THE BRITISH JOURNAL OF
EXPERIMENTAL PATHOLOGY

VOL. XVII FEBRUARY, 1936 No. 1

THE TYPHUS GROUP OF DISEASES IN MALAYA.—PART I: THE STUDY OF THE VIRUS OF RURAL TYPHUS IN LABORATORY ANIMALS. PART II: THE STUDY OF THE VIRUS OF TSUTSUGAMUSHI DISEASE IN LABORATORY ANIMALS.

R. LEWTHWAITE AND S. R. SAVOOR.

From the Division of Pathology, Institute for Medical Research, Kuala Lumpur, F.M.S.

Received for publication November 8th, 1935.

GENERAL INTRODUCTION.

The typhus-like diseases of Malaya are members of that large and ever-enlarging group of typhus-like fevers, one or more of which may exist within the confines of one country, and even of circumscribed areas of comparatively small extent. Thus, in the Federated Malay States, within a radius of twenty miles from Kuala Lumpur, three members of the group have frequently been encountered, namely, the rural type of tropical typhus, the urban type of tropical typhus and the tsutsugamushi disease.

In recent years successive discoveries of new endemic foci in the Old and New Worlds and in the Far East have added to the complexity of the typhus group, and given an impetus to research in this field, especially to that directed towards the elucidation of the degree of relationship between the various entities of the group.

During the past four year the authors have established in laboratory animals strains of the three typhus diseases of Malaya from human sources, have studied their characteristics, and the relationship of the three viruses with one another and with that of Rocky Mountain spotted fever; strains of typhus have been isolated from wild rats; the problem of transmission in nature has been investigated and, in part, elucidated. Preliminary reports have been published in successive annual reports from this Institute (1931, 1932, 1933 and 1934), and a résumé of one phase of the work read at the Ninth Congress, Far Eastern Association of Tropical Medicine (1934).

It is proposed to present the report of the above investigations as a consecutive series of papers in this Journal.

PART I: THE STUDY OF THE VIRUS OF THE RURAL TYPHUS* IN LABORATORY ANIMALS.

Rural typhus is an acute infectious disease that is characterized by onset of fever and severe headache; by a rash that appears about the fifth day; by nervous manifestations; and by termination by crisis or lysis about the end of the second week. Clinically it closely resembles typhus exanthematicus. In epidemiology, however, it differs entirely, for it does not flare up in devastating epidemics, nor is it louse-borne. To mark this similarity and contrast with the typhus of the western world, Fletcher and Lesslar (1925), who first recognized the disease to exist in Malaya in 1924, designated it as "tropical" typhus. In the following year these workers (1926) recognized a second type of tropical typhus to exist in Malaya, differing from the first type in serology and epidemiology. Insomuch as the incidence of the first type was exclusively amongst those who lived, worked or sought recreation in the countryside, while that of the second type was predominantly a disease of the house and the town, they called the first type the "rural" form of tropical typhus, the latter the "urban" form of tropical typhus.

The description of the clinical features of rural typhus, and successive reports of investigations into its serology, ætiology and epidemiology, will be found in previous publications from this Institute (see References, p. 20).

EXPERIMENTAL RURAL TYPHUS IN GUINEA-PIGS.

Previous Investigations.

The earliest attempts to infect guinea-pigs were those of Fletcher and Lesslar (1925). The disease being at that time but recently recognized, and the cases few and sporadic, opportunity for laboratory investigation occurred only late in the course of the disease, not earlier than the 12th day of fever. Of 6 cases in which guinea-pig inoculation was made, 4 gave a negative result in the first generation; in the other 2 a febrile reaction could not be reproduced beyond the second generation of guinea-pigs, and Fletcher and Lesslar regarded their results as inconclusive.

One of us (R. L.) has reported (1930) a larger series, in which 62 guinea-pig were inoculated, usually intra-peritoneally, with blood drawn from 43 patients at an earlier stage of the disease (4th–8th day); of these animals 3 only gave a febrile reaction. Guinea-pigs were inoculated also with brain, spleen and kidney emulsions from fatal human cases; none reacted. Attempts to propagate strains from the 3 reacting guinea-pigs failed at the 5th generation.

Anigstein (1933) reported the inoculation of 200 guinea-pigs with infected human blood or brain, and a further 240 with virus from passage animals and other sources; no more than 11 p.c. gave a febrile reaction, and in one instance only could a strain be maintained as far as the 12th generation. Attempts

* The "rural" form of tropical typhus is also variously referred to in the literature as "scrub-typhus" and as "K-typhus"; similarly, the "urban" form of tropical typhus is frequently referred to as "shop-typhus" and as "W-typhus". The first of the three designations will be adhered to in each case in this series of papers, and for brevity will be written as "rural typhus" and "urban typhus" respectively.

by him to infect guinea-pigs selected from a group known to be susceptible to typhus exanthematicus proved equally unsuccessful.

The Isolation and Maintenance of a Strain of Rural Typhus in Guinea-pigs.

At the end of 1931 the authors, after failure to infect guinea-pigs by intra-testicular inoculation and successive passage by this route, renewed the attempt to infect the guinea-pig with virus of human origin by using only vitamin-deficient guinea-pigs. For some days before and after inoculation they were fed on a diet consisting of water, autoclaved rolled oats, and skimmed milk, this being the diet used successfully by Zinsser, Castaneda and Seastone (1931) to increase the virulence of infection with the endemic typhus of the United States of America—based on the historic association of famine with enhancement of virulence in epidemics of typhus exanthematicus.

Numerous attempts were made by us; all failed but one. In the one exception 2 guinea-pigs that had been fed on a vitamin-deficient diet for 10 days were inoculated intra-peritoneally with blood from a patient (Seerangayee), who showed the clinical syndrome of rural typhus, and who came from an estate on which this disease has been endemic for many years. The Weil-Felix reaction proved subsequently to be positive to high titres. Both guinea-pigs reacted with fever, and from these a strain has been maintained by continuous passage, and is now in its 97th generation in the guinea-pig. The characteristics of this "Seerangayee" strain of rural typhus are as follows:

The incubation period following intra-peritoneal injection of virus varies between 5 and 18 days; in the majority its limits are 7 to 12 days. Fever follows, rising during 3 days from a normal morning temperature of 102° F., to reach a maximum of 104° F.–105° F. In those guinea-pigs that survive defervescence begins about the 5th to the 6th day, and is completed usually about the 9th day. Many variations of temperature curve are seen; the fever may range between 103° F. and 104° F. only; it may be as short as 7 days or as long as 15 days (Charts I and II). No swelling of the scrotum has been observed in any of the 300 or more male guinea-pigs that have been infected with this virus. The mortality amongst those guinea-pigs inoculated by the intra-peritoneal route is fully 90 p.c., death occurring on the 4th to 5th day, and being preceded by a sudden fall of the temperature to subnormal limits. Those that survive show extreme weakness.

The two cardinal post-mortem signs are enlargement of the spleen and the presence of ascites. The splenic enlargement is always striking after the second day; the spleen is increased in size three to four times, and its surface coarsened in most cases, in contrast to the smoothness of the surface of the spleen in guinea-pigs infected with urban typhus. The ascites develops rapidly from the 3rd day of fever onwards; the fluid is slightly viscid and fibrinous. In an animal that has been infected by the intra-peritoneal route it is invariably present, and usually abundant; if the route has been subcutaneous it is usually absent or scanty. If the guinea-pig dies or is killed late in the fever, a layer of fibrin is commonly present on the surface of the spleen, and appears to be a deposit from the ascitic fluid. *Rickettsia* are always present in the

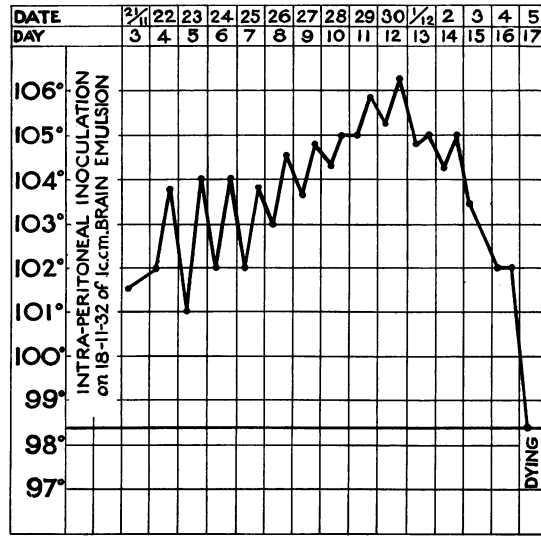


CHART I: RURAL TYPHUS.—Guinea-pig 1076. A passage animal of the "Seerangayee" strain of rural typhus. Inoculated by the intra-peritoneal route.

