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# Studies of the Rickettsias of the Typhus-Rocky-Mountain-Spotted-Fever Group in South Africa.

## III.—The Disease in the Experimental Animal. Cross-Immunity Tests.

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In this section, we shall deal with the effect of the various typhus strains on the guinea-pig, rabbit, rat, mouse, dog, sheep, and ox, and with cross-immunity tests carried out in the guinea-pig and in the sheep.

### Rat Typhus.

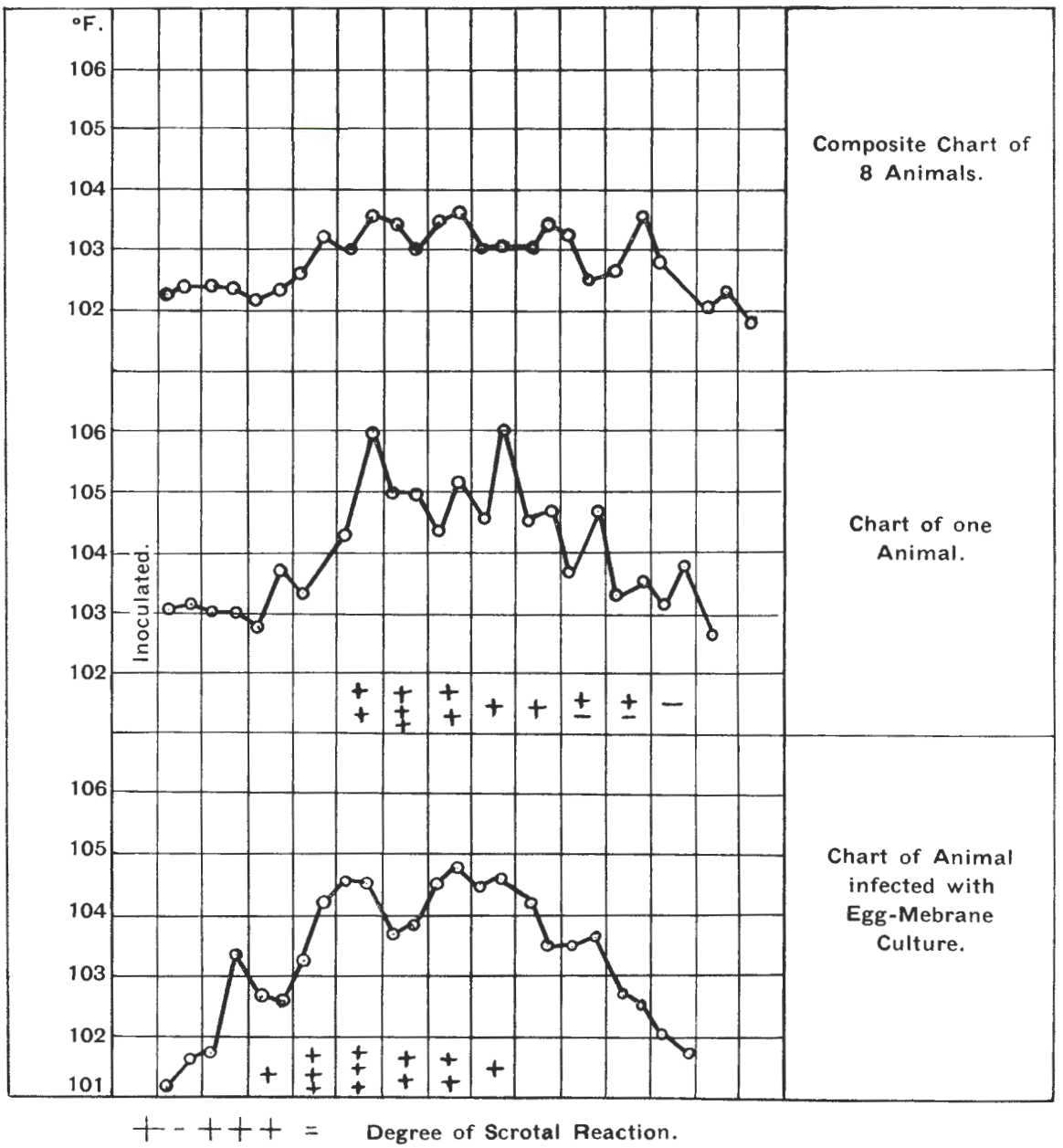
#### *In the Guinea-pig.*

No difficulty has been experienced in maintaining this rat typhus strain in guinea-pigs by brain to peritoneum passage every 9th or 10th day.

The type of reaction produced in the guinea-pig did not differ from that described by most workers in the typhus field. The first rise in temperature took place usually on the third, fourth or fifth day after infection and, generally, the disease (as manifested by the temperature reaction) was of a more protracted nature than that caused by "Robertson", "Hare" or *fièvre boutonneuse*. In common with our experience with the other four virus strains employed, a "saddle-back" type of curve was common; the initial rise was maintained for one or two days and was followed by a lower temperature during the next day or two and then by a secondary rise which returned to normal by lysis. Chart 1 shows a composite temperature curve of eight infected guinea-pigs, a typical curve of one animal and the reaction produced by an egg-membrane culture. The composite chart, although reproducing the

CHART 1.

Rat typhus in guinea-pigs.



general trend of the reaction, is not typical; each animal does not react to the same degree at the same time, so that a flattening of the curve is produced. This point must be kept in mind when composite charts are read. No deaths directly attributable to rat typhus occurred among the hundreds of guinea-pigs that received the virus (brain, tunica or egg-membrane) and, except for the temperature and scrotal reactions, the animals looked healthy.

### *The Scrotal Lesion.*

In sexually mature, male guinea-pigs, i.e. in those with descended, palpable testes, a tumefaction of the scrotum was an almost constant phenomenon. Of 89 males, 84 (94·4 per cent.) showed this reaction and if adults only had been inoculated, this percentage would have been almost one hundred. After 50 sub-inoculations (brain to peritoneum) this reaction occurred as regularly and as strongly as in the first passage carried out by us. The day of the appearance of the swelling in 38 guinea-pigs was as follows:—

| Day on which swelling was first noticed | 4 | 5 | 6 | 7 | 8 | 9 | 10 |
|---|---|---|---|---|---|---|---|
| Number of guinea-pigs involved | 5 | 15 | 7 | 4 | 5 | 1 | 1 |

The majority of the reactions were definite by the fifth day after infection; those appearing for the first time on or after the seventh day were atypical and were associated with a lengthened (temperature) incubation period. The rapidity with which a scrotum could swell was remarkable; often only a few hours elapsed between a doubtful and a " + + + " reaction. The maximum enlargement, once reached, was maintained for from two to four days; thereafter reduction occurred, the return to apparent normality occupying from three to seven days.

In character, the scrotal reaction in rat-typhus-infected guinea-pigs differed somewhat from that produced by the other strains. At the height of the reaction, the scrotum was tense, hard, and firm and lacked that fluctuating, oedematous feeling of the swelling produced by " Hare " or " Robertson ". As a rule, it was not possible to force the testicles into the abdominal cavity and, even at post-mortem examination, it was frequently impossible to draw them out of the scrotum. Actually, on many occasions, it was necessary to dissect them out in order to make scrapings from the tunica. The testicles themselves showed distension and ramiform injection of the blood vessels, petechiae, even ecchymoses up to 3 mm. in diameter, and, not uncommonly, small haemorrhages in the polar fat. An exudate, which was fairly copious and fluid in the early stages of the reaction, later thickened to form fibrinous sheets that covered nearly the whole testicle. These sheets, greyish-white in colour, could be scraped off and floated intact in saline. The final outcome was usually *restitutio-ad-integrum* but, on several occasions, some thickening of the scrotum persisted that left the testicle hard and attached to the sac.

*Post-mortem examination.*—Apart from the scrotal lesion, the only other constant macroscopical change was a swelling of the spleen, often to two or three times its normal size. Not infrequently, a tenacious greyish-white deposit or even a pseudo-membrane covered the surface of the spleen and liver.

