a normal man have not shown, with one exception, *Rickettsia* either in their excreta or in their stomach contents.

On 15 June, 1918, nits were taken from Box 163, the excreta from which were known to contain the virus of Trench Fever and numerous *Rickettsia*. The nits were divided into two batches, I and II.

Batch I. These were put into a clean box without being treated.

Batch II. These were put for three minutes into 1 % lysol and then washed in a current of tap water for several minutes, dried and put in another clean box. Both boxes were kept subsequently at about 30° C. and as soon as larvae were hatched out they were fed twice daily on a healthy man.

On 15 July the excreta from Box I were examined and one small flake crowded with *Rickettsia* was found, but no other parts of the film showed these forms. Three subsequent examinations of the excreta from Box I and three examinations of the excreta from Box II all gave negative results. It seems clear that the flake with *Rickettsia* was a portion of excreta carried over on the unwashed eggs.

When newly hatched larvae, descendants of the infected lice in Boxes 15 and 16, were fed twice daily on an infected man their excreta contained the parasites on the 8th and 5th days respectively, and not before.

Table III.

Showing the proportion of lice found on dissection to be infected with *Rickettsia* at different periods.

	Day after first infecting feed								
	0–7th day			8th–14th day			15th onwards		
	+	–	Total	+	–	Total	+	–	Total
Series 1	3	24	27	7	5	12	15	0	15
„ 2	0	3	3	5	4	9	43	17	60
Both series together	3	27	30	12	9	21	58	17	75

+ = *Rickettsia* found. – = No *Rickettsia* recognized.

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In Series 1 comprising lice from six boxes the lice only fed on an infected man during 24 hours, and afterwards on a healthy man. In Series 2 (eight boxes) the lice fed on an infected man from the first infecting feed onwards. When individual lice from a recently infected box are dissected and examined it is found that only a few are infected with *Rickettsia* in the first week after the first infecting feed, that during the second week about an equal number are infected and uninfected, and that after the second week the majority show parasites microscopically.

Table III gives the actual figures in a series of lice taken at different stages from 14 boxes of infected lice, and examined microscopically. The increase in the number of infected lice is very marked.

In microscopic sections of infected lice *Rickettsia* were seen crowding the region of the epithelial cells lining the alimentary canal, but there was no definite invasion of the cells. Appearances like those figured by da Rocha-Lima from sections of Typhus lice in which the epithelial cells show well defined areas which are badly stained and occupied by masses of *Rickettsia* were not seen.

EXAMINATION OF NORMAL LICE.

For comparison with these boxes of lice which have been infected by feeding on Trench Fever patients a number of boxes of uninfected lice have also been examined frequently, some daily for weeks, by making films of the excreta. Also a considerable number of normal lice have been dissected and films from the midgut examined, and serial sections have been cut of others. Only in one box on one occasion have forms closely resembling *Rickettsia* been found. Seeing how difficult or impossible it is to distinguish bacteria by their morphology, an occasional error is not surprising. When these organisms occur in an infected box they are almost always found on many successive days and not only on one single occasion, as was the case with the apparently exceptional occurrence among normal lice mentioned above.

In all 22 boxes of lice fed on normal persons have been examined repeatedly over periods lasting usually over two months and never less than 14 days. These normal persons on whom they have been fed are seven in number, and two stocks of normal lice from different sources are under observation.

Two other boxes of lice supposed at the time to be normal, besides the one referred to above, showed *Rickettsia*. They were both being fed on A. who had been also feeding infected lice for over five weeks and had been working daily with infected excreta in the laboratory. He developed Trench Fever on June 11th. The first box, A 12 *a*, showed *Rickettsia* in the excreta on June 8th, and of nine lice dissected on June 10th three gave a positive result, the other box, A 10, showed infected excreta on June 14th. It does not seem reasonable to include these findings in Boxes A 10 and A 12 *a* as positive results from normal lice.

Some other lice found on healthy civilians have been examined but none have been found to be infected with *Rickettsia*.

The total number of specimens of lice or excreta from boxes of lice which had been fed on men believed to be infected with Trench Fever was 253, and of these 150 showed *Rickettsia*, 83 gave a negative and 20 a doubtful result. Of these specimens of excreta collected during the first week after the first infective feed, 14 were positive and 73 were negative.