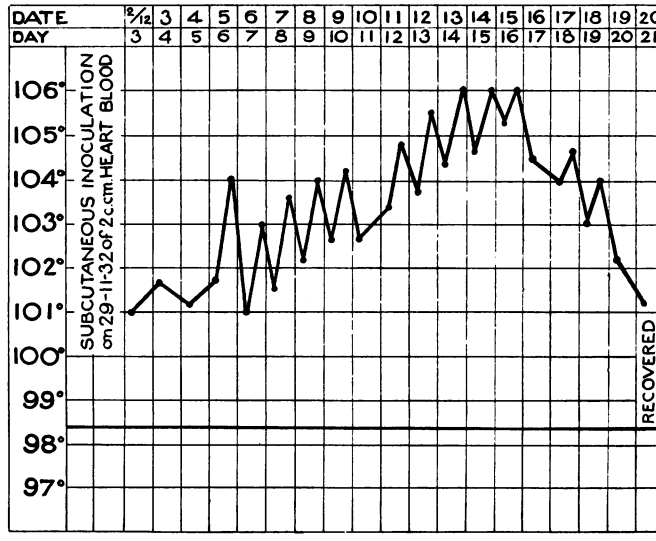


CHART II: RURAL TYPHUS.—Guinea-pig 1095. A passage animal of the "Seerangayee" strain of rural typhus. Inoculated by the subcutaneous route. As compared with the infection in guinea-pig 1076, note the longer incubation period, the more gradual evolution of fever, and recovery of the animal, three features that are characteristic of infection by the subcutaneous route.

ascitic fluid in numbers that increase with each succeeding day of fever ; in the fibrinous deposit on the spleen they are copious. Less frequent post-mortem signs are injection of the suprarenal glands, enlargement and injection of the glands of the groin and axilla, and punctiform subpleural hæmorrhages. " Typhus nodes " are to be found on histological examination of the brain, but are not a conspicuous feature. Perivascular collections of mononuclear cells are, however, a prominent feature in the mesentery, being readily demonstrable in fragments of this tissue that have been spread on to slides and stained by Giemsa's method.

The severity of the experimental infection is diminished considerably by employing the subcutaneous route of inoculation ; not only is the ascites less, or absent, but survival is the rule. The subcutaneous route of inoculation serves also to minimize troublesome secondary infection. As inoculum, heart-blood, ascitic fluid, and emulsions of brain, spleen or suprarenals are effective. Failure of a guinea-pig to react is extremely rare when brain-virus is injected by the intra-peritoneal route ; heart-blood is less efficacious than brain, the subcutaneous route less certain to secure infection than is the intra-peritoneal route.

The brain retains the virus for some few days after defervescence ; no special investigation to determine the number of days has been made, the point having merely been noted following use of the brain of a convalescent guinea-pig as an inoculum in an emergency. A series of about 30 infected brains, some in pure glycerine, others in 50 p.c. glycerine, others dry, were frozen in ice, or kept at a temperature of 0°–5° C. for periods of time varying from 2 to 30 days, with a view to determining the length of time during which the virus might remain virulent at these temperatures. Whereas control inoculations of the fresh brain were all positive, inoculation of the preserved brains showed that the virulence for the guinea-pig had been entirely lost. Bruynoghe and Jadin (1933) report much the same degree of failure in the case of material from guinea-pigs infected with a strain of " typhus murin ", being unable to preserve the virulence of brain-virus with certainty at 4° C. for more than two days. The method of preservation recommended by Laigret and Durand (1933), namely, to freeze material to a temperature of —10° to —12° C., has not been tried.

After convalescence guinea-pigs are immune for a period of at least 15 months.

Although, as has been stated above, failure of a passage animal to react is now very rare, this was far from being the case at the outset. During the first 9 or 10 generations the maintenance of the strain was always precarious—much more so than was the case in the initiation of a strain of urban typhus (as will be reported later). After that period, however, infection became more and more constant, and eventually the use of vitamin-deficient animals could be discontinued.

It was noted during periods of apparent diminution of virulence that resort to the inoculation of mixed emulsions of brains from 2 or more feebly-reacting guinea-pigs restored the febrile reaction to its usual intensity.

During the maintenance of this and other typhus strains in guinea-pigs, intercurrent infections have been a constant source of anxiety, especially

when the guinea-pigs were fed on a diet deficient in vitamin A. Three contaminants that have been encountered, *B. enteritidis* Gaertner, *Spirillum morsus muris* and a *Toxoplasma*, simulate typhus in the guinea-pig both in febrile reaction and post-mortem appearances, and may supplant the typhus virus. *B. paratyphosus B* and *B. whitmori* have also been isolated, the latter giving a scrotal reaction similar to that met with in some forms of experimental typhus in the guinea-pig. It is proposed to amplify our experiences of these contaminating infections later in this series of papers.

The Demonstration of Rickettsia.

Anigstein (1933) has reported the finding of *Rickettsia* in smears from the tunica vaginalis of a guinea-pig that reacted to inoculation with the virus of rural typhus.

In the passage animals of the " Seerangayee " strain *Rickettsia* are constantly to be found in the shed endothelial cells that lie in the ascitic fluid, increasing in number as the infection progresses. They are most abundant in the fibrin on the splenic surface (Figs. 1, 5 and 6); cultures of this fibrin on ordinary laboratory media are sterile in the large majority of cases. In morphology, staining reactions and distribution these *Rickettsia* do not differ in any way from those demonstrated by the authors (1934) in the endothelial cells lining Descemet's membrane of rabbits infected by the intra-ocular route (also *vide infra*). Stained by Giemsa's method the organisms appear as short rod-shaped bipolar-staining bacilli. With optimum differentiation they are seen to consist of two polar granules that are stained purple and are connected by a pale blue rod. Their most characteristic grouping is that of one or more clusters that lie in greatest concentration close to the nucleus; but occasionally they lie scattered beyond the ruptured cell margin, as though prolific multiplication within the cytoplasm has caused rupture of the cell. Average limits of dimensions may be stated as 0·8–2·0μ in length, and 0·3–0·5μ in width. Some variation in length and width is seen, but the essential structure is constant. They appear to be identical with the *Rickettsia orientalis* described by Nagayo and his collaborators (1931), and regarded by them as the causal organism of the tsutsugamushi disease, specimens of which were very kindly furnished to the authors for comparative study by Prof. Nagayo. They appear also to be identical with the diplococcal forms described by Anigstein (1933). [To facilitate appreciation of the dimensions of the *Rickettsia*, a photomicrograph of a smear of pneumococci in the blood of a mouse, taken at a magnification of 1700, is included (Fig. 10).]

EXPERIMENTAL RURAL TYPHUS IN RABBITS.

Previous Investigations.

It has previously been shown by Anigstein (1933) and by one of us (Lewthwaite, 1930) that the intra-peritoneal inoculation of the virus of rural typhus into rabbits provoked in the sera agglutinins (" O " type) to the " K " strain of *Proteus X.19*; agglutination has, however, been low in titre and fitful in

incidence. Thus Anigstein reported maximum titres of 1 : 125 to 1 : 250, and drew attention to the vagaries of the development of agglutinins, especially to the influence of the idiosyncrasies of individual rabbits in this respect, and to the influence of the origin and quantity of the inoculated virus, as stressed by Felix (1933). In the cases previously reported by one of us, 11 rabbits were inoculated intra-peritoneally with blood drawn from patients at the height of fever ; in 8 the Weil-Felix reaction was negative ; in the other 3 the titres were positive, but did not exceed 1 : 125. No fever occurred. Later, an attempt was made to initiate strains in rabbits by intra-testicular inoculation of human blood drawn early in the disease. No fever, orchitis or positive Weil-Felix reaction developed.

It was clear, then, that the rabbit was far from being an ideal animal in which to study the characteristics of the virus of rural typhus.

The work of Nagayo and his collaborators (1931), however, on the infection of the rabbit with the virus of the kindred tsutsugamushi disease by the intra-ocular route offered the prospect of much more promising results, insomuch as inoculation had resulted in the evolution of an intense iridocyclitis, easily observed in all its stages, that ultimately disappeared to leave the eye normal, the animal healthy and immune. Moreover, the causal organism of the tsutsugamushi disease was found in abundance in material from the eye. If it were found that the virus of rural typhus evoked a similar reaction, then a new criterion of infection would be available, and one that would enable cross-immunity experiments to be made with ease.

The Isolation and Maintenance of Strains of Rural Typhus in Rabbits by the Intra-ocular Inoculation of the Virus.

The method used was that practised by Nagayo and his collaborators, viz. the inoculation of virus into the anterior chamber of the eye of the rabbit. The virus used for initiation of the strain was defibrinated blood, drawn from the patient as early as possible in the course of the disease.

From 12 patients seen early in the disease it has been possible to initiate 4 strains ; a 5th strain has been initiated from the above-mentioned " Seerangayee " guinea-pig strain of rural typhus by inoculation of blood from the heart of a reacting guinea-pig. Maintenance of these 5 strains by intra-ocular inoculation of infected aqueous humour afforded no difficulty. They were carried on for 45, 29, 17, 7 and 4 generations respectively, *i. e.* sufficiently long to permit cross-immunity and other experiments to be made ; they were then discontinued because of the excessive drain on the available supply of rabbits. Our experience has been that in attempting to isolate a strain, at least 2, preferably 3 or 4, rabbits should be used, for success with a single rabbit is uncertain. Similarly, to secure reaction in the second and third generations, it may be necessary to pass the aqueous humour from a feebly reacting rabbit of the previous generation on more than one occasion into more than one rabbit, according as the intensity of the ocular signs dictates. Once a strain has survived the first three passages, failure of a rabbit to react to inoculation of passage virus is extremely rare—less than 1 p.c.