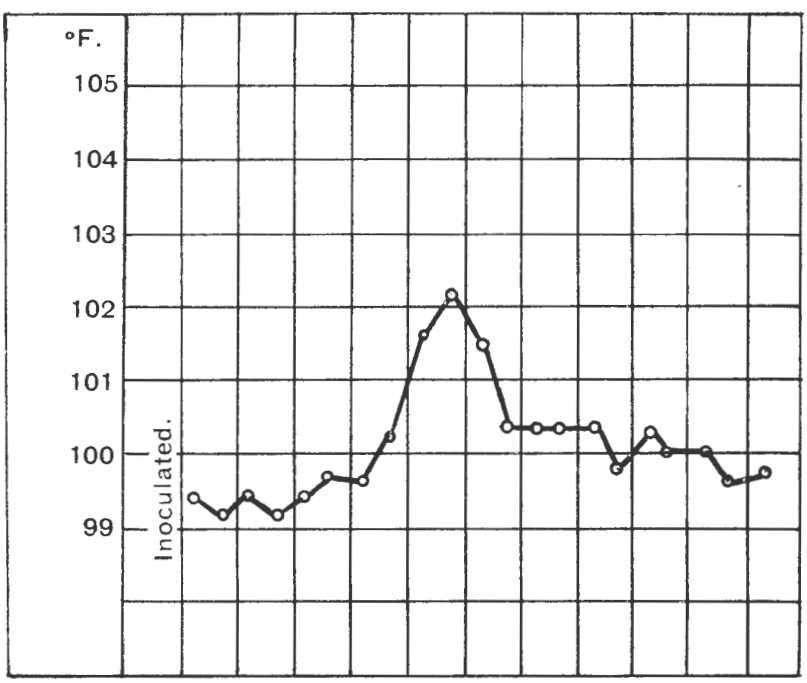
### *In the White Rat.*

No difficulty was experienced in maintaining this strain in the rat; 50 animals were used, 12 subinoculations were made and the experiment was terminated at this point. The brain of a reacting guinea-pig was the first inoculum and thereafter the brain of a rat, taken on the 8th to the 10th day after infection. At each passage, a portion of the rat brain was also inoculated into two indicating guinea-pigs. Unless a definite, easily recognizable reaction was noted, the guinea-pigs were retained for one month to undergo an immunity test.

Most rats showed a definite thermal rise that began on the 4th or 5th day (102° F. to 103° F.) and that was maintained for from two to four days, but at no time was a scrotal swelling observed. Chart 2 is a composite temperature curve of 10 infected rats.

CHART 2.

Rat typhus in rats. Composite chart of 10 rats.



Apart from the temperature reaction, many rats were visibly affected by the virus; some were poor, thin or even emaciated, and 8 died. This debilitating effect was most marked in those animals which received benzol and olive oil subcutaneously in addition to the virus inoculation (see Zinsser and Castaneda, 1930). In all rats, the chief post-mortem lesion was an enlargement of the spleen which was sometimes three to five times the size of that of the normal animal. In smears taken from the surface of such an enlarged spleen, rickettsias were plentiful and in one preparation there were dozens of infected cells per microscopic field.

### *In the Mouse.*

The disease was passed (brain to peritoneum at 8 to 9 day intervals) through 3 lots of mice only, the 4th and subsequent passages being negative. The mice showed no symptoms or macroscopic post-mortem lesions and, as the temperature of normal mice can rise or fall several degrees in a few hours, infection could be judged only by the use of indicating guinea-pigs.

### *In the Dog.*

Durand (1933) showed that murine typhus caused an inapparent disease in dogs, that the virus, in some instances, could be recovered in guinea-pigs from the blood, brain or spleen, and that the Weil-Felix reaction became positive (chiefly OX19). However, the disease could not be passaged from dog to dog. Combiesco and Angelesco (1933) also set up a " maladie inapparente " and 14 days later recovered the virus from the brain. However, to our knowledge, no indication has been given that the dog plays a rôle in the spread of typhus. Our results confirm this; in fact, we could do no more than demonstrate a survival and translocation of the virus.

Eight young dogs, obtained from a pound, were used. Dog 1 (1972) inoculated intraperitoneally with the brain of an infected guinea-pig, did not react. Guinea-pigs which received an intraperitoneal injection of the blood (3 c.c. to 4 c.c.) of this dog on the 4th, 7th, and 15th day after the attempted infection did not react and, tested later, were not immune.

Dog 2 (2089) was infected in the same way as dog 1, and, 9 days later, its brain was removed and a portion injected, intraperitoneally, into dog 3 (2093) and into guinea-pigs. None reacted. After 8 days, the brain of dog 3 was injected into dog 4 (2097) and into guinea-pigs without a reaction being produced. Finally no reaction occurred when the brain of dog 4 was inoculated into guinea-pigs.

In the 3rd experiment, 4 dogs (1967, 1969, 1970 and 2030) were infected with guinea-pig brain by the intraperitoneal route. No apparent reaction occurred. Table I summarizes the results of injecting the blood and the brain of these dogs into guinea-pigs.

TABLE 1.

*The Infectivity for Guinea-pigs of the Blood and Brain of Rat-typhus-infected Dogs.*

| Inoculum. | Days after Infection. | From Dogs. | Result in Guinea-pigs. |
|---|---|---|---|
| Blood: 3–4 c.c. i.p. | 4th | 5, 6, 7, 8 | Positive. |
| | 6th | 5, 6, 7, 8 | Negative. |
| | 10th | 5, 6, 8 | Negative. |
| | 14th | 5, 6, 8 | Negative. |
| | 19th | 5, 6 | Negative. |
| Brain: i.p. | 7th | 7 | Positive. |
| | 14th | 8 | Negative. |
| | 19th | 6 | Negative. |

The foregoing results show that the dog was not easily infected with typhus and that the disease could be carried on in it. Virus was demonstrable in the blood on the 4th but not on the 6th day after infection and in the brain on the 7th but not on the 14th day.

*In the Sheep.*

About half of the sheep used reacted with an elevation of temperature (see Chart 3) when infective guinea-pig brain was injected intravenously, and the disease could be carried on in sheep for one passage only, when blood, taken at the height of the thermal reaction, was the inoculum. The reaction of the recipient sheep was much milder than that of the donor. No symptom, other than the temperature, was noticed and in no instance did death occur. Attempts to infect guinea-pigs with the blood or brain of sheep taken at the height of the thermal reaction were unsuccessful.

*In the Rabbit.*

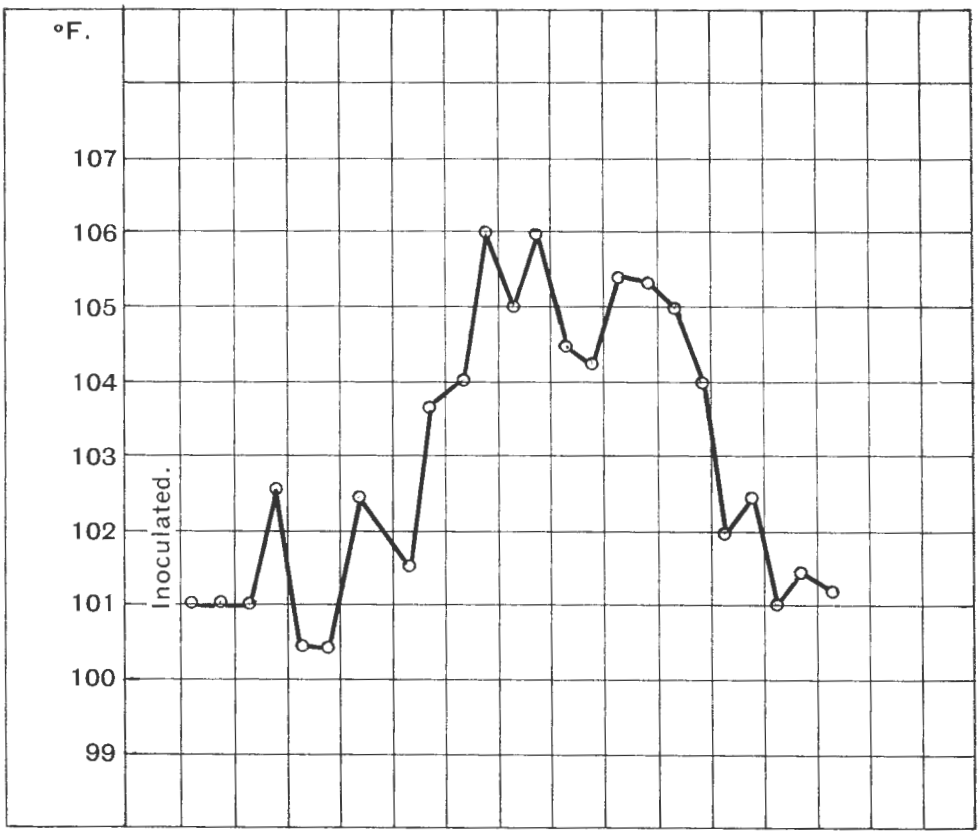
The effect of the rat typhus and the other virus strains on this animal will be discussed under " The Weil-Felix reaction ".