Second week, 75 were positive and 27 were negative.

Third week, 61 were positive and 3 were negative.

Of 245 specimens from 22 boxes of normal lice fed on seven healthy persons, only one was positive (if the four specimens from A's two boxes mentioned above are excluded), 234 gave a negative and 10 a doubtful result.

CORRELATION OF THE PRESENCE OF *RICKETTSIA* AND TRENCH FEVER VIRUS IN THE LOUSE.

The association of *Rickettsia* and infectivity for man of the lice containing them is very striking; *Rickettsia* and the virus of Trench Fever also have certain properties in common.

1. The size of the *Rickettsia* is such that one would expect them to be held back by a good Berkefeld filter; they nevertheless approach the lower limits of size of known bacteria.

In an experiment by McNee with filtered and unfiltered blood plasma, the unfiltered alone transmitted the disease. The American Medical Research Committee (1918) in France have been able to transmit the disease by means of filtered material. They have also found that plasma, freed from cells by centrifuging, is still virulent. In experiments with emulsion of louse excreta in salt solution, we have found that only prolonged centrifuging at high speed (2500–3000 revolutions) for 20–30 minutes will produce a definite deposit of *Rickettsia*. They may therefore be separable from blood corpuscles by fractional centrifuging in blood plasma also, but we have been unable by centrifuging to demonstrate their presence in more than small numbers in citrated plasma of Trench Fever patients diluted 1 in 5.

2. The blood in Trench Fever is infective by direct inoculation during a febrile attack, and, at any rate sometimes, when there is no fever. Lice can also be infected with Trench Fever virus over a long period, whether the patient is febrile or not; they also become infected with *Rickettsia* when fed on patients during similar periods, *e.g.* the excreta of lice (Box 163) fed on the patient St. when non-febrile on the 27th to 28th day of the disease gave Trench Fever to several volunteers and contained large numbers of *Rickettsia*; the lice in Box 134 fed on another patient G. on the 79th day of his disease, gave Trench Fever to a volunteer by means of excreta collected on the 12th day from the infecting feed. The excreta from this box first showed *Rickettsia* on the 8th day.

3. Lice fed on patients are able to transmit the disease if their excreta or midgut contents are rubbed into scratches or inoculated subcutaneously. They, however, appear not to become infective (two series of experiments)

till the 5th to the 12th day after the infecting feed. In the same way *Rickettsia* is not in our experience recognizable in lice or their excreta when kept at 27° C. till the 5th to the 12th day (usually the 7th to the 10th day) after the first infecting feed on a patient.

4. In an infected box not every louse appears to contain the virus of Trench Fever as tested by inoculation of man, neither does every louse contain *Rickettsia* in recognizable form or amount. Only 12 out of 21 examined in the second week from the infecting feed showed these parasites. In one experiment two lice were selected from an infected box (A 16 *a*) on about the 13th day after the first infecting feed. The midguts were dissected out and emulsified separately. It was shown by microscopic examinations of both the gut contents and the excreta of these lice that the one, A, contained many *Rickettsia* and the other, B, did not. Inoculation of the emulsions separately into two volunteers by scarification, produced Trench Fever in the one inoculated with A and not in the one inoculated with B.

5. A box once infected appears to remain so for two or three weeks, both as regards the virus of Trench Fever and the presence of *Rickettsia*, *i.e.* during the life-time of the infected generation of lice.

6. The high infectivity of louse excreta is associated with the presence of enormous numbers of *Rickettsia*, whereas the difficulty of finding these bodies in blood films is well known.

7. Lice or the excreta of lice which have been proved to contain virus, as the result of successful inoculation, have in most cases, when examined, been shown to contain large numbers of *Rickettsia*. Certain exceptions to this have occurred. It is however not surprising that a small sample of excreta should occasionally fail to show the parasite which may be present in large numbers in another part of the same excreta. On the other hand, if uniformly distributed, *Rickettsia* might escape recognition though present in considerable numbers.

