

THE EXPERIMENTAL INFECTION OF THE HUMAN BODY LOUSE,  
PEDICULUS HUMANUS CORPORIS, WITH MURINE AND  
EPIDEMIC LOUSE-BORNE TYPHUS STRAINS

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PLATES 1 TO 3

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The rôle of the human body louse, *Pediculus humanus corporis*, in the transmission of typhus fever was discovered in 1909 by Nicolle, Comte, and Conseil (1). Their work opened the way for extensive investigations of typhus-infected lice. In 1916 Da Rocha-Lima (2), by using a normal colony of lice, succeeded in elucidating the nature and characteristics of the causative organism of typhus fever, which he named *Rickettsia prowazeki*, in honor of two investigators who died of typhus in pursuit of their studies of the disease. Da Rocha-Lima's work was extended and confirmed by several workers (references cited in 3).

In the course of the many experiments bearing on the etiology of typhus fever, it became apparent that the louse was exceptionally susceptible to infection with *R. prowazeki*. In Weigl's words, "My experiments have shown that the louse is extremely susceptible to typhus. We may thus certainly assume, without committing a serious error, that only one germ or at most a few are sufficient to infect the louse" (4, p. 50). Weigl's statement is supported by other evidence, such as the comparison of the ease with which strains of rickettsiae can be obtained from patients suffering from typhus fever. To establish a strain of epidemic louse-borne typhus in guinea pigs it is necessary to inject them with several cubic centimeters of human blood, or ground clot. It is a common observation that even under the most favorable circumstances this large volume of blood fails to produce the infection in guinea pigs in one out of every four or five attempts. By contrast, a very high percentage of lice can be infected by 1/900th of a cc. of human blood (4, p. 50). Wolbach, Todd, and Palfrey state that "With the recognition of the conditions favorable for infection of lice, we were able to secure almost uniformly positive results. Rickettsiae appeared in lice in each of the last thirteen consecutive feeding experiments . . ." (5, p. 43).

Furthermore, according to Weigl, infection of the louse with *R. prowazeki* without exception results in the invasion and destruction of all the cells of the mid-intestine, a condition which inevitably produces the death of the louse (6, p. 358; 7, p. 1591). Usually the typhus-infected lice die in a few days, but occasionally 20 to 25 days must elapse before all of the cells of the mid-intestine become packed full of rickettsiae.

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This high degree of susceptibility would seem to qualify the louse as nearly ideal for various types of laboratory studies with *R. prowazeki*, but the potential usefulness of the louse has not been fully exploited because of the difficulties peculiar to the handling of typhus-infected lice. For example, it has been considered necessary to feed lice at least once daily on human beings, and those persons who act as louse feeders, unless they are immune to typhus fever, are very likely to contract the disease from their exposure to infected lice. Another obstacle has been the method for the experimental infection of lice. The standard procedure has been Weigl's ingenious intrarectal injection technique (6) which is a delicate and time-consuming operation. Although considerable quantities of vaccine have been produced by Weigl's method over a period of years in Poland, China, and elsewhere, the disadvantages of the procedure have prevented its application to other laboratory aspects of typhus research.

In attempts to simplify the handling of infected lice, several investigators observed that human lice would feed on a variety of animals, for example, monkeys (8, 9), guinea pigs (10), dogs, cats, rats, mice (11), young pigs (12), and rabbits (13, 14). Some of these experiments with small laboratory animals are particularly interesting and pertinent to our subject.

In 1916 Nöller (12), appreciating the need for a convenient laboratory method, tried a young pig as a host. He took human body lice from typhus fever patients and nourished these infected lice for 2 days on the young pig. He found many rickettsiae in these lice, which, when inoculated into guinea pigs, produced characteristic typhus infection. In another experiment he fed newly hatched larvae as well as adult lice twice daily for 7 days on the ear of a 6 weeks old pig, and noted only a slight loss of life. Furthermore, he allowed normal human body lice to feed once, for a very short period only, on the shaved skin of a typhus-infected guinea pig, and thereafter nourished them for 6 days by feedings twice daily on the ear of a young pig. He found rickettsiae in these lice and he stated that pig's blood was, therefore, not harmful to typhus rickettsiae (12).