" HARE."

*In the Guinea-pig.*

Once established, no difficulty was experienced in maintaining the " Hare " strain in guinea-pigs (71 brain to peritoneum passages in 20 months at 8 to 10 day intervals). A composite temperature curve and a typical thermal reaction are reproduced in Chart 4. Usually the first rise was noted between the 4th and 6th days after inoculation, was maintained for from 4 to 7 days and slowly, with small fluctuations, returned to normal. It was unusual for a temperature of 106° F. to be recorded for more than 24 hours; generally the fluctuations were between 104° F. and 105° F. No symptoms, other than the elevated temperature and a scrotal swelling were observed.

CHART 3.
Rat typhus in sheep.




*The scrotal lesion.*—In the early passages, a scrotal swelling occurred very commonly in guinea-pigs; of 244 males, 211 (86·5 per cent.) showed this lesion and probably the percentage would have been higher if fully mature animals only had been used. In more recent passages, this reaction has been seen in only about 30 per cent. of cases, although the temperature curve has not varied in the slightest degree. The day of the appearance of the swelling in 34 guinea-pigs was as follows:—

| | | | | | |
|---|---|---|---|---|---|
| Day on which swelling was first noted | 4 | 5 | 6 | 7 | 10 |
| Number of guinea-pigs involved | 12 | 7 | 9 | 5 | 1 (late reactor). |

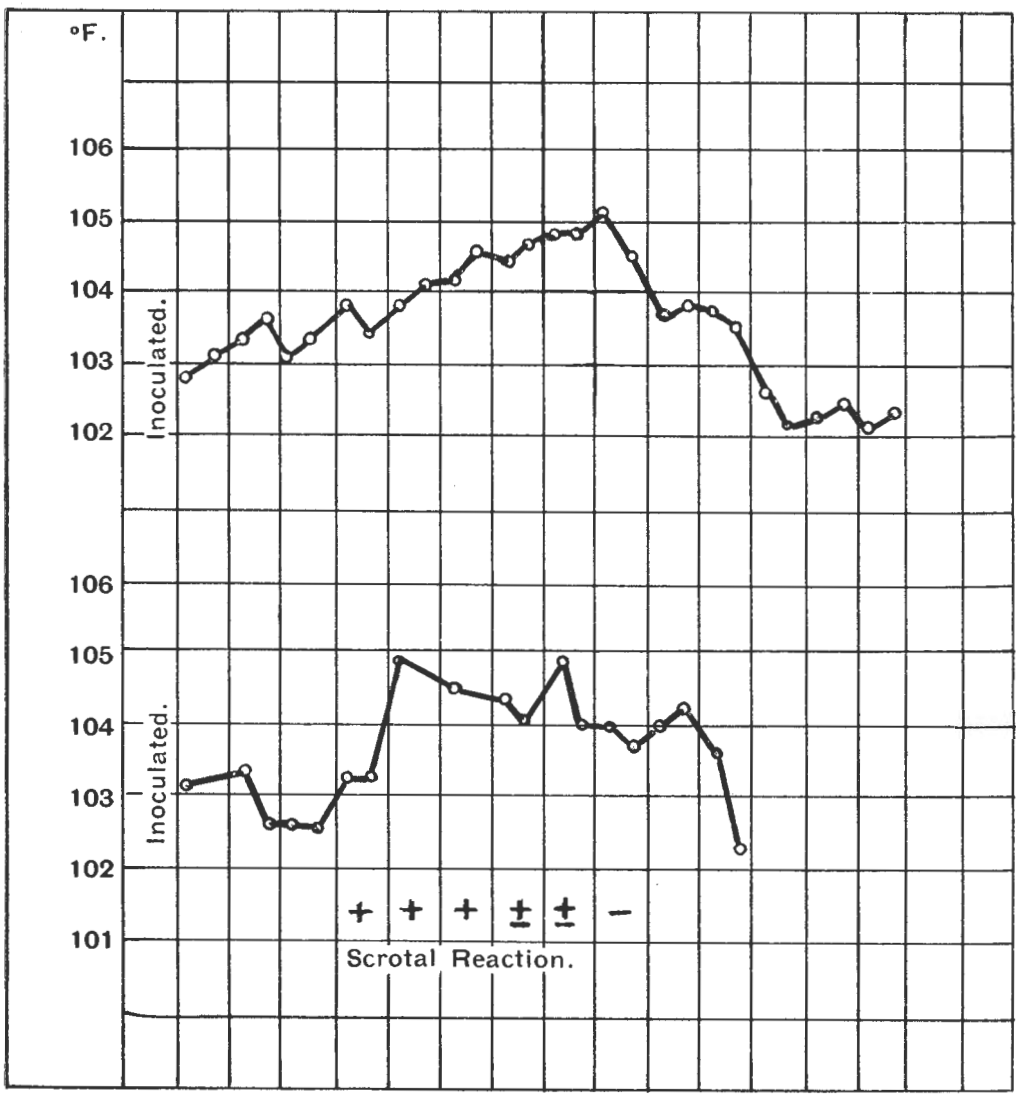
The enlarged scrotum felt soft and oedematous, and was not hard and firm as in rat-typhus-infected animals. Usually the testicle could be forced into the abdominal cavity and no thickening or hardness remained after the swelling had subsided. At post-mortem examination, in the early stage of the disease, the testes were hyperaemic and surrounded by a small quantity of greyish translucent exudate. Later, this hypermaemia became more marked,

but distinct haemorrhages in the testicle or polar fat were very rare. The exudate increased in amount, became viscid in consistency and whitish in colour and although flakes about 1 mm. in diameter could be scraped from the testicle, fibrinous sheets were not formed.

*Post-mortem examination.*—The remarks made under "rat typhus" apply to "Hare" with the reservation that the spleen was not usually so greatly enlarged (and occasionally was not apparently enlarged at all) and that a deposit on the spleen and liver was uncommon.

CHART 4.

"Hare" in guinea pigs. Composite chart of 8 guinea pigs (top). Chart of one animal (bottom).



*In the White Rat.*

Two attempts were made to adapt the " Hare " virus to rats, one involving 7 and the other 5 passages. As with the rat typhus rat passages, indicating guinea-pigs were inoculated at each sub-inoculation. Neither the rats nor the guinea-pigs reacted; the latter were later shown to be susceptible to a test inoculation of virulent brain.

*In the Mouse.*

No success attended two attempts to infect this animal. The brains of the mice of even the first generation (removed 9 days after the intraperitoneal inoculation of virulent guinea-pig brain) failed to cause a reaction in indicating guinea-pigs. Subsequent passages were also negative.