Technique.—The details of technique given below do not differ in essentials from those set forth by Nagayo and his co-workers, on which they have been modelled. The rabbit is anæsthetized by ether, an eye speculum inserted to retract the eyelids, and the eye washed successively with solutions of 1 p.c. phenol and boric acid. The fine needle of a 1 c.c. syringe is inserted into the anterior chamber, as great a part of its course as possible being directed intracorneally in order to minimize efflux of the inoculated fluid when the needle is ultimately withdrawn. To steady the eyeball counter-pressure is applied by a pair of forceps from the opposite side. Approximately 0·1 c.c of the normal aqueous humour is withdrawn, the barrel of the syringe is then detached from the needle, is replaced by the barrel of another syringe containing 0·1 c.c. of blood-virus or infected aqueous humour ; this fluid is inoculated and the syringe with its needle withdrawn. Infected aqueous humour, when required as an inoculum, is obtained from a reacting rabbit by a similar technique. One eye only is inoculated ; the other serves as a control.

The ocular reaction.—The evolution of the reaction, which is an acute iridocyclitis, is as follows :

(*a*) An incubation period of 4–15 days, being fairly constant at 4–7 days after the first few generations. Within 24 hours of the inoculation of infected blood, during the incubation period of the true reaction there occurs a non-specific reaction—a degree of circumcorneal injection and injection of the iris—which subsides in 3 to 4 days. Where the inoculum is passage virus, *i. e.* infected aqueous humour, this immediate reaction is very slight or even absent, and does not exceed 48 hours in duration.

(*b*) The appearance of circumcorneal injection, and, one day (or two) later, of iris injection and turbidity of the aqueous humour ; these signs progress for 6 or 7 days to a marked degree of intensity, and then the appearance of pannus marks the beginning of retrogression ; the turbidity of the aqueous humour is the last sign to disappear. The duration of active signs is 12–16 days usually, though occasionally longer.

In reacting rabbits the aqueous humour is infective from the first appearance of ocular signs until at least 2 days after pannus has become evident. The maximum infectivity is present from the 4th to 6th day of iris injection and turbidity of the aqueous humour. The evolution of signs is constant, and has been identical in all the 5 strains of rural typhus. It corresponds in all respects to that described by Nagayo and his collaborators in the case of the tsutsugamushi disease.

The infected rabbits show no sign of generalized ill-effects, and all recover. Febrile reaction is fitful or absent entirely. The infected aqueous humour is almost invariably sterile on ordinary laboratory media ; in the very rare instances in which secondary infection has appeared, it has disappeared in the passage animals of the next one or two generations. After convalescence the rabbits are immune to reinfection for a period of at least 12 months. (A detailed report of a large series of cross-immunity experiments will appear in a later paper.)

Demonstration of the virus.—The procedure of Nagayo was again followed. From time to time infected rabbits were killed at the fastigium of the ocular reaction, the cornea of the reacting eye was excised, and scrapings made of the endothelium covering Descemet's membrane. Stained by Giemsa's method these scrapings reveal within the endothelial cells clusters of short rod-shaped bipolar-staining bacilli, that in morphology, staining characteristics and distribution cannot be distinguished from the *Rickettsia* noted above as being present in infected material from the " Seerangayee " guinea-pig

strain of rural typhus, from those present in reacting eyes of rabbits infected in this laboratory with strains of the tsutsugamushi disease (*vide infra*), and from the *Rickettsia orientalis* of Nagayo (Figs. 2, 3 and 7). Whereas in some scrapings many cells containing *Rickettsia* are found in every microscopic field, in others long search may be required before they are found ; but they are invariably to be found.

The Weil-Felix Reaction in Rural Typhus.

It is proposed to devote a separate report in this series to the Weil-Felix reaction in rural typhus, urban typhus and the tsutsugamushi disease, and detailed discussion is therefore deferred. The findings in rabbits inoculated with human and passage virus of rural typhus are here given in brief. In all, sera were submitted for the Weil-Felix reaction from 55 inoculated rabbits. Twenty-nine of these had been inoculated by the intra-peritoneal route ; most of them with brain or other tissue virus from the " Seerangayee " strain of rural typhus, in order to confirm, by the result of the Weil-Felix reaction, that the signs of reaction in passage guinea-pigs were still those of typhus, and were not due to one or other of the contaminants that in the guinea-pig may simulate typhus and supplant the typhus virus. Of these 29, in 18 the sera showed significant agglutination, maximum titres lying between limits of 1/50–1/200 in 16 and 1/200–1/300 in 2. The remaining 26 rabbits had been inoculated by the intra-ocular route, and of these, 12 showed significant agglutination, maximum titres lying between 1/50–1/200 in 9, 1/200–1/300 in 1, and 1/300–1/400 in 2. Agglutination was of the " OXK " strain of *Proteus X.19*, never of the " Warsaw " strain (the " OX19 " strain in use in this laboratory).

The presence of significant agglutination was judged not merely by the titres attained, but also by the form of the curves of these agglutination titres, which must show the waxing and waning features to which Fletcher and Lesslar (1926) draw attention as constituting conclusive proof of active infection with the virus of tropical typhus.

Infection of Rabbits by the Intradermal Inoculation of Virus.

As part of a series of experiments designed to elucidate the non-occurrence of an initial ulcer in rural typhus in man, and its occurrence in the tsutsugamushi disease in man, rabbits and monkeys were inoculated intradermally with virus from strains of rural typhus. Monkeys proved to be the more susceptible, so that detail of the method is deferred until the reaction in monkeys is described. The following is a summary of the results obtained in rabbits : Six rabbits were inoculated on separate occasions with the virus of rural typhus, 5 of them with infected peritoneal fluid from a reacting guinea-pig, 1 with material from an infected eye of a reacting rabbit. Macules, rapidly progressing to papules, developed at the sites of inoculation in all 6 ; in 4 central necrosis ensued, to give the characteristic appearance of the primary eschar

of the tsutsugamushi disease in man (*vide* Fig. 11). Control reactions carried out with saline and with the aqueous humour of normal rabbits yielded no trace of skin reaction.

The Weil-Felix reactions showed a slight rise of agglutination titres of the " OXK " strain in sera from 2 of the 6 rabbits, but not to a conclusive degree.

EXPERIMENTAL RURAL TYPHUS IN THE WHITE RAT.

During the past seven years numerous attempts have been made in this laboratory to infect wild (brown) rats with the virus of rural typhus, attempts prompted by the discouraging outcome of attempted infection of guinea-pigs. The results have proved unsatisfactory, the incidence of fever being fitful, and, when obtained, of no more than 2 or 3 days' duration. Moreover, recent work by Anigstein (1933) has shown that in a considerable proportion of wild rats trapped in the countryside, the serum yields a positive Weil-Felix reaction ; while the authors (1931) have isolated in guinea-pigs two strains of tropical typhus from wild rats trapped in areas in which cases of human tropical typhus had occurred. Use of wild rats for isolation or maintenance of strains of rural typhus is therefore fraught with difficulties.

Nicolle (1933) recently drew attention to a distinction between the virus of Old World typhus (*le typhus historique*) and the virus of rat typhus (*le typhus murin*) that is founded on their differing behaviour to attempted propagation in the white rat ; whereas the former could not be maintained by him beyond the 13th generation in three separate series of white rats, the latter can be maintained experimentally in the white rat indefinitely.

An attempt was made to classify the " Seerangayee " strain of rural typhus by this experimental criterion. Hitherto white rats had not been used in experimental typhus work in this laboratory, since they had proved difficult to rear in the damp heat of this country. But a batch obtained in 1933 from India throve sufficiently to promise an adequate supply for the experiment, and two parallel series of strains were initiated in white rats by intra-peritoneal inoculation of brain virus from a reacting passage guinea-pig of the rural typhus strain. As in the brown rat, the incidence of febrile reaction proved fitful, and the duration of any such reaction was 2 to 3 days only. No scrotal swelling occurred. The optimum time for passage in each individual case was determined by the febrile reaction of a control guinea-pig.

At the 7th generation the white rat of one series died overnight ; and, on account of considerable decomposition, it was deemed advisable to continue the two parallel series by inoculation of virus from the rat of the remaining series into two fresh rats. Both series were maintained successfully until the 11th generation, when the control guinea-pig of one series was febrile for one day only. Lest this should have been a rare example of insusceptibility of an individual guinea-pig, passage of virus from the corresponding rat was continued. Again, the control guinea-pig showed only a short febrile reaction. In two further passages the control guinea-pig entirely failed to react, so that, in this series, the maintenance of the virus failed at the 13th generation. The other

of the two parallel series, however, was maintained successfully, with no evidence of any abatement in virulence, for 21 generations ; at this point the investigation was arbitrarily terminated for reasons of economy.

Rabbits that were inoculated intra-peritoneally with virus from white rats or control guinea-pigs at various stages during the maintenance of this strain in the white rat have, in many instances, yielded significant agglutination of the " OXK " strains of *Proteus X.19*. Further, smears from the tunica vaginalis of male white rats have revealed, usually after long search, characteristic examples of intra-cellular *Rickettsia* indistinguishable from those found in material from infected guinea-pigs of the rural typhus strain. At post-mortem examination of infected rats the only constant finding was a marked enlargement of the spleen ; but frequently ascites and a fibrinous deposit on the surface of the spleen were noted, smears of the latter yielding *Rickettsia*. Of those rats not killed to provide emulsions for passage, the majority died late in the second week or early in the third week.