Frickhinger in 1916 asserted that he had maintained human lice on a guinea pig for several weeks (10) but other observers found that human lice succumbed after a short period of feeding on guinea pigs (15, 8, 9).

Davis and Hansens (14) described experiments in which human lice were nourished on a rabbit through a complete cycle of development, though their colony of rabbit-fed lice did not thrive.

Thus, human lice have been successfully nourished for short periods on several different species, but it was generally observed that the only practical way to obtain a healthy, rapidly multiplying colony of human body lice was by feeding the lice on human volunteers (9, 15, 16).

Attempts to nourish or to infect lice by inducing them to ingest a meal from various artificial membranes such as sausage skins (9) were unsuccessful until the method of Pshenichnov and Raikher (17) was devised. They permit lice to feed into a mixture composed of human defibrinated blood and a suitable suspension of rickettsiae (from infected animal tissues). The membrane through which the lice feed is obtained by applying boiling water to the skin of a cadaver.<sup>1</sup> The Russian workers report that

<sup>1</sup> The authors are indebted to Dr. A. A. Smorodintseff for this information.

they are able to make vaccine from infected lice with far less trouble using this technique than was required with Weigl's intrarectal injection technique. The Russian method involves a considerable amount of manipulation, however, and is not without certain disadvantages.

It is the purpose of the present report to describe very simple methods for the experimental infection and subsequent feeding of lice. In principle our methods are similar to those in Nöller's experiments (12) except that rabbits were used instead of young pigs. These methods eliminate the feeding of *infected* lice on human beings at any time, and make it possible to infect large numbers of lice in a few minutes, without resort to the tedious, time-consuming intrarectal injection procedure, or to devices with membranes.

### Methods

The experiments in this study are described in three parts: (a) A normal stock colony of lice was maintained on a human volunteer. (b) The infection of lice was accomplished in two ways. In the "bleb technique" the lice ingested a mixture containing rickettsiae from a bleb produced by the inoculation of the infective mixture into the skin of the rabbit. In the "I.V. technique" the lice fed anywhere on the skin of a rabbit which had been inoculated intravenously with a suitable suspension of rickettsiae. (c) The infected lice were thereafter nourished only on a rabbit. These steps are described in detail below.

(a) *Normal Stock Colony of Lice.*—A normal stock colony of lice was maintained on a human volunteer by feeding twice daily in the customary manner (9, pp. 2-8; 16, p. 102). The colony was brought to the Cairo laboratory from the National Institute of Health in Bethesda, Maryland.<sup>2</sup> The method of feeding the normal colony is shown in Fig. 1. It was checked several times during the course of these experiments by smears and animal inoculations. It was found to be free of rickettsiae, both pathogenic and non-pathogenic, throughout this study.

(b) *Procedures for Experimental Infection of Lice.*—The normal stock colony was drawn upon to provide lice for the infection experiments. The two methods of infecting lice were based on our observation that human lice would feed quite promptly on the freshly shaved skin of rabbits after the area was covered with a small amount of human saliva or human perspiration.<sup>3</sup> If the application of saliva or perspiration was omitted, the lice often did not begin to feed for long periods of time even though they had previously been starved for several hours to increase their hunger. It was found desirable not to use soap in shaving the rabbit since traces of soap on the skin tended to keep the lice from feeding.

"*Bleb technique.*"—In this method a small amount of infective material was injected into the skin of the rabbit, either on the abdomen or the tip of the ear. When the former site was used, it was sometimes observed that blood from the rabbit's capillaries had oozed into the inoculum, and that the inoculum fluid tended to diffuse out of the bleb area before the feeding period of the lice was completed. When the bleb was made on the tip of the rabbit's ear a long hemostat was clamped entirely across the ear just proximal to the bleb, tightly

<sup>2</sup> The authors wish to express appreciation to Dr. R. E. Dyer, who made the louse colony available, to Brigadier General Leon A. Fox, who brought it to Cairo, and to Lieutenant Commander A. Yeomans, Major E. S. Murray, Sergeant L. Stephens, and Corporal Stearman who nourished the colony for several months.