*In the Dog.*

One experiment only was carried out in this animal and although infection was not produced, we do not feel that the result is of great value. The pups, reared in tick-free surroundings, received infective guinea-pig brain intraperitoneally and showed no thermal or other reaction. Their pooled blood, taken at different times between the 2nd and 14th day after the injection did not infect guinea-pigs and these animals were not immune at a subsequent immunity test. However, a considerable number of the guinea-pigs died within one to four days after the blood injection and most of the remainder had severe temperature reactions beginning within 24 hours of the test. It has been our experience that a guinea-pig which has a non-specific temperature rise of this type does not develop typhus and, when tested later, is not immune. The lack of dogs, reared in tick-free surroundings, (and in work of this kind, it is essential to be certain of this point) has prevented our repeating the experiment.

*In the Sheep.*

What has been said about rat typhus in sheep applies also to " Hare ".

*In the Ox.*

A bovine that received the tunica scrapings of a " Hare " infected guinea-pig intravenously did not react.

*In the Rabbit.*

See " Rat typhus ".

" APPLETON ".

*In the Guinea-pig.*

From the start difficulty was encountered in carrying this strain in guinea-pigs. A sustained rise in temperature (104° F. and above) occurred in about 30 per cent. of animals and a scrotal swelling was infrequent (about 10 per cent.). In an attempt to " acclimatize " the virus to guinea-pigs, we fed them on a vitamin-deficient diet

(autoclaved oats). This procedure proved to be disadvantageous because not only was the reaction no better but the guinea-pigs became thin and some died.

*The Scrotal Lesion.*

Although a scrotal swelling was uncommon, it occurred more frequently in the guinea-pigs of the earlier than of the later passages. The day of its appearance in 29 males was as follows:—

| Day on which swelling was first noticed | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 |
|---|---|---|---|---|---|---|---|---|
| Number of guinea-pigs involved | 1 | 6 | 8 | 1 | 7 | 4 | 1 | 1 |

In character, the scrotal swelling produced by the Appleton strain was similar to that caused by " Hare ".

The post-mortem lesions were the same as those noted under " Hare ".

At the time when we discontinued the passage of this strain (65th guinea-pig passage) we had no experience in the use of the chick chorio-allantoic-membrane-method of cultivating typhus rickettsias. The use of this technique with a tick-bite fever (" Robertson ") and a *fièvre boutonneuse* strain indicates that an egg-membrane culture of " Appleton " rickettsias would probably have produced satisfactory reactions in guinea-pigs.

*In the White Rat.*

This strain was passed through eleven generations of rats (29 in all), at which stage the experiment was terminated. A period of 8 to 9 days was allowed to elapse between each passage. A rise in temperature to 102° F. or 102·5° F. (from normal of 99° F. to 100° F.) occurred on about the 3rd or the 4th day, was maintained for from 1½ to 3 days and thereafter fell fairly rapidly to normal. Indicating guinea-pigs inoculated with rat brain at each passage also reacted and were immune at a subsequent immunity test. Rats living on a deficient diet (autoclaved oats) and/or into which benzol and olive oil were injected subcutaneously, had no better reactions than those receiving a normal diet. The only apparent post-mortem lesion was an enlargement of the spleen. In Chart 5, a composite curve of the temperatures of 14 rats is given.

*In the Dog.*

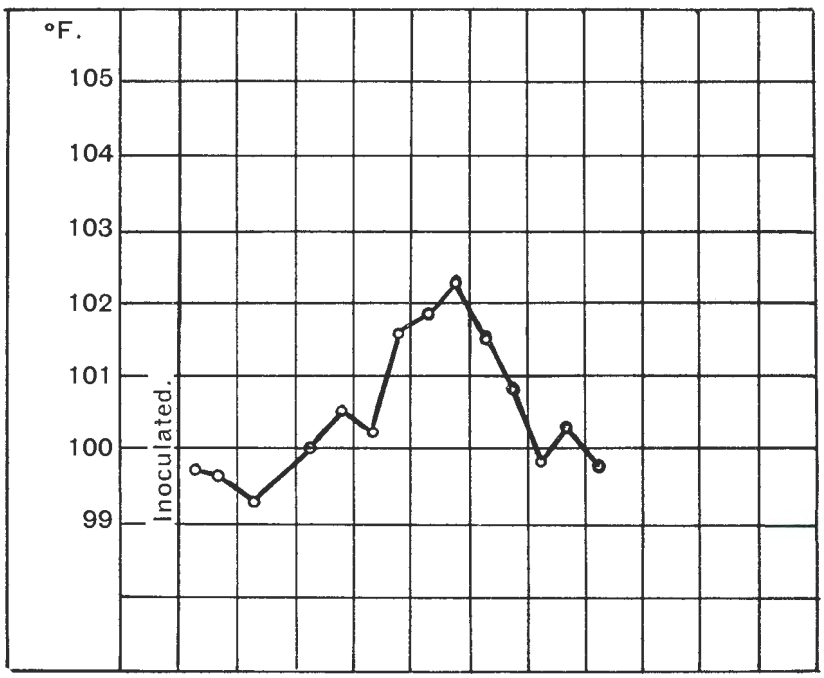
One dog (1935) inoculated intraperitoneally and another (1936) inoculated intravenously with the brain mush of two infected guinea-pigs showed no thermal or other reaction. As the dogs originated from a pound, we have no knowledge of their history; they may have been immune and, as no subinoculations were made from them into guinea-pigs, we do not know if they underwent an inapparent infection. However, it should be borne in mind that this strain originated from a dog, which was suffering, at least, from an inapparent infection.

*In the Sheep.*

See " Hare ".

CHART 5.

"Appleton" in rats. Composite chart of 4 rats.




"ROBERTSON."

*In the Guinea-pig.*

This, the only tick-bite fever strain of human origin with which we worked produced reactions (thermal and scrotal) only slightly better than those of "Appleton" when brain to peritoneum (8 to 10 day intervals) was the method of passage. As a routine measure we used three guinea-pigs per passage and as a rule one of these reacted fairly well, one poorly and one very slightly or not at all. The brain of such an apparently non-reacting animal taken on the 9th day after infection proved, on inoculation, to be just as virulent as that of a guinea-pig which had a good reaction. In general, the first thermal rise was late, very often not before the 6th or 7th day after infection. A temperature much exceeding 105° F. was seldom maintained for more than a day or two and generally the febrile period lasted for not more than 2 to 5 days and usually 3 to 4 days. Even in sexually mature male guinea-pigs, a scrotal swelling was uncommon, and occurred in not more than 10 per cent. of the animals. However, in spite of the poor reactions we succeeded in maintaining this tick-bite fever strain in guinea-pigs for more than a year (46 brain to peritoneum passages).

With the use of egg-membrane cultures we obtained much more definite reactions, both thermal and scrotal. In Chart 6 we reproduce a good temperature reaction produced by this means and include

a curve caused by the inoculation of brain. This chart, typical of many, shows that the infected egg-membrane caused an early temperature rise and an earlier-appearing scrotal lesion due, most probably, to the large number of rickettsias inoculated. This reaction was got in about 70 per cent. of sexually mature animals and followed much the same course as that of " Hare "; perhaps the only difference was that the scrotum seldom attained the same large dimensions. The lesions at post-mortem examination were the same as those given for " Hare ".

*In the White Rat.*

The first rats were infected with egg-membrane culture and thereafter 10 successful subinoculations (at 8 to 10 day intervals) were made by brain to peritoneum passage. The reactions were almost superimposable on those obtained with the " Appleton " strain.

*In the Rabbit.—See " Rat Typhus ".*

FIÈVRE BOUTONNEUSE.

*In the Guinea-pig.*

We soon realised that it would be difficult to maintain this strain in guinea-pigs by brain to peritoneum passage. Even after 10 sub-inoculations (at 8 to 10 day intervals), there was no indication that the virus was adapting itself; reactions of the " Appleton " type were the rule. When a rise in temperature did occur, it was late in appearance (7th to 9th day), seldom rose much above 104·5° F. and was not maintained for more than 2 or 3 days. Of 31 inoculated animals, 15 had a thermal rise high enough to be considered significant and only one developed a scrotal swelling (small, appearing on the 7th day). Because we had two main objects in view—the study of the causative rickettsias and cross-immunity work—we decided to abandon brain to peritoneum passage and to resort to egg-membrane culture which had given such good results with another " weak " virus, that of " Robertson ".