It having proved possible to maintain a strain of rural typhus in white rats for 21 generations with unabated virulence, there would seem to be no reason to doubt that propagation of the strain in the rat could have proceeded indefinitely ; and that, therefore, by Nicolle's criterion, rural typhus is a murine strain. In another investigation, to be described in a later contribution in this series, the authors have isolated from wild (brown) rats two strains of rural typhus ; serologically they did not differ from strains isolated from human cases ; cross-immunity experiments were carried out with one of them, and showed this rat strain and the human strains to be immunologically identical. This evidence points strongly to the rat as being the reservoir of rural typhus, as maintained by Fletcher and his co-workers, and by Anigstein. The above successful propagation in white rats of the laboratory strain of rural typhus not only affords additional evidence as to the rodent reservoir, but also accords with the conclusion of Nicolle that typhus virus of rat origin can be maintained indefinitely in rats, in the laboratory as in nature.

A further point of interest emerging from the propagation of this rural typhus strain in white rats was the failure to evoke a scrotal swelling in any of those 32 control guinea-pigs that were males. Pinkerton (1931) noted a periodicity in the occurrence of scrotal swelling in guinea-pigs in the case of the " Wolbach " strain of Old World typhus. Further, he found that he could cause re-appearance of the scrotal swelling during a period of its non-occurrence by interpolating the inoculation of a white rat in his series of passages, and continuing the strain in guinea-pigs by intra-peritoneal inoculation of spleen and testicular emulsions from this rat.

It was hoped to find a similar phenomenon in the case of the rural typhus strain, which, unlike the urban strain to be described later, has never yet afforded an example of scrotal swelling ; and so not only to gain this additional valuable diagnostic sign of infection with the virus, but also to furnish a rich supply of *Rickettsia* that might serve as a basis for attempted production of a prophylactic vaccine against rural typhus. But although inoculation of guinea-pigs with emulsions of spleen and/or testicular washings of the rats constantly provoked the febrile reaction, in none of the 32 males was any degree of scrotal reaction evident.

EXPERIMENTAL RURAL TYPHUS IN MONKEYS.

The authors (1934) have summarized the many common features shared by rural typhus and the tsutsugamushi disease ; in particular, their lethality, epidemiology, serology, behaviour of the respective viruses in the guinea-pig and the rabbit, and the apparent identity of the respective *Rickettsia*. The clinical syndrome of the two infections in man is closely similar ; but, as noted earlier, there exists one outstanding distinction, clear-cut in all but one of a large number of cases seen by the authors, that serves as a differential criterion, namely, the presence in the tsutsugamushi disease and the absence in rural typhus of a small round ulcer, characteristic in its black necrotic centre and surrounding red areola, and an attendant painful bubo of the nearest group of lymphatic glands.

To furnish experimental data regarding this distinction, intradermal inoculation of monkeys and rabbits with the respective viruses was practised. Comparison of the results obtained is deferred to a later paper ; it is proposed here to report only the features of experimental rural typhus in monkeys.

One gibbon and 3 macacus monkeys were inoculated on different occasions. To provide inoculum for the gibbon and 1 macacus monkey, a rabbit strain of rural typhus was established by the intra-ocular inoculation of infected heart-blood from the " Seerangayee " guinea-pig strain ; and aqueous humour and scrapings of the endothelium lining Descemet's membrane were secured at the height of the ocular reaction from passage rabbits. As inoculum for the two remaining macacus monkeys, peritoneal fluid and fibrin from the surface of the spleen were obtained from a reacting guinea-pig of the " Seerangayee " rural typhus strain. Seven to 9 intradermal injections, spaced along either side of the mid-line of the abdomen, were made in each case. Cultures of the various inocula were sterile on ordinary laboratory media in every case.

In all 4 animals active infection followed. Following an incubation period of 5 days in 3 of the animals and 10 days in the other, a febrile reaction occurred that persisted for 9 days in the case of the gibbon and for 4, 14 and 2 days respectively in the case of the 3 macacus monkeys (*vide* Charts III and IV).

At every site of inoculation in all 4 monkeys lesions developed that passed through a macular to a papular stage. In each monkey some of the papules progressed further to end as small circumscribed ulcers, with the black necrotic centre and surrounding hyperæmic areola that are typical of the initial lesion of the tsutsugamushi disease in man, this final necrotic stage being secured with more certainty by inoculation of teased endothelium covering Descemet's membrane rather than aqueous humour alone ; by inoculation of fibrin rather than peritoneal fluid alone (Figs. 12 and 13). The macular stage, usually only faintly discerned, appeared one day prior to the papular stage, which appeared variously from the 3rd to the 7th day after inoculation. The black necrotic centre, where present, was clearly evident 3 to 7 days after the first appearance of the papule.

Swelling of the lymphatic glands of the groin was seen in 3 of the 4 cases. In the gibbon it was especially prominent as a large, raised, tender bubo, of the size of a walnut, that appeared on the 8th day, reached its maximum on the 10th day, to recede on the 11th day (Fig. 12).

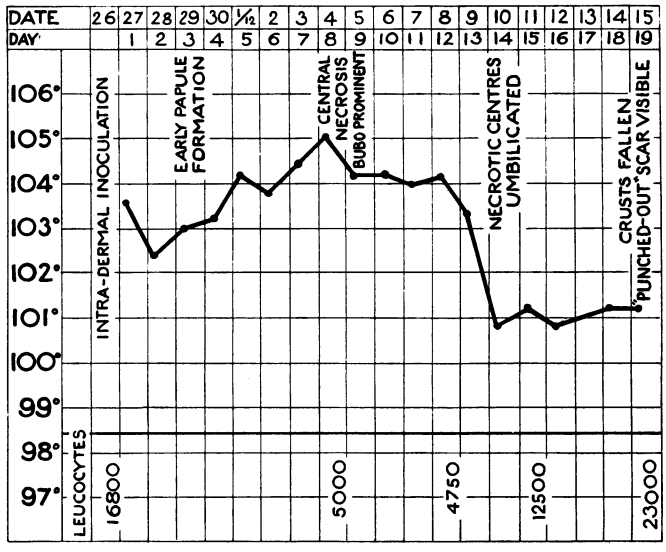


CHART III: RURAL TYPHUS.—Gibbon No. 2. Inoculated intradermally on November 26th, 1934, with infected material from the eye of a passage rabbit of a strain of rural typhus. For dermal lesions, see Fig. 12.

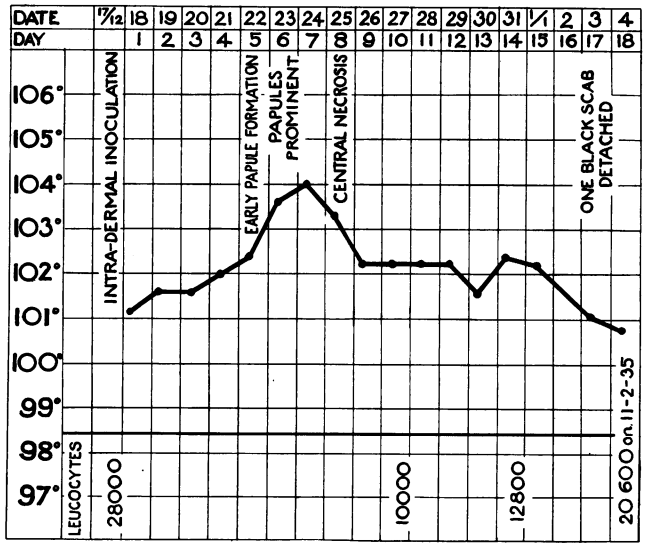


CHART IV: RURAL TYPHUS.—Monkey No. 4 (a macacus.) Inoculated intradermally on December 17th, 1934; source of inoculum, and strain, as for gibbon No. 2. For dermal lesions, see Fig. 13.

Leucopenia, characteristic in rural typhus and the tsutsugamushi disease in both man and monkey, was a well-defined feature in all 4 of these monkeys infected with rural typhus, as the following table indicates:

	(0).	(8).	(12).	(15).	(19).
Gibbon	16,800	5000	4750	12,500	23,000
	(0).	(10).	(15).	(56).	
First macacus	28,000	10,000	12,800	20,500	
	(0).	(10).	(19).	(24).	
Second macacus	22,500	16,400	15,000	20,000	
	(0).	(9).	(16).		
Third macacus	12,500	8750	17,000		

(Figures in brackets = number of days after inoculation.)

The three macacus monkeys suffered only a mild illness, and survived the infection. The gibbon was more seriously affected; during the febrile reaction it crouched in the corner of its cage, inactive and disinclined to eat. For some days after defervescence it improved, but it died from a cellulitis, that accompanied a flaring-up of an old skin disease, on the 13th day after defervescence and 26th day after inoculation (*vide* Chart III).