<sup>3</sup> It was observed that the saliva of C. M. W., a non-smoker, was much more effective than that of two other workers both of whom smoked cigarettes or a pipe.

enough to prevent the passage of blood or lymph into the bleb fluid, or the loss of bleb fluid by diffusion.

The bleb method is suitable for experiments in which the volume of infective material is small, or in which it is desired to infect only a few lice.

When the skin of the abdomen was used for the bleb, it was found desirable to place a small glass ring over the bleb in order to restrict the area available for feeding to the central portion of the bleb.

*"Intravenous technique."*—In this method the infective inoculum, a suspension of yolk sac (18), was injected into the ear vein of the rabbit so that wherever the lice fed on the rabbit they ingested a mixture of the rabbit's blood and rickettsiae. The rabbit was anesthetized with intravenous pentobarbital before the yolk sac suspension was introduced. This technique is suitable for experiments in which large numbers of lice are to be infected. The minimal amount of infective material which is necessary to assure infection of all the lice which feed on the rabbit subsequent to the intravenous injection of the rickettsial suspension has not been determined. A very high percentage of infection of lice was obtained by the injection of 8 cc. of a 20 per cent yolk sac suspension in saline into a rabbit weighing approximately 700 gm. The entire amount of yolk sac was injected in less than 3 minutes. On smears these suspensions showed only a few rickettsiae in each oil immersion field and were not regarded as rich suspensions. Four of five rabbits thus inoculated died several days after the injection of the yolk sac suspension, but no rickettsiae were observed in the smears of the organs of the two rabbits which were examined for the presence of rickettsiae at autopsy. On the basis of this observation it was assumed that multiplication of rickettsiae in these rabbits was not extensive.

In some of the tests, the lice were permitted to feed twice daily on the inoculated rabbits until the latter died. In other trials, the lice were permitted only one feeding on the inoculated rabbits and subsequently were nourished only on normal rabbits. New lots of lice were placed on the inoculated rabbits for their first infective meal at various intervals after the inoculation of infected yolk sac suspension. The intervals ranged from a few minutes to 3 days.

In two experiments normal stock lice were fed on uninoculated rabbits as control groups.

*(c) Nourishment of Infected Lice.*—After taking one or more infective meals as described in section (b), the infected lice were nourished by feedings twice daily on a normal rabbit. They were left on the rabbit for 10 or 15 minutes at each feeding. In most instances it was not necessary to anesthetize the rabbits for routine feedings. Most of the rabbits remained quiet for an hour or more, although the bites of large numbers of lice tended to make them restless. The interval of 10 to 15 minutes was sufficiently long in most instances to give all the lice an adequate opportunity to feed. Between feedings the lice were kept on a small circular cloth in an open-mouthed specimen jar (2 ounce size) in an incubator maintained between 30 and 32°C. During the feedings the lice were restrained away from the shaven area by the application of a cup-shaped device made by cutting a hole in the bottom half of an ordinary ointment tin. This shield, being open at the top, permitted easy observation or manipulation of the colony while the feeding was in progress. It was found desirable to strap the shields firmly in place with thin strips of adhesive tape. These details are illustrated in Figs. 2 and 3 which are photographs showing the lice and the shields on the abdomen of a rabbit.

The skin of the rabbit was carefully cleaned with 95 per cent ethyl alcohol after each feeding in order to remove any louse feces which might have fallen on the area. A new rabbit was employed for each experiment.

After a few days of feeding on a rabbit a variable number of lice inevitably succumbed from causes other than infection with typhus. Usually about three-fourths of the original

number survived for 10 days. By increasing the initial number of lice to make allowance for this expected loss, a satisfactory number of survivors was regularly obtained.