In Table IV are shown all the experiments made by inoculating volunteers with the excreta or body contents of lice in which the specimen has also been examined microscopically. The result of such examination is shown in column 3 and of the inoculation experiment in column 5. The 6th column states whether there is agreement (A.), disagreement (Dis.), a doubtful result (?) or a reason for agreement not being expected (O.).

Fifty-six specimens are included in Table IV. Fifty-three were samples from boxes of lice which had had a feed on an infected patient, of these 50 were samples of excreta and three were emulsions of single lice. Three further specimens of excreta are included in the Table—two from boxes of normal lice fed on healthy men and both examined microscopically and inoculated into volunteers, as control experiments; the third specimen is from a box containing the offspring of infected lice which were examined to test the hypothesis of hereditary transmission of the virus of Trench Fever or of *Rickettsia*. Out of the 53 specimens from boxes of lice which had been

Table IV.

Showing the results of microscopical examination and inoculation of 54 specimens of louse excreta and of three single lice.

1 No. of Box of Lice	2 Source of infecting feed: Name	2 Source of infecting feed: Date	3 *Rickettsia* in specimen	4 Days from infecting feed	5 Inoculation of man: No. of Experiment	5 Inoculation of man: Date	5 Inoculation of man: Result	6 Agreement or disagreement	7 Incubation in man. Day	Notes
Mixed	...	...	+ + +	...	10	9. ii.	+	A.	6th	
Mixed	...	...	+ + +	...	11	9. ii.	+	A.	7th	
Mixed	...	...	+ + +	...	12	9. ii.	+	A.	9th	
Mixed	...	...	+ + +	...	20	5. iii.	+	A.	9th	
Mixed	...	...	+ + +	...	21	16. iii.	+	A.	8th	
134	G.	20. iii.	−	1st	23	21. iii	−	A.	...	
134	G.	20. iii.	−	3rd	24	23. iii.	−	A.	...	
134	G.	20. iii.	−	4–5th	25	25. iii.	−	A.	...	
134	G.	20. iii.	+*	6–8th	26†	28. iii.	−	Dis.	...	
134	G.	20. iii.	− +	12th 13th	27	1. iv.	+	A.	8th	
Mixed	...	...	+ + +	...	28	28. iii.	−	O.	...	Immunity
Mixed	...	...	+ + +	...	29	30. iii.	−	O.	...	Immunity
134	G.	20. iii.	− +	23rd 24th	30	13. iv.	+	A.	7th	
Mixed	...	...	+ + +	...	32	6. iv.	+	A.	8th	Excreta untreated
Mixed	...	...	+ + +	...	33	6. iv.	+	A.	8th	Excreta heated 56° C.‡
Mixed	...	...	+ + +	...	34	6. iv.	−	O.	...	Excreta heated 80° C.§
150	J.	15. iv.	−	1st	39	17. iv.	−	A.	...	
150	J.	15. iv.	−	6th	41	21. iv.	−	A.	...	
150	J.	15. iv.	+ +	7th	42	22. iv.	+	A.	13th	
150	J.	15. iv.	−	8th	43	23. iv.	−	A.	...	
150	J.	15. iv.	+	9th	45	26. iv.	+	A.	9th	
150	J.	15. iv.	+	10th	46	26. iv.	+	A.	7th	
Mixed	...	...	+	...	51	7. v.	+	A.	8th	
150	J.	15. iv.	+	5th	53	2. v.	+	A.	16th	Few *Rickettsia*; none seen in 2nd film
150	J.	15. iv.	−	6th	41 54	2. v. 2. v.	− −	A.	...	
150	J.	15. iv.	+ +	7th	55	2. v.	+	A.	13th	
150	J.	15. iv.	+	9–10–11th	58	10. v.	+	A.	12th	
150	J.	15. iv.	+	11th	59	2. v.	+	A.	8th	
163	S.	7. v.	+ + +	11–12–13th	64	20. v.	+	A.	7th	
163	S.	7. v.	+ + +	21–22–23rd	70	1. vi.	+	A.	7th	
157	W.	23. iv.	+?	26–31st	71	31. v.	+	?	8th	One louse
155 164	T.	...	−	11–24th	72	1. vi.	+	Dis.	10th	
163	S.	7. v.	+ + +	11–14th	73	2. vi.	+	A.	9th	
163	S.	7. v.	+ + +	11–24th	74	5. vi.	−	O.	...	Excreta treated with lysol 2 %

A. = results agree. Dis. = results disagree. O. = results not comparable.

* A very few *Rickettsia* were found in only one out of three films.