In all 4 monkeys the Weil-Felix reaction was carried out with 5 to 10 successive samples of blood, drawn from the day of inoculation onwards until late in convalescence. In all 4 the reaction was positive, agglutination reaching a significant degree, and showing the waxing and waning titres indicative of active infection. In the case of the 3 macacus monkeys the maximum titres were, respectively, 1/141 on the 14th day, 1/125 on the 19th day, and 1/125 on the 28th day after inoculation; agglutination was of the "OXK" strain of *Proteus X.19*, never of the "W" (= "Warsaw") strain (the "OX19" strain in use in this laboratory). In the gibbon, agglutination of the "OXK" strain was also obtained to a maximum titre of 1/170 on the 21st day, but a predominant "O" type agglutination of the "W" strain also occurred in a maximum dilution of 1/1925 on the 15th day. In view of the interest of this dual agglutination, a strain that had been established in rabbits by intra-ocular inoculation of heart-blood from this gibbon was maintained through several generations. The rabbit of the first generation yielded a characteristic ocular reaction, and a positive Weil-Felix reaction of the "W" type only; it proved immune to reinoculation with virus of the parent "Seerangayee" (*i. e.* "K") strain of rural typhus—a result that is in entire contrast to the complete and invariable lack of cross-immunity between the viruses of rural (*i. e.* "K") and urban (*i. e.* "W") typhus, as will be shown later. Converse cross-immunity experiments between parent and daughter strain also showed complete cross-immunity to exist. Subsequent rabbits inoculated with the daughter strain failed to give a Weil-Felix reaction of the "W" type, but gave ocular reactions typical of infection with virus of rural strains, and, in one instance, a positive Weil-Felix reaction of the "K" type. Thus, in this instance, the passage of virus of a rural typhus strain from the guinea-pig, through the rabbit, to the

gibbon, caused a temporary change in its serological properties, but no change in its immunological character. Later, to afford further data, a second gibbon was inoculated intradermally ; this gibbon had three months previously reacted to intradermal inoculation of the virus of the tsutsugamushi disease with febrile reaction, leucopenia, dermal lesions, an agglutination of the " OXK " strain of *Proteus X.19*, to a titre of 1/3850, and absence of any trace of agglutination of the " W " strain. At the time of reinoculation the " K " agglutinins had fallen to zero. Peritoneal fluid from a reacting guinea-pig of the parent " Seerangayee " rural typhus strain was inoculated at multiple sites into this second gibbon, and into a macacus monkey as a control. In the gibbon, leucopenia alone resulted ; no fever or dermal lesions occurred ; there was no rise of agglutinins of the " K " strain ; and no trace of agglutination of the " W " strain, such as would have been expected to develop had the " Seerangayee " rural typhus strain contained any " W "-type virus. The control macacus monkey reacted with fever, dermal necrotic ulcers, leucopenia, and significant agglutination of the " K " strain of *Proteus X.19*, but no trace of agglutination of the " W " strain.

This rare appearance of agglutination of the " W " strain by the sera of animals infected with a virus that almost invariably provokes agglutination of the " K " strains only, with or without concomitant agglutination of the " K " strains of *Proteus X.19*, has been encountered more than once by the authors in the course of experimentation in this laboratory (I.M.R. Annual Report, 1932). A similar unexpected result is recorded by Anigstein (1933). Several human cases of tropical typhus that have shown high agglutination (" O "-type) of both " K " and " W " strains have been noted. Experiments to elucidate this paradox are in progress, and will be reported later in this series of papers, in the discussion on the inter-relationship of the Malayan typhus fevers.

PART II : THE STUDY OF THE VIRUS OF THE TSUTSUGAMUSHI DISEASE IN LABORATORY ANIMALS.

Previous reports from this Institute have dealt fully with the clinical and epidemiological characteristics of the tsutsugamushi disease in man, as encountered in Malaya, and have stressed their close similarity with those of rural typhus. This similarity amounts almost to identity, save for the occurrence of the primary ulcer and bubo in the tsutsugamushi disease. Until, in a later paper in this series, the results of cross-immunity experiments have been reported, it would be premature to discuss differential criteria ; and this present account will be restricted to the behaviour of the virus in the guinea-pig, rabbit and monkey.

ATTEMPTS TO INFECT GUINEA-PIGS WITH THE VIRUS OF THE TSUTSUGAMUSHI DISEASE.

Attempts to infect guinea-pigs by the intra-peritoneal inoculation of infected human blood have been few in number through lack of opportunity, the incidence of the disease as compared with that of rural typhus being infrequent. Fletcher and Field (1927) report three such attempts ; the authors have made

eight, the blood being taken between the 5th and 10th days of fever. In all, continued maintenance of a strain has proved impossible, in so far as febrile reaction is regarded as a criterion of successful infection. Usage of the vitamin-deficient diet, described earlier in connection with rural typhus, has not availed to overcome the marked degree of insusceptibility of the guinea-pig. Occasionally an early halting success, amounting to a precarious maintenance of a strain for a few generations, would be obtained; but invariably failure ensued. The following is a record of one such experience:

From a patient showing the typical clinical syndrome of the disease, and whose serum subsequently developed high agglutination of the "OXK" strain of *Proteus X. 19*, blood was drawn on the 7th day of fever, and inoculated into 2 guinea-pigs by the intra-peritoneal route and into 2 rabbits by the intra-ocular route. Whereas in the rabbits a strain was isolated and maintained without difficulty, in the guinea-pigs success was short-lived. Precariously, and by extravagant use of guinea-pigs, the strain was maintained for 9 generations. In the 9th generation 6 out of 8 animals failed to react; in the 10th generation none reacted. Those guinea-pigs of the earlier generations that reacted with fever after an incubation period of 8 to 13 days showed the characteristic features of the "Seerangayee" strain of rural typhus, viz. absence of scrotal swelling; the presence of subpleural hæmorrhages, ascites, and enlargement of the spleen occasionally; and, less often, a deposit of fibrin on the surface of the spleen, smears of which, when stained by Giemsa's method, revealed abundant *Rickettsia* indistinguishable in morphology, distribution and staining characteristics from *Rickettsia orientalis*.

THE ISOLATION AND MAINTENANCE OF STRAINS OF THE TSUTSUGAMUSHI DISEASE IN RABBITS BY THE INTRA-OCULAR INOCULATION OF VIRUS.

The authors have attempted thus to isolate strains of this disease from 5 patients seen by them before defervescence. Two of the 5 were not seen until the second week of fever, at a period in the evolution of the disease at which the prospect of success was remote; and the attempts failed. The other 3 patients came under observation during the first week of fever, at which period the prospect of success could be regarded as favourable, and from 2 of them strains were isolated.

Blood was drawn from the first patient on the 4th day of his fever, and inoculated into one rabbit by the intra-peritoneal route, and into 4 rabbits by the intra-ocular route. The rabbit inoculated intra-peritoneally gave a positive Weil-Felix reaction, its serum agglutinating the "OXK" strain of *Proteus X.19* only. Of the 4 rabbits inoculated intra-ocularly, 2 failed to develop either ocular signs or agglutinins in the serum to strains of *Proteus X.19*; the third developed agglutinins in the serum to the "OXK" strain, and a mild ocular reaction that could not be reproduced on passage; the fourth developed similar agglutinins, and also the ocular signs described by Nagayo and his collaborators as characteristic of the tsutsugamushi disease in rabbits thus inoculated.

Blood was drawn from the second patient on the 7th day of his illness and inoculated into 2 rabbits by the intra-ocular route. Both developed agglutinins to the "OXK" strain of *Proteus X.19*; a characteristic ocular reaction developed in one, but failed to develop in the other.

By intra-ocular passage of infected aqueous humour, precisely as described

above in the case of strains of rural typhus, these two strains of the tsutsugamushi disease were maintained without difficulty. One strain, called the " Raub " strain, has been maintained as a stock laboratory strain since its isolation in June, 1932, and is now in its 99th generation in the rabbit. The other strain, called the " Kepong " strain, was maintained sufficiently long (9 generations) to allow cross-immunity experiments to be completed, and was then discarded.

The evolution of the ocular reaction in the case of the tsutsugamushi disease is identical in every feature with that described above in the case of strains of rural typhus, and need not be re-described. As also noted above, the infected rabbits survive the infection, showing no generalized ill-effects ; febrile reaction is insignificant or absent. After convalescence, the rabbits are immune to reinfection for a period of at least 12 months.

Again the virus may be demonstrated to be rickettsial in nature precisely as described in the case of rural typhus ; and in abundance, distribution, morphology and staining characteristics the *Rickettsia* cannot be distinguished from the *Rickettsia* of rural typhus and from the *Rickettsia orientalis* of Nagayo (Figs. 4, 8 and 9).

The Weil-Felix reactions are also similar, significant agglutination being of the " OXK " strains, occasionally to a very high titre ; the " W " strains are never agglutinated. The following examples are given here as representative of the maximum degree of agglutination that has been attained :

Tsutsugamushi strains: Rabbit No. 670—1/385 on 34th day.
,, ,, 918—1/440 on 60th day.
,, ,, 1012—1/500 on 10th and 15th day.
,, ,, 1041—1/1925 on 42nd day.

INFECTION OF RABBITS BY THE INTRADERMAL INOCULATION OF THE VIRUS OF THE TSUTSUGAMUSHI DISEASE.

Complementary to corresponding inoculations of the virus of rural typhus (*vide supra*), rabbits and monkeys were inoculated intradermally with the virus of the tsutsugamushi disease.