*Technique Used in Making Smears of Lice.*—A single transverse or slightly diagonal cut was made across the upper portion of the abdomen of the louse by means of a sharp blade. The cut surfaces of the two segments were quickly touched to a glass slide several times. In some instances the remaining segments of the louse were ground in saline for animal inoculations. The smears were dried in air, lightly heat-fixed, and stained with Macchia-vello's stain (19). The smear was called "definitely positive" only when numerous clear-cut, morphologically typical, red-staining organisms were demonstrable. It is obvious that this method of examination of lice was likely to give negative results when a louse contained only a few rickettsiae, and that there may have been more infected lice than the smears indicated.

#### PROTOCOLS

Eight different experiments were completed successfully, three with the bleb technique, four with the intravenous technique, and one with both techniques. The details of each test are listed below.

#### *Experiment 1*

*May 5, 1944:* 53 normal lice fed on bleb on abdomen of rabbit at 10:00 a.m. Bleb made by injecting mixture of 0.5 cc. of saline peritoneal washings (from a gerbille (20) infected with Breinl strain rickettsiae) and 1.5 cc. of freshly defibrinated human blood. *May 6, 1944:* Second infectious meal at 10:00 a.m.; 51 of the original lot of lice fed on a bleb of same composition as on May 5. Thereafter the lice were permitted to feed directly on a normal rabbit twice daily; the last meal was in the morning of May 14. Daily observations are tabulated below:—

Date	Fed	Dead	Sacrificed	Ratio: Positive smears to total examined
May 5, a.m.	53 (bleb)			
May 6, a.m.	51 (bleb)	0	2	0/2
p.m.	51	0	0	
May 7, a.m.	47	0	4	0/2
p.m.	46	1	0	0/1
May 8, a.m.	40	3	3	0/3
p.m.	40	0	0	
May 9, a.m.	36	1	1	2/2
p.m.	33	1	1	2/2
May 10, a.m.	29	2	4	4/4
p.m.	28	1	0	1/1
May 11, a.m.	21	3	4	2/2
p.m.	20	1	0	
May 12, a.m.	16	0	3	1/1
p.m.	9	0	0	
May 13, a.m.	8	3	3	1/1
p.m.	8	0	0	
May 14, a.m.	5	0	3	1/1
May 15		1	1	2/2

Some of the lice which were sacrificed were placed in fixative solution and not examined by direct smear. Feces of the lice were examined for rickettsiae on May 7, negative; on May 9, few; on May 10, positive; on May 11, positive.

*Results of Animal Inoculations.*—Those lice which died between May 8 and 14 were stored

in the +4°C. box until May 15. Three lice which were still alive on May 15 were added to the collection which was then ground with sterile sand in 1.5 cc. nutrient broth. Two gerbilles (species *Gerbillus gerbillus*) were inoculated intraperitoneally with 0.75 cc. of this suspension. Smear of the inoculum fluid: no definite rickettsiae seen. On May 19 one gerbille was sick; it was sacrificed and definite rickettsiae were seen intracellularly in the peritoneal exudate. The second gerbille died on May 20; peritoneal exudate showed rickettsiae "3 plus" intracellularly; brain of this gerbille used to prepare inoculum for Experiment 2, group *c*, and for inoculation of eggs. Culture of the brain in nutrient broth: no growth at end of 7 days. Rickettsiae demonstrated in yolk sac of egg inoculated with the brain, 6 days after inoculation.

### *Experiment 2*

*May 20, 1944:* Rabbit anesthetized with pentobarbital *via* ear vein. Blebs made on ear.