With egg-membrane cultures, no difficulty was experienced in producing good thermal and scrotal lesions. In Chart 7 two typical reactions are given. It will be noticed that the temperature rise occurred early and although it was not maintained for long, it was definite. The scrotal swelling, which occurred in about 60 per cent. of the male animals, usually appeared early and could not be distinguished from that of " Hare ". The post-mortem picture was similar to that recorded for " Hare ".

SUMMARY OF REACTIONS IN ANIMALS.

The results just presented show that rat typhus could be maintained, without any difficulty, in the guinea-pig and rat, and that in the former animal it nearly always caused a scrotal swelling. It could be passaged three times only in the mouse and not at all in

the dog. The sheep proved susceptible when inoculum was virulent guinea-pig brain, but the virus could not be passed serially through this animal. The single ox used proved to be insusceptible.

" Hare ", alone of the other four viruses, was easily passaged in guinea-pigs; it caused readable temperature reactions and a scrotal swelling in a large percentage of cases. It did not infect the rat and the reactions in sheep were the same as those produced by rat typhus.

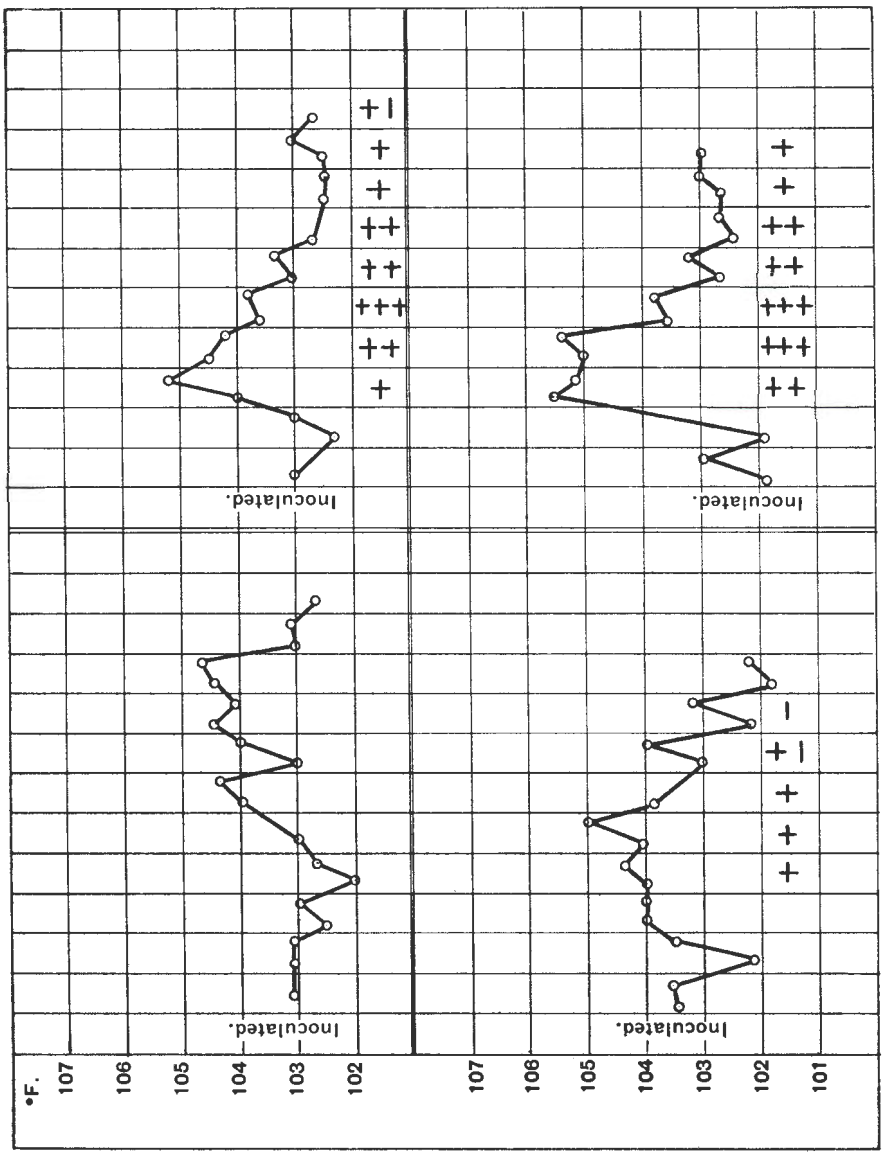



CHART 6.

"Robertson" in guinea-pigs. Chart of one animal (brain inoculum) (top). Chart of one animal (egg-membrane inoculum) (bottom).

CHART 7.

Fièvre boutonneuse charts of 2 guinea-pigs infected with egg membrane.

" Appleton " and " Robertson " and *fièvre boutonneuse* occasioned great difficulty when attempts were made to maintain them in guinea-pigs by the brain to peritoneum method of passage. However, when infected egg-membrane (in the case of " Robertson " and *fièvre boutonneuse*) was the inoculum, good temperature and scrotal reactions were obtained. Both " Appleton " and " Robertson ", although, as will be shown later, belonging to the same group as " Hare ", were able to infect the rat.

## The Weil-Felix Reaction.

### *In the Rabbit.*

Rabbits were inoculated intraperitoneally with virulent guinea-pig brain (rat typhus and " Hare ") or with egg-membrane culture (" Robertson ") in an attempt to produce agglutinins to a proteus X strain. None reacted as the direct result of the inoculation and the serum of none, taken prior to the infection, agglutinated proteus OX2, OX19 or OXK at a dilution of 1:20 or higher. In carrying out the tests, living saline suspensions of non-motile OX2, OX19 and OXK were used. The cultures were grown according to the instructions sent out by Dr. Felix and every endeavour was made to prevent motile " H " variants appearing. This was never necessary with OX2, only very occasionally with OX19 and quite frequently with OXK. The tubes were incubated at 37° C. for four hours and left overnight at room temperature before being read.

Table 2 summarises the results of agglutination tests with serum taken at different times after infection.

A Weil-Felix reaction was obtained with the sera of 10 rabbits infected with rat typhus; in no instance was proteus OX2 agglutinated; OX19 was agglutinated by all and one case (rabbit 6) both OX19 and OXK were agglutinated. The sera of two of nine rabbits, inoculated with " Hare ", became positive; that of rabbit 14 agglutinated OX2, did not affect OXK and produced a doubtful reaction with OX19 and that of rabbit 15 agglutinated OX19 only.

The serum of one of seven rabbits inoculated with " Robertson " caused a doubtful agglutination of OX19, at a dilution of 1:20; the sera of the remaining six animals were negative.

### *In the White Rat.*

The sera of 7 rats infected with rat typhus, and taken 8 to 36 days after the inoculation were tested with the 3 proteus X strains. Two were positive, both for OX19. That of one rat (27 days after infection) was postive at a dilution of 1:160 and that of the other (16 days after infection) at 1:40.