Three rabbits were inoculated, the inoculum consisting of infected aqueous humour and scrapings of the endothelium lining Descemet's membrane, secured at the height of the ocular reaction from reacting rabbits of the " Raub " strain. One rabbit, inoculated with infected aqueous humour only, failed to react. Each of the other 2 rabbits was inoculated with both aqueous humour and scrapings of endothelium. In one of these papules developed on the 4th day at all four sites of inoculation, but did not progress to central necrosis ; agglutination of the " OXK " strain of *Proteus X.19* also developed to a significant titre. In the other of the two, macules developed on the 2nd to 5th days at four of five sites of inoculation, progressed to form papules on the 3rd to 5th days, and underwent central necrosis on the 5th to 6th days ; the rabbit died of intercurrent infection on the 11th day, so that development of a positive Weil-Felix reaction was precluded.

INFECTION OF MONKEYS BY THE INTRADERMAL INOCULATION OF THE VIRUS OF THE TSUTSUGAMUSHI DISEASE.

The inoculum was as described in the preceding paragraph. Two monkeys were used, one a gibbon and one a macacus ; both reacted, and proved to be much more susceptible to the virus than were the rabbits.

In the gibbon, inoculation of infected aqueous humour at three sites on the abdominal wall resulted in the development of a macule on the 4th day at two sites, and a papule at the third site on the 11th day ; the macules persisted for 2 days only and the papule for 4 days. At two other sites teased infected endothelium from Descemet's membrane was inoculated ; and at both, papules developed on the 7th and 11th days that persisted for 9 and 4 days respectively ; no necrosis ensued.

In the macacus monkey 6 inoculations were made, 2 with an inoculum of infected aqueous humour, 4 with an inoculum of infected endothelium ; macule formation occurred at three sites, papule formation at all 6 sites on the 2nd and 3rd days ; and, in 5 of these 6, necrosis ensued on the 5th to 7th days after inoculation (Fig. 14). The central necrotic scabs persisted for many days before being shed ; 18 days after their first appearance, punched-out depressions marking their sites were clearly visible. The necrotic ulcers appeared identical with those described above in experimental rural typhus, and with the initial lesion of the tsutsugamushi disease in man. Control inoculations with an inoculum of aqueous humour and teased Descemet's membrane of normal rabbits were made at the same time, and yielded no trace of dermal reaction.

In both animals a febrile reaction occurred ; in the gibbon it was of 6 days' duration, following an incubation period of 18 days ; in the macacus monkey it was of 2 days' duration only, following an incubation period of 10 days (*vide* Charts V and VI). Swelling of the inguinal glands coincided with the development of necrosis in the case of the macacus, but was absent in the gibbon. Leucopenia was a feature of both infections, being especially marked in the gibbon, as the following figures show :

	(0).	(10).	(14).	(23).
Gibbon	14,500	12,500	6500	12,500
	(0).	(5).	(11).	(15).
Macacus	18,750	12,500	13,500	17,000

(Figures in brackets = number of days after inoculation.)

Neither animal appeared seriously ill during the infection.

In both the Weil-Felix reaction was positive, the " OXK " strain of *Proteus X.19* alone being agglutinated. The titres showed the waxing and waning features characteristic of active infection ; in the gibbon the pre-inoculation titre of 1/17 rose to 1/3850 on the 28th day after inoculation ; in the macacus monkey the initial titre of 1/50 rose to 1/385 on the 20th day.

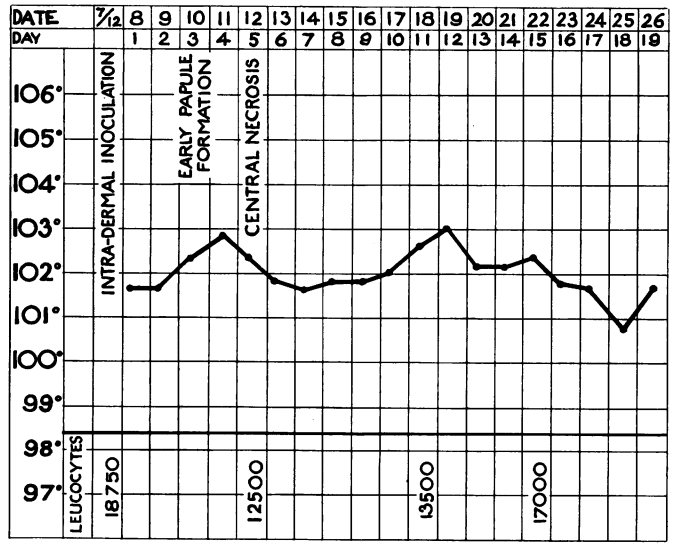


CHART V: TSUTSUGAMUSHI.—Monkey No. 3 (a macacus). Inoculated intradermally on December 7th, 1934, with infected material from the eye of a passage rabbit of a strain of the tsutsugamushi disease. For dermal lesions see Fig. 14.

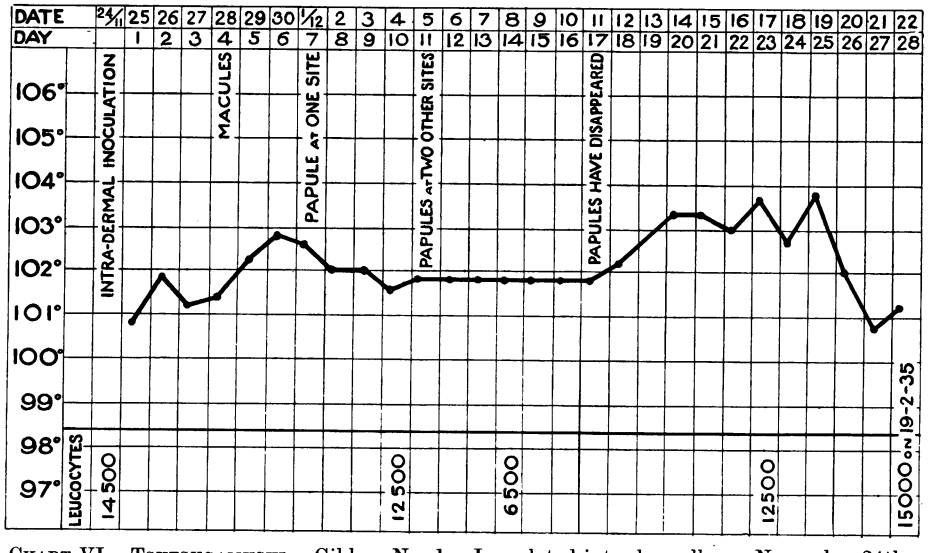


CHART VI: TSUTSUGAMUSHI.—Gibbon No. 1. Inoculated intradermally on November 24th, 1934. Source of inoculum, and strain, as for Monkey No. 3.

SUMMARY.

1. A strain of the rural form of tropical typhus has been established and maintained in guinea-pigs, and is now in its 97th generation. The history and characteristics of this strain are given. The clinical criterion of infection is a well-marked febrile reaction. Scrotal swelling does not occur. Ascites invariably follows intra-peritoneal inoculation of passage virus.

2. In more than one hundred other such attempts made in this laboratory it has proved impossible to maintain a strain beyond a few generations. Similarly all attempts to maintain the virus of the tsutsugamushi disease in guinea-pigs have failed. The guinea-pig must, therefore, be regarded as very insusceptible to the viruses of the rural typhus and tsutsugamushi group of fevers of Malaya.

3. Infection of rabbits by the intra-ocular inoculation of the virus of rural typhus and of the tsutsugamushi disease has been readily secured. The method, based on that of Nagayo *et al.*, and the criteria of infection, are described in detail.

4. The white rat is readily infected with the virus of rural typhus, the infection being of the " inapparente " form. That the virus could be maintained with unabated virulence for 21 generations indicates, by the criterion of Nicolle, that rural typhus is a murine strain.

5. Monkeys have been successfully infected by the intradermal route with the virus of rural typhus and of the tsutsugamushi disease. At the sites of inoculation, in the case of both viruses, necrotic ulcers have developed that appear to be identical with one another, and with the initial lesion of the tsutsugamushi disease in man ; in all other features the experimental infections in the monkey appear to be identical. Rabbits have been similarly infected.

6. The results of the Weil-Felix reactions of sera from rabbits and monkeys convalescent from experimental infection with rural typhus and the tsutsugamushi disease are summarized.

7. Methods of demonstration of *Rickettsia* from infected guinea-pigs and rabbits are described. In morphology, distribution and staining characteristics the *Rickettsia* demonstrable in material from animals infected with rural typhus and with the tsutsugamushi disease are identical, and do not appear to differ from the *Rickettsia orientalis* of Nagayo.

8. The experimental data secured indicate that, provided that intra-ocular inoculation is practised, the rabbit is the laboratory animal of choice in the case of the rural typhus and tsutsugamushi group of fevers (it being assumed that expense and scarcity make extensive use of monkeys impracticable). Further, these data stress the remarkable similarity of the behaviour of these two viruses in experimental laboratory animals—a similarity that, as will be set forth in a later paper, is fully supported by cross-immunity experiments.

REFERENCES.

ANIGSTEIN, L.—(1933) " Studies on Tropical Typhus," *Stud. Inst. med. Res. F.M.S.*
BRUYNOGHE, R., AND JADIN, J.—(1933) *C. R. Soc. Biol.*, Paris, **113**, 1522.
FELIX, A.—(1933) *Trans. R. Soc. trop. Med. Hyg.*, **26**, 365.