Three groups of lice were used: (*a*) Six lice fed on mixture of human serum and yolk sac infected with Breinl strain rickettsiae. Final concentration of yolk sac was 3 per cent, of serum 67 per cent; physiological saline constituted the remainder. These lice, after feeding into the bleb, showed no trace of having ingested any rabbit blood. (*b*) Six lice fed on the same bleb as those in (*a*), but during the feeding they picked up some rabbit blood which oozed into the bleb fluid. (*c*) Nine lice fed on a bleb made by inoculating a mixture of two parts human serum and one part of a suspension of 3 per cent gerbille brain in saline (Breinl strain; this gerbille was described in Experiment 1). The three groups were permitted to feed twice daily on a normal rabbit, except in the afternoon on May 25 and 28 when the feeding was omitted. The last meal was May 31 in the morning. No lice died or were sacrificed until May 26; one smear from each group in the afternoon showed no rickettsiae. No further smears were made in this experiment. On May 30 there were two lice still alive in group *a*, 4 in group *b*, and 5 in group *c*. Guinea pigs were inoculated on May 31 as follows: two living lice and one dead louse from group *a* were ground in saline and inoculated intraperitoneally into guinea pig 5-40. This guinea pig developed fever and a positive complement fixation test in its serum against Breinl antigen (21).<sup>4</sup> Four living lice from group *b* were ground in saline and injected into guinea pig 5-49. This pig died on June 7 of an intercurrent infection (pneumonitis). At autopsy a fibrinous exudate was present over the spleen. Smear from the surface of the spleen taken under this exudate: numerous cells were observed in which typical rickettsiae were seen. Five living lice and two dead lice from group *c* were ground in saline and injected intraperitoneally into guinea pig 5-46. This pig underwent a febrile period consistent with typhus infection. Serology and immunity test were not performed.

### *Experiment 3*

*May 22, 1944:* Rabbit anesthetized with pentobarbital intravenously. Bleb made on abdomen. Bleb fluid composed of 0.1 cc. saline washings of tunica of *Gerbillus gerbillus* infected with Wilmington murine strain, plus 0.9 cc. human serum. Needle left in place in order to maintain continuous pressure in bleb during the feeding of the lice. Seven lice fed on the bleb. Thereafter lice permitted to feed on a normal rabbit twice daily except in the afternoon on May 25 and 28. The last meal was given on May 31, a.m. One louse was sacrificed for smear on May 26: no definite rickettsiae seen. On June 1, four living lice and one dead louse (24 hours at +4°C.) were ground in broth and inoculated into guinea pig 5-22. This pig reacted with fever and scrotal swelling on June 5, 6, and 7. It was sacrificed on June 7. Smear of tunica: rickettsiae "3 plus" intracellularly. Two second generation guinea pigs inoculated with tunica washings of No. 5-22 developed positive complement fixation tests in their sera against both Wilmington and Breinl antigens, higher against the former.

<sup>4</sup>The authors wish to express their thanks to Captain C. J. D. Zarafonitis and Sergeant Dworkowitz who performed the serologic studies on the animals used in these tests.

*Experiment 4*

July 30, 1944: Approximately 700 gm. rabbit anesthetized with intravenous pentobarbital (artificial respiration required for a few minutes). 8 cc. of 20 per cent yolk sac suspension in saline inoculated intravenously at 5:15 p.m. Sixth egg passage of "N 230" strain of louse-borne typhus, isolated from blood of a patient in Naples in Jan., 1944, was used for the inoculation of the rabbit. Yolk sac suspension was centrifuged lightly to remove large tissue particles and yolk. Smear of yolk sac: rickettsiae 1 to 2 plus. Four groups of lice were fed on the inoculated rabbit: (a) twenty-two lice fed in the 15 minute period immediately following the intravenous injection of the infected yolk sac suspension; (b) twenty-two lice fed in the interval from 15 to 30 minutes after the intravenous injection; (c) twenty lice were permitted their first meal from the inoculated rabbit 16 hours after the intravenous injection; (d) twenty lice were permitted their first meal from the inoculated rabbit 40 hours after the intravenous injection. Subsequent to their first infective meal as noted, the lice in each group were nourished on the same rabbit until it died on Aug. 4. Thereafter all four groups were fed on a normal rabbit. At autopsy the rabbit had a few grayish red lesions scattered through both lungs, 2 to 3 mm. in diameter. On smears the cut surfaces of the lung lesions did not show any rickettsiae or bacteria. Smears of other organs were negative. The last meal given to the lice in this experiment was in the afternoon of Aug. 6. Daily notes are arranged below:—