† This man was inoculated three times with negative result (Exps. 43 and 63).

‡ Dry heat for 20 mins.

§ Moist heat for 10 mins.

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Table IV—*continued.*

Showing the results of microscopical examination and inoculation of 54 specimens of louse excreta and of three single lice.

1 No. of Box of Lice	2 Source of infecting feed: Name	2 Source of infecting feed: Date	3 *Rickettsia* in specimen	4 Days from infecting feed	5 Inoculation of man: No. of Experiment	5 Inoculation of man: Date	5 Inoculation of man: Result	6 Agreement or disagreement	7 Incubation in man. Day	Notes
163	S.	7. v.	+ + +	11–24th	75	8. vi.	+	A.	10th	
170	C.	14. v.	+	12–20th	76	9. vi.	–	O.	...	Immunity
163	S.	7. v.	+ + +	11–13th	77	11–13. vi.	+	A.	11th	
163	S.	7. v.	+ + +	11–24th	80	14. vi.	+ ?	?	12th	Influenza (?)
163	S.	7. v.	+ + +	11–24th	81	14. vi.	+	A.	10th	
163	S.	7. v.	+ + +	14–36th	82	14. vi.	–	O.	...	Excreta heated to 70° C. 20 mins.—moist
171	B.	15. v.	+ + +	11–19th	85	16. vi.	–	O.	...	48th day of disease—immunity
177	P.	29. v.	–	12–20th	86	18. vi.	+	Dis.	9th	
173	T.	17–18–19. v.	±	14–17th	90	19. vi.	–	?	...	Immunity
150	J.	15. iv.	–	8th	91	19. vi.	–	A.	...	
155 } 164 }	T.	...	–	11–24th	92	22. vi.	–	A.	...	
173	T.	17–18–19. v.	±	14–17th	93	22. vi.	–	?	...	
163	S.	7. v.	+ + +	11–36th	94	2. vii.	–	O.	...	Excreta heated to 99·9°C. 20 mins.—dry
A 18	A.	20. vi.	+ + +	8–22nd	96	19. vii.	+	A.	7th	2 mg. excreta sub-cut.
A 18	A.	20. vi.	+ + +	8–22nd	97	19. vii.	+	A.	11th	0·1 mg. excreta sub-cut.
Mixed	R.	...	+ + +	...	98	23. vii.	–	O.	...	Immunity—excreta kept 154 days
171	B.	15–18. v.	+ + +	11–19th	99	23. vii.	+	A.	9th	
A 16 *a*	A.	13. vii.	–	11th	100	24. vii.	–	A.	...	One louse
A 16 *a*	A.	13. vii.	+ + +	11th	101	24. vii.	+	A.	9th	One louse
122	Healthy man		–	...	22	18. iii.	–	A.	...	Offspring of infected lice
127–9	Healthy man		–	...	17	12. iii.	–	A.	...	Lice were fed on a healthy man as a control experiment
127–9	Healthy man		–	...	18	12. iii.	–	A.	...	

A. = results agree. Dis. = results disagree. O. = results not comparable.

fed on Trench Fever patients, three gave uncertain results from microscopic examination and in one the reaction following inoculation was of a doubtful nature, as the attack of fever was attributed to influenza, nine are excluded from the results because agreement between the results of the two methods of examination was not to be expected, either on account of the disinfection of the excreta by lysol or heat, or because the volunteer had previously gone through an attack of Trench Fever and was probably immune—the inoculation being in fact given as a test for immunity.

Of the 40 remaining, 27 gave positive results, both as regards the presence of *Rickettsia* and the virulence of the samples, 10 gave negative results from both tests, and three different results from the two tests.

There was therefore agreement as to the presence or absence of *Rickettsia* and virus in 37 out of the 40 samples which gave a decided answer, *i.e.*

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92·3 % of agreement. In one of the three samples in which there was disagreement the microscopic examination was positive, and the virulence test negative; in the other two the reverse was the case.