FLETCHER, W., AND FIELD, J. W.—(1927) *Bull. Inst. med. Res. F.M.S.*, No. 1 of 1927.
Idem AND LESSLAR, J. E.—(1925) *Ibid.*, No. 2 of 1925.—(1926) *Ibid.*, No. 1 of 1926.
FLETCHER, W., LESSLAR, J. E., AND LEWTHWAITE, R.—(1928) *Trans. R. Soc. trop. Med. Hyg.*, **22**, 161.—(1929) *Ibid.*, **23**, 57.
LAIGRET, J., AND DURAND, R.—(1933) *Arch. Inst. Pasteur Tunis*, **22**, 499.
LEWTHWAITE, R.—(1930) *Bull. Inst. med. Res. F.M.S.*, Nos. 1 and 3 of 1930.
Idem AND SAVOOR, S. R.—(1931–2–3–4), *Rep. Inst. med. Res. F.M.S.*—(1934) *Trans. 9th Congress, Far Eastern Ass. trop. Med.*, Nanking, **1**, 249.
NAGAYO, M., MIYAGAWA, Y., MITAMURA, T., *et al.*—(1931) *Jap. J. exp. Med.*, **9**, 87.
NICOLLE, CH.—(1933) *Arch. Inst. Pasteur, Tunis*, **21**, 349.
PINKERTON, H.—(1931) *J. exp. Med.*, **54**, 181.
ZINNSER, H., CASTANEDA, R., AND SEASTONE, C. V.—(1931) *Ibid.*, **53**, 333.

COLOURED PLATES.

(All preparations were stained by Giemsa's method.)

FIG. 1.—Guinea-pig 1635—a passage animal of the " Seerangayee " strain of rural typhus, maintained in guinea-pigs by the intra-peritoneal inoculation of virus. Shows smear of the fibrin covering the spleen. A group of mononuclear cells is seen ; the cytoplasm of the cells contains numerous *Rickettsia*. Two features characteristic of the *Rickettsia* of rural typhus are readily seen, viz. the presence of two well-defined polar coccoid elements, in some instances slightly elongated, and the tendency of the organisms to occur in clusters close to the nucleus. × 1700.

FIG. 2.—Rabbit 600—a passage animal of the " Munian " strain of rural typhus, maintained in rabbits by intra-ocular inoculation of virus. Shows smear of scrapings from the endothelium covering Descemet's membrane in the reacting eye. *Rickettsia* are plentiful ; their characteristics do not differ from those of the *Rickettsia* demonstrated in material from guinea-pigs infected with the virus of rural typhus. × 1800.

FIG. 3.—Rabbit 485—a passage animal of a series of rabbits infected with the virus of the " Seerangayee " strain of rural typhus. Maintained in guinea-pigs, this strain may be established in rabbits with ease by intra-ocular inoculation of heart-blood from an infected guinea-pig. Source of smear—as for Fig. 2. The typical clumping of *Rickettsia* close to the nucleus is well marked. × 2600.

FIG. 4.—Rabbit 1057—a passage animal of the " Kepong " strain of the tsutsugamushi disease, maintained in rabbits by the intra-ocular inoculation of virus. Source of smear as for Fig. 2. *Rickettsia* are plentiful ; in morphology, distribution and staining characteristics they do not differ from those *Rickettsia* demonstrable in infected material from guinea-pigs and rabbits infected with the virus of rural typhus. × 1300.

PHOTOMICROGRAPHS.

FIG. 5.—Guinea-pig 2303—a passage animal of the " Seerangayee " strain of rural typhus. Shows smear of the fibrin covering the spleen. Three characteristics of the *Rickettsia* of rural typhus are well defined, viz. the presence of two polar elements, the more faintly staining inter-connecting rod, and the distribution within the cytoplasm as one or more clusters that lie close to the nucleus. × 1750.

FIG. 6.—Guinea-pig 2306—strain and source of material as for Fig. 5. Extra-cellular *Rickettsia* are abundant ; other features noted above are well-marked. × 1600.

FIG. 7.—Rabbit 1144—a passage animal of a series of rabbits infected with the virus of the " Seerangayee " strain of rural typhus (*vide* notes on Fig. 3). Source of smear as for Fig. 2. Characteristic intra-cellular *Rickettsia* are seen. × 1600.

FIG. 8.—Rabbit 1055—a passage animal of the " Kepong " strain of the tsutsugamushi disease. Source of smear as for Fig. 2. Nucleus is somewhat degenerate. Intra-cellular *Rickettsia* are plentiful, and their morphological identity with the *Rickettsia* of rural typhus is evident. × 2000.

FIG. 9.—Rabbit 1163—a passage animal of the " Raub " strain of the tsutsugamushi disease, maintained in rabbits by the intra-ocular inoculation of virus. Source of smear as for Fig. 2. The typical clumping of *Rickettsia* at a point in the cytoplasm close to the nucleus is here well seen. × 1500.

FIG. 10.—Pneumococci in the blood of a mouse. × 1700. (This figure is given to afford comparison of the dimensions of pneumococci with those of the *Rickettsia* of rural typhus and the tsutsugamushi disease.)

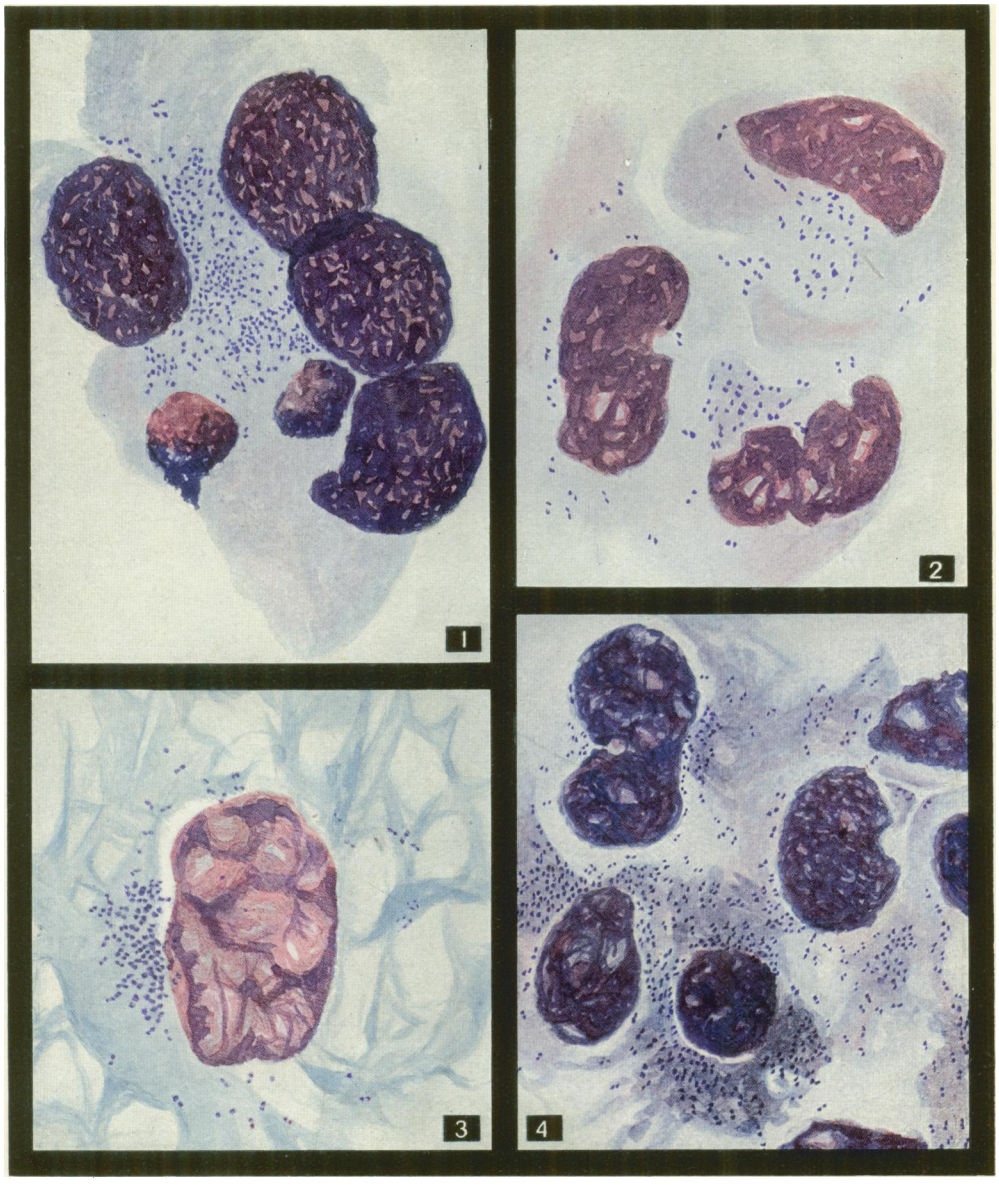
PHOTOGRAPHS.

FIG. 11.—Rabbit 1125—inoculated intradermally with material from the reacting eye of an infected rabbit of the " Seerangayee " strain of rural typhus. Shows the ulceration, with early necrosis, that followed inoculation, at two sites on each flank, of scrapings of the endothelium covering Descemet's membrane. Tenth day after inoculation. The less virulent aqueous humour from the same eye was inoculated intradermally at sites posterior to the above ; and, at three of these sites, a feeble reaction is just perceptible—at the lower margin of the photograph.