### *In the Guinea-pig.*

In an attempt to isolate a specific proteus from guinea-pigs infected with rat typhus, " Hare " or " Appleton ", we cultured the intestinal contents of 26 of them and from 6 obtained a swarming proteus, the " O " antigens of which were not serologically related

## Table 2.

### Weil-Felix Reaction in Rabbits.

| Typhus Strain. | Proteus Strain. | Rabbit. | | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | 1 | | | 2 | | 3 | | 4 | | | 5 | | 6 | 7 | | 8 | | 9 | 10 | |
| | | Days after Infection. | | | | | | | | | | | | | | | | | | | |
| | | 11 | 20 | 38 | 11 | 17 | 11 | 17 | 12 | 15 | 25 | 10 | 23 | 20 | 9 | 13 | 9 | 13 | 11 | 15 | 32 |
| Rat Typhus..... | 2 | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — |
| | 19 | ?20 | — | — | 160 | 160 | 320 | 40 | 160 | 80 | 20 | ?20 | — | 160 | 20 | 40 | 20 | 40 | 160 | 20 | — |
| | K. | — | — | — | — | — | — | — | — | — | — | ?20 | — | 80 | — | — | — | — | — | — | — |

| Typhus Strain. | Proteus Strain. | Rabbit. | | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | 11 | | 12 | 13 | | 14 | | 15 | | 16 | | | 17 | | 18 | | 19 | | | |
| | | Days after Infection. | | | | | | | | | | | | | | | | | | | |
| | | 9 | | 10 | 9 | | 9 | 17 | 9 | 15 | 9 | 15 | 28 | 12 | 32 | 9 | 27 | 7 | 23 | | |
| "Hare"..... | 2 | — | | — | — | | — | 80 | — | — | — | — | — | — | — | — | — | — | — | | |
| | 19 | — | | — | — | | — | ?20 | — | 80 | — | — | — | — | — | — | — | — | — | | |
| | K. | — | | — | — | | — | — | — | — | — | — | — | — | — | — | — | — | — | | |

| Typhus Strain. | Proteus Strain. | Rabbit. | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | 20 | | 21 | 22 | | 23 | | 24 | | 25 | | 26 | | |
| | | Days after Infection. | | | | | | | | | | | | | |
| | | 9 | 17 | 9 | 10 | 16 | 10 | 16 | 10 | 14 | 10 | 16 | 14 | | |
| "Robertson".... | 2 | — | — | — | — | — | — | — | — | — | — | — | — | | |
| | 19 | — | — | — | ?20 | — | — | — | — | — | — | — | — | | |
| | K. | — | — | — | — | — | — | — | — | — | — | — | — | | |

— No agglutination at 1 : 20 serum dilution ; 20, 40, etc. = reciprocal of the highest dilution of serum which caused agglutination.

to those of OX2, OX19 or OXK. It would appear that a proteus infection of the gut of guinea-pigs is not uncommon, because, even with the use of only a small number of animals, we obtained a 25 per cent. infection rate.

Giraud and Tannenbaum (1937, [1&2]) state that guinea-pigs do not develop agglutinins for proteus X during or after a typhus infection because they do not harbour banal proteus in their intestines; if this microbe is implanted in their alimentary canal by the oral and rectal routes and a typhus infection then set up, the Weil-Felix reaction develops.

To ascertain if proteus X agglutinins would develop if a banal proteus was implanted in the gut of guinea-pigs, we repeated Giraud and Tannenbaum's work. A heavy suspension of a swarming proteus [(R5H6), isolated from the small intestine of a rat and not serologically related to the " O " antigen of OX2, OX19 or OXK)] was administered to 6 guinea-pigs (1·0 c.c. fed through stomach tube and 1·0 c.c. per rectum) on 30.10.37 and thereafter, for 15 days, 10 c.c. was added to the food and 1·0 c.c. given per rectum. On the 16th day each animal received 10·0 c.c. per os and 1·0 c.c. per rectum. Three of these animals and three fresh untreated controls were infected, intraperitoneally, with typhus and three proteus-infected guinea-pigs left uninfected with typhus to see the effect of the proteus alone on agglutinin production. A Weil-Felix test, using living suspensions of OX2, OX19, OXK and R5H6, was put up with the serum of each animal on the day before the first proteus feeding, on the day of the infective inoculation and thereafter weekly for 5 weeks.

The results did not confirm those of Giraud and Tannenbaum. The serum of no animal, taken prior to the infection by proteus and/or rat typhus, agglutinated any of the four proteus strains used; those which were infected with rat typhus only remained negative throughout the experiment. The sera of the remaining 6 guinea-pigs (proteus alone and proteus plus typhus) at no time agglutinated OX2, OX19 or OXK, but all were positive for R5H6 (" H " agglutination) 17 days after the first proteus feeding and remained so for a further 4 to 5 weeks. The highest serum titre was 1:320.

### *Attempts to Isolate a Proteus from Guinea-pigs.*

The brain, heart-blood, urine, tunica vaginalis, liver, and spleen of 238 guinea-pigs, infected with " Hare ", " Appleton " or rat typhus were inoculated into broth and on to nutrient agar and observed for 14 days at 37° C. About 1·0 c.c. of blood was deposited in a sterile tube and the serum, after separating, was removed and replaced with about 15 c.c. of broth. Small portions of brain, liver, tunica, and spleen were placed in broth, and about 1·0 c.c. of urine was cultured in the same medium. The agar cultures were made by smearing slopes heavily with the blood or urine or the organ mush. Any Gram-negative bacilli which grew were isolated in pure culture and tested against sera prepared against OX2, OX19, OXK and against the sera of sheep recovered from " Hare ", " Appleton "

or rat typhus. Eighteen such organisms were isolated—2 from the brain and 1 from the urine of "Hare" guinea-pigs, 1 from the brain, 1 from the urine and 1 from the liver of "Appleton" guinea-pigs, and 6 from the brain, 3 from the blood and 1 each from the urine, liver, and tunica of rat typhus animals. None were agglutinated by any of the sera used.

These results are in agreement with those of most workers who have tried to isolate a specific proteus X from guinea-pigs infected with one or other of the typhus strains.

## The Duration of Infectivity of the Brain, Blood, and Tunica vaginalis of infected Guinea-pigs.

Guinea-pigs were infected by the intraperitoneal inoculation of virulent brain. At intervals after infection, the infectivity of the pooled citrated blood (3 c.c. to 4 c.c. intraperitoneally) of 4 to 6 of them was tested, and the infectivity of the brain and tunica scrapings of one animal was determined. Two guinea-pigs were inoculated intraperitoneally with the brain emulsion (half-a-brain per animal) and two with the tunica scraping suspension. If the indicating animal did not react typically, it was held for one month after the temperature had returned to normal and tested for immunity. The results of the experiments are collected in tables 3, 4 and 5.

Systematic work has not been carried out on the infectivity of the tunica or blood of "Robertson" or button fever infected guinea-pigs or of the tunica of "Hare" animals. However, on many occasions, we have demonstrated the infectivity of the tunica taken at the height of the scrotal reaction. In addition, the liver and spleen of rat-typhus animals have been proved capable of transmitting the disease, but, in these experiments, no attempt was made to remove the blood from the organs.

The results given in tables 3, 4 and 5 may be briefly summarized.

*Rat Typhus*:

*Brain.*—Virulent from the 2nd to the 22nd day after inoculation but not on the 1st day or on the 26th day or later.

*Blood.*—Virulent from the 3rd to the 21st day inclusive, but not before or after.

*Tunica.*—Virulent from the 2nd to the 12th day inclusive, but not before or after.

"*Hare*":

*Brain.*—Virulent from the 5th to the 15th day inclusive, but not before or after.

*Blood.*—Clear-cut results were not obtained, but it was infective on the 4th, 5th, 8th, 10th, and 13th days after infection.

TABLE 3.