The specimens of excreta from which results showing agreement were obtained were in some instances inoculated into more than one man. There are eight such observations recorded which were repetitions of former experiments. In seven of these the two results were positive and in one negative. In every case the excreta when used for a second experiment gave the same result as on the first occasion. Excluding the repetitions the total number of experiments is 32 of which 29 showed agreement (90·6 %) and three disagreement.

8. There is evidence that the virus of Trench Fever is not inherited in the louse, since excreta of the offspring of infected lice fed on healthy men from the egg have failed on inoculation to produce Trench Fever; the excreta of the offspring of the infected lice in several boxes have on examination not shown *Rickettsia*. One apparent exception to this statement occurred (see above).

CONCLUSIONS.

1. The intimate association in lice of *Rickettsia* with the virus of Trench Fever appears to have been amply proved.

2. The examinations of lice which have fed on healthy civilians in England have given negative results in a sufficiently uniform manner to constitute a significant negative control, but further examinations of lice from normal civilians are desirable.

3. Lice from soldiers who have been in France, or who have mixed with men from France in this country, would not afford a satisfactory control, since the infection of Trench Fever with the power of infecting lice with Trench Fever virus and with *Rickettsia* may be very long lasting.

4. Whether *Rickettsia* constitute the virus of Trench Fever or are in some way produced by it remains undecided because *Rickettsia* cannot be cultivated on artificial media.

5. It is conceivable that *Rickettsia* are not living microorganisms, but their appearance certainly suggests that they are bacteria, and their remarkable association with Trench Fever virus in the louse further suggests that they are the causal agent of Trench Fever.

REFERENCES.

American Red Cross Medical Research Committee. Report of Commission on Trench Fever (1918). Oxford University Press.

Bacot, A. (26. xi. 1917). A contribution to the bionomics of *Pediculus humanus* (*vertimenti*) and *Pediculus capitis*. *Parasitology*, ix. p. 228.

Byam, W. and others (v. 1918). Trench Fever, a Louse-borne Disease. *Trans. Soc. of Trop. Med. and Hygiene*, May 1918, xi. No. 7.

Couvy, L., Dujarric, R. and de la Rivière (12. i. 1918). Note sur l'étiologie de la fièvre des Tranchées. *C. rend. Soc. Biol.* lxxxi. p. 22.

CSERNEL (28. xii. 1916). Ueber die Morphologie des Fleckfiebererregers. *Wien. klin. Wochenschr.* No. 52, p. 1643.

DAVIES, F. C. and WELDON, R. P. (3. ii. 1917). A preliminary contribution on "P.U.O." (Trench Fever). *Lancet*, I. p. 183.

HIS, W. (3. vii. 1916). Ueber eine neue periodische Fieber-erkrankung (Febris Wolhynica). *Berlin. klin. Wochenschr.* p. 738.

JUNGMANN, P. (iii. 1916). Zur Aetiologie des Febris Wolhynica. *Berlin. klin. Wochenschr.* p. 323.

JUNGMANN, P. and KUCZINSKI, M. H. (22. iii. 1917). Zur Klinik und Aetiologie der Febris Wolhynica (His-Wernersche Krankheit). *Deutsche med. Wochenschr.* p. 359.

KORBSCH, R. (5. x. 1916). Zur Kenntnis der Febris Wolhynica. *Deutsche med. Wochenschr.* p. 1217.

MCNEE, J. W., RENSHAW, A. and BRUNT, E. H. (12. ii. 1916). "Trench Fever," A Relapsing Fever occurring with the British Forces in France. *Brit. Med. Journ.* p. 225.

MUNK, F. and DA ROCHA-LIMA, H. (30. x. 1917). Klinik und Aetiologie des sogen. Wolhynischen Fiebers (Werner-Hissche Krankheit). II. Ergebnis der ätiologischen Untersuchungen und deren Beziehungen zur Fleckfieberforschung. *München. med. Wochenschr.* p. 1422.

NÖLLER, W. (10. vii. 1916). Beitrag zur Flecktyphusübertragung durch Läuse. *Berlin. klin. Wochenschr.* p. 778.