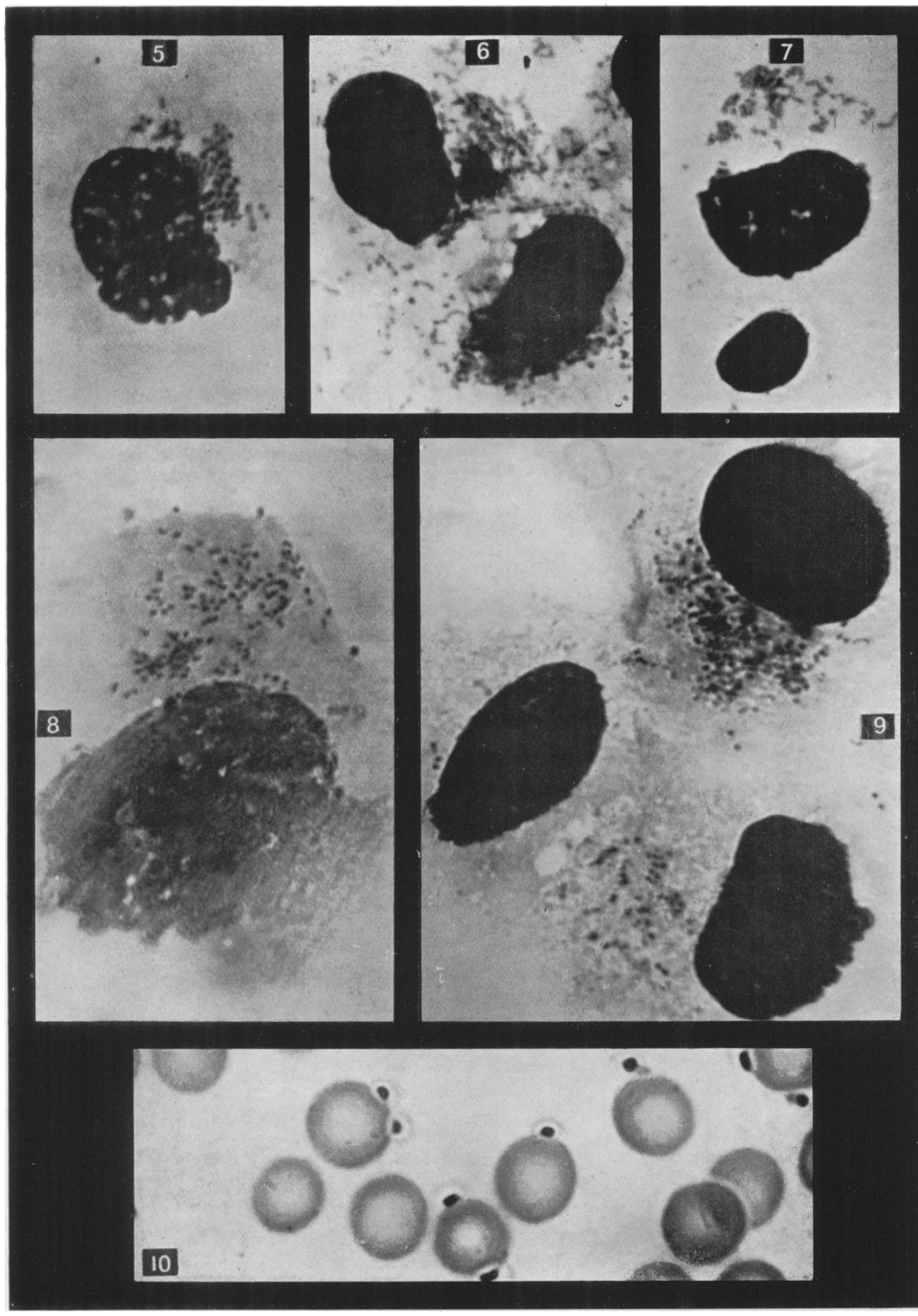
FIG. 12.—Gibbon No. 2—inoculated intradermally with material from the reacting eye of an infected rabbit of the " Seerangayee " strain of rural typhus. Inoculations of both aqueous humour and teased endothelium from Descemet's membrane were made severally into the skin of both sides of the abdomen. This photograph, taken on the 12th day after inoculation, shows two small areas of necrosis at sites respectively above and below the umbilicus, to the right of the mid-line ; the lower is the more distinct. On the left side there was development of papules that did not progress to the stage of necrosis. A large bubo in the left groin is prominent ; the skin over it, tightly stretched and œdematous, has torn.

FIG. 13.—Monkey No. 4 (a macacus)—inoculated intradermally with material from the reacting eye of an infected rabbit of the " Seerangayee " strain of rural typhus. Photograph taken on the 12th day after inoculation. Shows five ulcers, each with well-marked central necrosis, in the skin of the abdomen at five sites of inoculation of teased endothelium from Descemet's membrane. Note the identity of these dermal lesions, caused by the virus of rural typhus, with those of Fig. 14, caused by the virus of the tsutsugamushi disease.

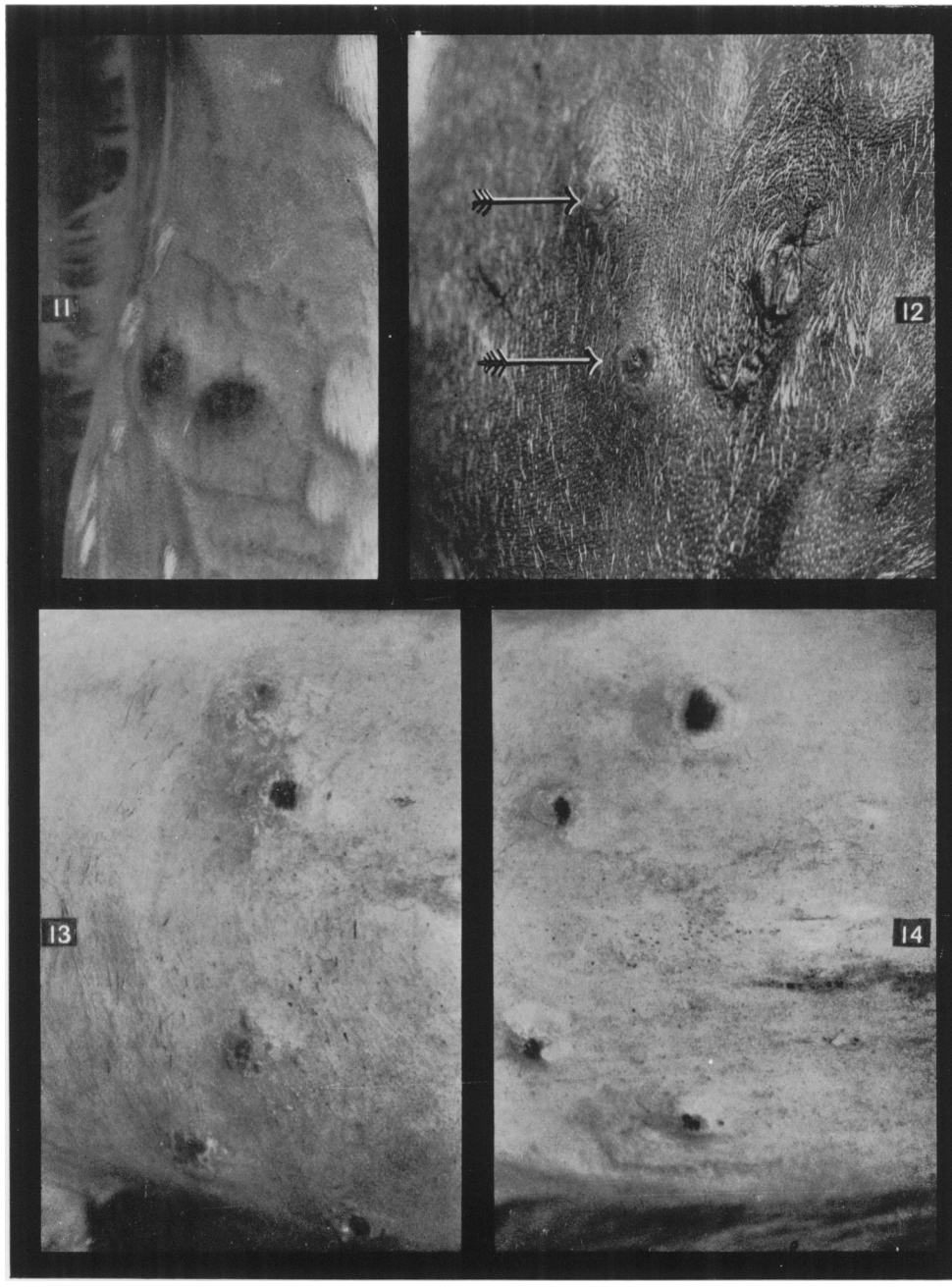
FIG. 14.—Monkey No. 3 (a macacus)—inoculated intradermally with material from the reacting eye of an infected rabbit of the " Raub " strain of the tsutsugamushi disease. Photograph taken on the 8th day after inoculation. Shows four ulcers, with well-marked central necrosis, in the skin of the abdomen at four sites of inoculation of teased endothelium from Descemet's membrane. (*Cf.* Fig. 13.)



Lewthwaite and Savoor.



Lewthwaite and Savoor.



Lewthwaite and Savoor.