*Infectivity of Brain and Tunica of Rat-typhus-infected Guinea-pigs (Exp. 109).*

BRAIN.

| | Days after Infection. | | | | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 1 | | 2 | | 3 | | 4 | | 5 | | 6 | | 7 | | 19 | | 22 | | 26 | | 30 | | 33 | |
| Result of direct test..... | N | N | † | P | P | P | P | P | † | † | P | P | P | P | P | P | P | P | N | N | N | N | N | N |
| Result of immunity test..... | P | P | | N | N | N | N | N | | | ND | | ND | | N | N | N | N | P | P | P | P | P | P |
| Infectivity of brain..... | − | | + | | − | | + | | | | + | | + | | − | | + | | − | | − | | − | |

TUNICA.

| | Days after Infection. | | | | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 1 | | 2 | | 3 | | 4 | | 5 | | 6 | | 7 | | 9 | | 10 | | 12 | | 13 | |
| Result of direct test..... | ?N | ?N | N | N | ?P | ? | P | ? | P | P | P | P | P | P | P | P | † | ?P | P | P | † | N |
| Result of immunity test. | P | P | N | P | N | P | ND | N | ND | | ND | | ND | | ND | | N | | ND | | P | |
| Infectivity of tunica..... | − | | + | | + | | + | | + | | + | | + | | + | | + | | − | | − | |

| | Days after Infection. | | | | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 16 | | 19 | | 22 | | 26 | | 30 | | 33 | | | | | | | | | | | |
| Result of direct test..... | ?P | ?P | N | † | N | N | N | N | N | N | N | N | | | | | | | | | | |
| Result of immunity test. | P | | P | | P | P | P | P | P | P | P | P | | | | | | | | | | |
| Infectivity of tunica..... | − | | − | | − | | − | | − | | − | | | | | | | | | | | |

( N = no reaction ; P = positive reaction ; † = died ; ND = not done ; + = infected ; − = not infected).

Duration of Infectivity of Brain = 22 days.

Duration of Infectivity of Tunica = 12 days.

## Table 4.

*Infectivity of Brain and Blood of "Hare"-infected Guinea-pigs (Exp. 19).*

**Brain.**

| | Days after Infection. | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | 5 | 6 | 10 | 13 | 15 | 18 | 24 | 25 |
| Result of direct test | N N | N N | N N | N N | N P | P ? | P P | ?P † | N N | N N | N ? | N † |
| Result of immunity test | P P | P P | P P | P P | P N | N P | ND | N | N P | P P | P P | P |
| Infectivity of brain | − | − | − | − | + | + | + | + | + | − | − | − |

**Blood.**

| | Days after Infection. | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 13 | 15 | 18 | 25 |
| Result of direct test | ? N | N ? | N ? | N N | ? N | ?P P | P ?P | ? ? | ?P ?P | ?P ?P | N ?P | P ? | N N | N † |
| Result of immunity test | ? † | P P | N N | N N | P † | ?N P | N P | P P | P N | ?P P | P N | P P | ?P ?P | P |
| Infectivity of blood | ? | − | + | + | − | ? | + | − | + | ? | + | − | ? | − |

(N = no reaction; P = positive reaction; † = died; ND = not done; + = infected; − = not infected).

Duration of infectivity of brain = 15 days.

Duration of infectivity of blood = 13 days.

TABLE 5.

*Infectivity of Blood of Rat-typhus-infected Guinea-pigs (Exp. 95).*

| | Days after Infection. | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 | 21 | 22 | 23 | 24 | 25 |
| Result of direct test..... | N N | P † | P N | P P | N N | P P | P P | P P | † P | P P | P P | P P | P P | ? † | N N | N N | N N | N N | N N |
| Result of immunity test..... | P P | N | N P | N N | P P | N N | N N | N N | | N N | N N | † N | N N | P | P | N P | P P | P P | P P |
| Infectivity of blood..... | — | + | + | + | — | + | + | + | + | + | + | + | + | — | + | — | — | — | — |

( = no roaction ; P = positive reaction ; † = died ; ND = not done ; + = infected ; — = not infected).

Duration of infectivity of blood = 21 days.

The results obtained with the brains of rat-typhus-infected guinea-pigs do not differ greatly from those obtained by Nicolle and Laigret (1933). With a Toulon murine strain, the virus survived for 41 but not for 44 days and with a Mexican strain for 34 but not for 44 days. However, Philip and Parker (1938) were able to recover virus from the brains of infected guinea-pigs 120 days, but not 150 days, after inoculation of virulent material.

It is interesting to note that 4 examples of an inapparent infection were got in these experiments (rat typhus table 3, tunica, 2nd day; rat typhus, table 5, blood, 21st day; " Hare ", table 4, brain, 15th day; " Hare ", table 4, blood, 4th day). These results, and a few others that will not be discussed, support Nicolle's (1934) contention that an immunity test is essential in work of this kind.

## The Minimal Infective Dose of the Brain of Rat-typhus-infected Guinea-pigs.

### *Technique.*

The brain was thoroughly emulsified in a measured volume of saline and the emulsion treated in the following way:—

1. Dilutions made in saline and injected intraperitoneally into guinea-pigs.

2. Emulsion spun at 4,000 r.p.m. (Ecco-Superior-H) and the supernatant fluid or dilutions of this fluid injected intraperitoneally or intracerebrally into guinea-pigs.

3. The supernatant fluid of (2) centrifuged at 14,000 r.p.m. (Ecco Ultimo) and the supernatant fluid or dilutions of this fluid injected intracerebrally into guinea-pigs.

Table 6 summarizes the results.

The results given in Table 6 show that as little as 1/2000 of a brain contained sufficient virus to infect a guinea-pig, whereas the virus had been diluted below an infecting dose in 1/4000 of a brain. The supernatant fluid of brain mush, spun at 4,000 and 14,000 r.p.m., whilst greatly reduced in titre as compared with whole brain, was still infective (the equivalent of 1/200 of a brain, in terms of supernatant fluid, for the 4,000 r.p.m. material and the equivalent of 1/50 of a brain for the 14,000 r.p.m. sample).

## Immunity Experiments.

### 1. In Vitro *Neutralization.*

Parker and Davis (1933) were able to neutralize the virus of Rocky Mountain spotted fever, contained in the serum of infected guinea-pigs, with convalescent serum from guinea-pigs and rabbits. The virus and serum were mixed, left for half-an-hour at room temperature, and injected intraperitoneally into guinea-pigs. By a similar technique, Monteiro (1934 1 & 2) was not able to neutralize Sao Paulo typhus virus with epidemic typhus convalescent serum. Zia and Wu (1936) found that the serum of a horse hyperimmunized against typhus by Dr. Zinsser passively protected guinea-pigs against typhus.

TABLE 6.

*The M.I.D. of Brains of Rat-typhus-infected Guinea-pigs.*

| Material Injected. | Route. | Fractions of Brain Injected. | Result. |
|---|---|---|---|
| 1. Saline emulsion of brain..... | i.p. | 1/100 (see note) | + |
| | | 1/1000 | + |
| | | 1/1600 | + |
| | | 1/2000 | + |
| | | 1/4000 | — |
| 2. Supernatant prepared by spinning (1) at 4,000 r.p.m. for ½ hour | i.p. | 1/4 (see note) | + |
| | | 1/20 | + |
| | | 1/200 | + |
| | | 1/2000 | — |
| | i.c. | 1/100 | — |
| | | 1/150 | + |
| | | 1/300 | + — (*a*) |
| | | 1/400 | — |
| | | 1/600 | — |
| | | 1/800 | — |
| | | 1/1600 | — |
| | | 1/2400 | — |
| 3. Supernatant prepared by spinning (2) at 14,000 r.p.m. for ½ hour | i.c. | 1/50 (see note) | + — — — — (*b*) |

(i.p. = intraperitoneally; i.c. = intracerebrally; + = positive reaction; — = no reaction and not immune at subsequent test; (*a*) = 2 experiments with 2 different brains; (*b*) = 5 experiments with 5 different brains.

*Note.*—The fractions given for (1) represent the actual fractions of one whole brain injected. For (2) and (3) the equivalents of 1/20, 1/50, etc. of a whole brain in terms of supernatant fluid are given.

Tests carried out by us with infective guinea-pig brain as virus and the sera of recovered guinea-pigs, rabbits or sheep as antibody were very unsatisfactory. On some occasions, 5·0 c.c. of serum was given intraperitoneally to guinea-pigs, followed in 24 hours by a small dose of brain. Little if any protection was afforded even with the use of homologous serum. The same type of result was got when brain mush and serum were mixed *in vitro* and then injected intraperitoneally. Further, no definite neutralization of the virus contained in the supernatant fluid of a brain emulsion (spun at 4,000 r.p.m. for half-an-hour) could be demonstrated when this was mixed with serum *in vitro* and the mixture injected intraperitoneally.