RICKETTS, H. T. (30. i. 1909). A Microorganism which apparently has a specific relationship to Rocky Mountain Spotted Fever. *Journ. Amer. Med. Assoc.* LII. p. 379.

RICKETTS, H. T. and WILDER, R. M. (23. iv. 1910). The etiology of the Typhus Fever (Tabardillo) of Mexico City. *Journ. Amer. Med. Assoc.* LIV. p. 1373.

RIEMER (16 i. 1917). Beiträge zur Frage des Erregers des Fünftagefiebers. *München. med. Wochenschr.* LXIV. p. 92.

DA ROCHA-LIMA, H. (22. v. 1916). Zur Aetiologie des Fleckfiebers. *Berlin. klin. Wochenschr.* LIII. p. 567.

SERGENT, EDM., FOLEY, H. and VIALLATTE, C. (13. vi. 1914). Sur des formes microbiennes abondantes dans le corps de poux infectés par le typhus exanthématique et toujours absentes dans les poux témoins, non typhiques. *Compt. rend. Soc. Biol.* LXXVII. p. 101.

STINTZING, R. (30. i. 1917). Ueber Febris quintana. *München. med. Wochenschr.* p. 155.

STRISOWER, R. (30. iv. 1918). Experimentelle u. klinische Beiträge zur Febris quintana. *Ibid.* LXV. p. 476.

TÖPFER, H. (20. iii. 1916). Zur Aetiologie des Febris Wolhynica. *Berlin. klin. Wochenschr.* p. 323.

—— (12. x. 1916). Der Fleckfieber-erreger in der Laus. *Deutsche med. Wochenschr.* XLII. No. 41, p. 1251.

—— (17. x. 1916). Zur Ursache und Uebertragung des wolhynischen Fiebers. *München. med. Wochenschr.* No. 42, p. 1495.

—— (24. vi. 1917). Ursache und Uebertragung der Kriegsnephritis. *Medizin. Klinik*, p. 678.

WERNER, H., BENZLER, J., and WIESE, O. (19. ix. 1916). Zur Aetiologie des Fünftagefiebers. *München. med. Wochenschr.* p. 1369.

WERNER, H., and BENZLER, J. (22. v. 1917). Zur Aetiologie und Klinik der Febris quintana. *Ibid.* p. 695.

WOLBACH, S. B. (1916–17). The etiology of Rocky Mountain Spotted Fever. *Journ. Med. Research*, XXXIV. No. 1, p. 121, XXXV. No. 1, p. 147.

DESCRIPTION OF PLATES II AND III.

Figs. 1 and 3. Films of excreta of infected lice from Box A 16, made 30. vi. 18 on the 15th day from the first infecting feed on a Trench Fever patient.

Fig. 2. Film of excreta of infected lice from Box A 18, made 30. vi. 18 on the 10th day from the first infecting feed. Stained Giemsa. × 1000. Showing pure "culture" of *Rickettsia*.

Figs. 4 and 5. Films made from the fore-gut of an infected louse from Box 171, made 10. vi. 18 on the 26th day from the first infecting feed. Showing numerous *Rickettsia*—pure.

Fig. 6. Film of excreta of normal lice fed on healthy man; showing bacteria which are sometimes found in the excreta of normal lice. Stained Giemsa. × 1000.

Figs. 7 and 8. Sections of the hind-gut of an infected louse from Box 160, killed 4. v. 18, the 7th day after the first infecting feed. Stained Giemsa. × 1000. Showing *Rickettsia* on the surface of the epithelium. *a*, body-cavity. *b*, gut-wall. *c*, lumen of gut containing altered blood, and, close to the epithelial cells, *Rickettsia*.