At one time, we thought that egg-membrane cultures would prove very satisfactory as sources of virus, but later work did not justify this view. The membranes were thoroughly emulsified in 0·85 per cent. salt solution and the emulsion spun at 1,500 r.p.m. for 4 minutes to deposit large particles. The supernatant fluid was then mixed with serum (equal parts for the intracerebral test, 0·3 c.c. injected; 1·0 c.c. virus and 1·0 c.c. or 5·0 c.c. serum for the

intraperitoneal test, the whole injected) and, after standing for half-an-hour at room temperature, the mixture was inoculated into guinea-pigs. Thirteen such tests were conducted with rat typhus, " Robertson ", and " Hare " egg-membrane cultures and the sera of recovered guinea-pigs. The results were not consistent; in one test, for example, rat typhus serum neutralized rat typhus virus and " Hare " serum failed to do so, whilst at the following test (using the same batch of serum and the succeeding generation of egg-culture virus) partial protection was produced by both sera. This method appeared to have possibilities that are, perhaps, worth exploring, but in our hands had no advantage over the standard technique of directly testing the resistance of recovered guinea-pigs.

We realize that the sera with which we worked were probably of very low titre and that repeatable results would doubtless be obtained with the use of high value sera such as that produced in a horse by Zinsser and Castaneda (1933).

### 2. *Cross-immunity Experiments in Guinea-pigs.*

The method of conducting the cross-immunity tests was that used by most workers in the typhus field. Guinea-pigs, three to six weeks after recovery from one typhus strain, received, intraperitoneally, another strain (brain or egg-membrane) and were then observed for a further two or three weeks. We wish to stress the importance of using more than one or two animals and the necessity for many tests in work of this kind. The result of six tests on two animals per test is, in our opinion, of much greater significance than the result of one test on twelve animals. A virus, slightly " weaker " than usual, may fail to produce sufficiently good reactions to justify the separation of two strains immunologically, but it is unlikely that a " weak " virus will be used on three to six occasions. Further, it is essential that an adequate number of controls be inoculated at each test and that each react typically. As a rule, we tested three or four immunized and two or three normal guinea-pigs at one time. We have not included the controls in the tables because no experiment is recorded unless they reacted satisfactorily. Our interpretation of the results was as follows. If the temperature and scrotal reaction of the guinea-pig under test were the same or nearly the same as those of the controls, the animal was taken to be non-immune to the test-virus; if a slight rise in temperature (no scrotal swelling) took place and the rise was not maintained for more than 24 or 36 hours, the result was recorded as doubtful, and if no reaction of any kind occurred the guinea-pig was considered to be immune.

### 3. *Cross-immunity Experiments in Sheep.*

A comparatively small number of tests was carried out in sheep, but, as will be seen from the results given in Table 8, they confirmed those obtained in the guinea-pig. Virulent guinea-pig brain inoculated intravenously was the inoculum both at the time of the infection and test. A period of from three to six weeks after the return of the temperature to normal was allowed to elapse before

the immunity test was carried out. The result was judged on the temperature reaction only; by the method of test, scrotal swellings did not occur and no " doubtful " thermal rises were recorded.

TABLE 7.

*Summary of Cross-immunity tests in Guinea-pigs.*

| Tested with. | Immune to. | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Rat Typhus. | | | Hare. | | | Robertson. | | | Appleton. | | | Fièvre boutonneuse. | | |
| | R. | ? | NR. | R. | ? | NR. | R. | ? | NR. | R. | ? | NR. | R. | ? | NR. |
| Rat typhus..... | 0 | 0 | 10 | 10 | 1 | 0 | 10 | 0 | 0 | 16 | 0 | 2 | 16 | 2 | 2 |
| Hare..... | 5 | 3 | 12 | 0 | 0 | 12 | 0 | 0 | 10 | 0 | 0 | 8 | 0 | 0 | 12 |
| Robertson..... | 3 | 0 | 13 | 0 | 1 | 9 | 0 | 0 | 10 | N.D. | | | 1 | 0 | 26 |
| Appleton..... | 6 | 5 | 12 | 0 | 1 | 3 | N.D. | | | 0 | 0 | 9 | N.D. | | |
| Fièvre boutonneuse | 1 | 1 | 10 | 0 | 0 | 10 | 3 | 0 | 20 | N.D. | | | 1 | 0 | 22 |

R = reaction; ? = doubtful reaction; NR. = no reaction; N.D. = not done.

TABLE 8.

*Cross-immunity tests in Sheep.*

| Tested with. | Immune to. | | | | | |
|---|---|---|---|---|---|---|
| | Hare. | | Appleton. | | Rat Typhus. | |
| | R. | NR. | R. | NR. | R. | NR. |
| Hare..... | N.D. | | 0 | 2 | N.D. | |
| Appleton..... | 0 | 2 | N.D. | | N.D. | |
| Rat Typhus..... | N.D. | | N.D. | | 0 | 2 |

R = reaction; NR. = no reaction; N.D. = not done.

The results summarized in tables 7 and 8 permit the immunological grouping of the diseases in the following manner:—

*Group* 1: *Rat Typhus.*

(*a*) Immunizes solidly against itself.

(*b*) Immunizes to a great extent against " Hare ", " Robertson ", " Appleton " and *fièvre boutonneuse.*

*Group 2: "Hare", "Robertson", "Appleton" and fièvre boutonneuse.*

(*a*) "Hare" and "Appleton" cross-immunize.

(*b*) "Hare", "Robertson" and *fièvre boutonneuse* cross-immunize.

(*c*) None of the four strains immunizes against rat typhus.

SUMMARY.

1. The effect of the rickettsias of rat typhus, tick-bite fever, *fièvre boutonneuse*, and of two other tick-bite-fever-like diseases ("Hare" and "Appleton") on the guinea-pig, rat, mouse, rabbit, dog, sheep, and ox is recorded. Rat typhus could be maintained in the guinea-pig and rat but not in the sheep, dog or mouse. "Hare", alone of the other four strains, could be easily passaged in guinea-pigs. Only by the use of cultures from the chorio-allantoic membrane of the developing chick could consistently readable reactions be got in guinea-pigs with the tick-bite fever and *fièvre boutonneuse* strains.

2. A Weil-Felix reaction was obtained with the sera of rabbits inoculated with rat typhus, "Hare" and tick-bite fever. With rat typhus, OX19 was agglutinated by the sera of ten infected animals, OX2 by none and OXK by one serum only; with "Hare" one of nine sera agglutinated OX2, doubtfully OX19 and not OXK; one other serum agglutinated OX19 only. The serum of one of seven tick-bite-fever rabbits agglutinated, in a doubtful fashion, OX19 only. The serum of rats, infected with rat typhus, agglutinated OX19 only and that of guinea-pigs, infected with rat typhus and carrying a banal proteus in their intestine did not agglutinate any one of the three proteus OX strains.

3. Details are given of the duration of infectivity of the brain, blood, and tunica vaginalis of guinea-pigs infected with rat typhus and "Hare". The brain of rat-typhus-infected guinea-pigs was virulent after 22 days, the blood after 21 days, and the tunica after 12 days. The brain of "Hare"-infected guinea-pigs was virulent after 15 days and the blood after 13 days.

4. One two-thousandth but not 1/4000 of the brain of a rat-typhus-infected guinea-pig was virulent. Much of the virus could be deposited from brain by centrifugation at 4,000 r.p.m. for half-an-hour, and all but a trace was removed at 14,000 r.p.m. for half-an-hour.

5. Attempts to neutralize the various rickettsias *in vitro* gave unsatisfactory results.

6. Cross-immunity experiments in guinea-pigs permitted the grouping of the 5 typhus strains in the following manner:—

(*a*) *Rat-typhus.*—Immunized against itself and to a great extent against the other four typhus-like diseases.

(*b*) *"Hare", tick-bite fever, "Appleton" and fièvre boutonneuse.*—Gave almost complete reciprocal cross-immunity, but did not immunize against rat-typhus.



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