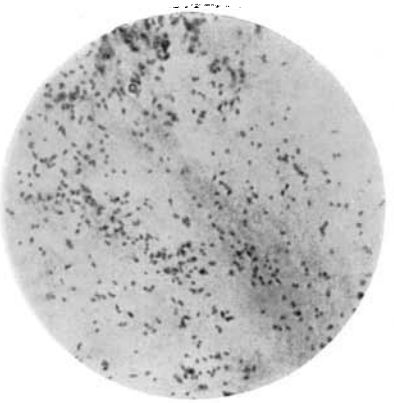

Fig. 1

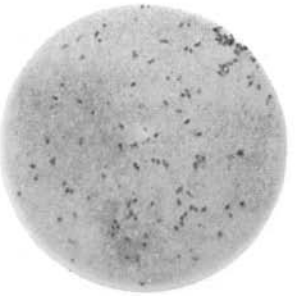

Fig. 2

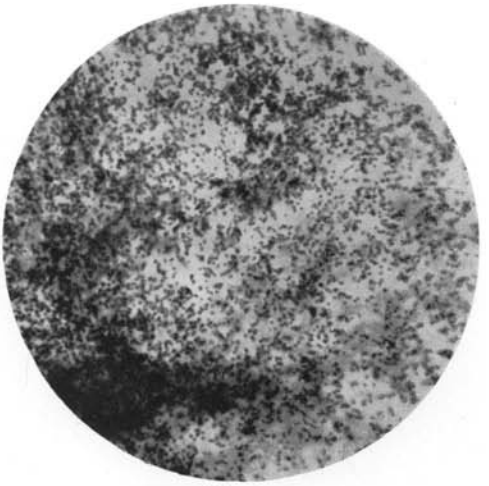

Fig. 3

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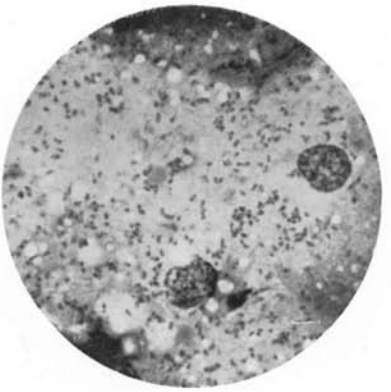

Fig. 4

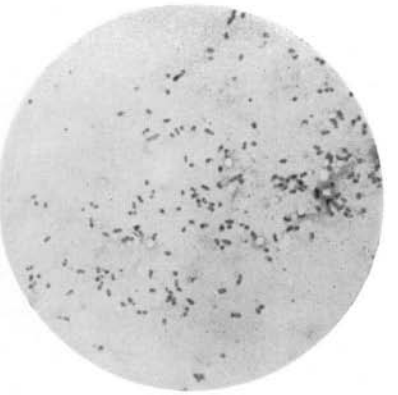

Fig. 5

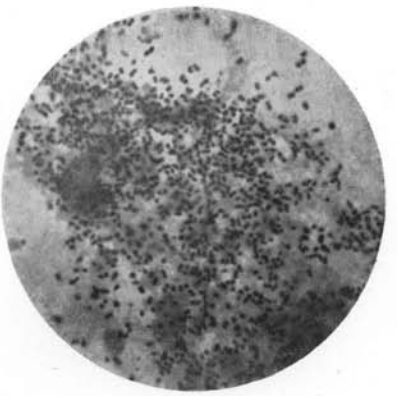

Fig. 6

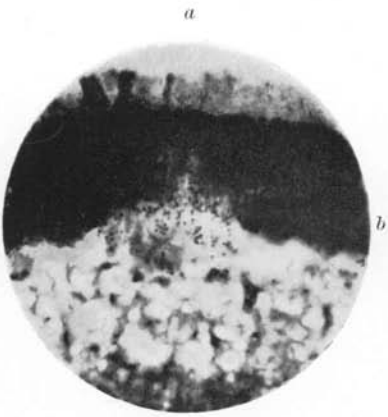

Fig. 7

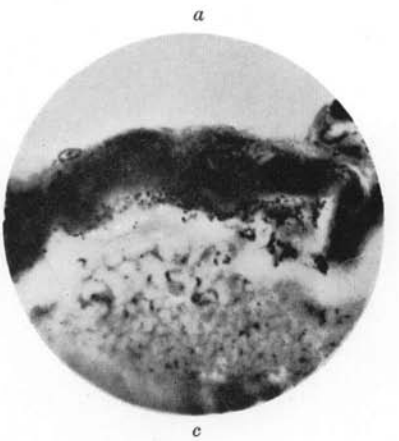

Fig. 8

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