Date	Group a				Group b				Group c				Group d				
	F*	D*	S*	R*	F	D	S	R	F	D	S	R	F	D	S	R	
July 30, p.m.	22				22												
July 31, a.m.	21	1	0	0/1	21	1	0	0/1	20								
p.m.	16	1	0	0/1	17	0	0		19	0	0						
Aug. 1, a.m.	20	0	0		12	2	0	0/2	12	3	0	0/2	21				
p.m.	19	1	0	1/1	13	3	0	2/3	15	1	0	0/1	18	0	0		
Aug. 2, a.m.	16	3	0	3/3	8	4	0	4/4	11	3	0	0/3	18	1	0	0/1	
p.m.	15	1	0		9	3	0	0/1	10	2	0	0/2	18	0	0		
Aug. 3, a.m.	13	0	0		9	0	0		9	1	0	0/1	15	3	0	0/3	
p.m.	13	0	1	1/1	8	1	1		8	0	0		16	0	0		
Aug. 4, a.m.	12	1	0	1/1	7	1	0	1/1	6	1	1	0/1	13	3	0	0/3	
p.m.	9	0	0		7	0	0		6	1	0	0/1	13	2	0	0/2	
Aug. 5, a.m.	9	2	0	2/2	7	0	0		5	0	0		8	0	0		
p.m.	9	0	0		7	0	0		2	1	0	0/1	9	3	0	0/3	
Aug. 6, a.m.	8	1	0		6	0	0		4	0	0		9	0	0		
p.m.	8	0	0		5	1	0	1/1	5	0	0		9	0	0		
Aug. 7, a.m.		1	7	8/8													
Aug. 8, a.m.					5	5	5		5	4	5		9	8	9		

\* Symbols indicate same column headings as in table in Experiment 1.

No animal inoculations were made with the lice in this experiment.

*Experiment 5*

Aug. 9, 1944: Approximately 700 gm. rabbit anesthetized with intravenous pentobarbital at 10:35 a.m. Inoculum same as described in Experiment 4. 7.5 cc. yolk sac suspension injected into ear vein 10:38 to 10:41 a.m. Four groups of lice were fed on the inoculated rabbit: (a) twenty-two lice, in the first 15 minutes after intravenous injection; (b) twenty-two lice, in the interval from 15 to 30 minutes after intravenous injection; (c) twenty lice, 22½ hours after intravenous injection; (d) twenty lice, 46 hours after intravenous injection. In this experiment only one meal was permitted on the inoculated rabbit. All subsequent feedings were made on a normal, uninoculated rabbit. Daily notes are tabulated below:—

Date	Group a				Group b				Group c				Group d			
	F*	D*	S*	R*	F	D	S	R	F	D	S	R	F	D	S	R
Aug. 9, a.m.	22				22											
p.m.	18	0	0		18	0	0									
Aug. 10, a.m.	17	0	0		18	0	0		20							
p.m.	19	1	0	0/1	17	0	0		13	0	0					
Aug. 11, a.m.	15	5	0	3/5	16	5	0		17	0	0		20			
p.m.	15	0	0		14	1	0	0/1	20	0	0		12	0	0	
Aug. 12, a.m.	16	0	0		15	1	0	1/1	20	0	0		14	0	0	
p.m.	16	0	0		12	0	0		20	0	0		17	0	0	
Aug. 13, a.m.	16	0	0		14	0	1		20	0	0		13	0	0	
p.m.	14	0	0		13	0	0		20	0	0		16	3	0	0/3
Aug. 14, a.m.	14	2	0	2/2	12	2	0	1/2	19	0	0		16	0	1	0/1
p.m.	13	1	0	1/1	9	2	0	2/2	18	1	0	1/1	15	0	0	
Aug. 15, a.m.	6	6	0	6/6	7	2	0	2/2	18	1	0	0/1	15	0	0	
p.m.	5	1	0	1/1	5	2	0	2/2	16	2	0	0/2	13	0	0	
Aug. 16, a.m.	0	5	1	5/6		6	6/6		12	3	0	1/3	11	1	0	0/1
p.m.									12	1	0	0/1	13	1	0	0/1
Aug. 17, a.m.									9	2	0	1/2	11	3	0	3/3
p.m.									9	1	0	0/1	10	1	0	0/1
Aug. 18, a.m.									6	3	0	2/3	5	5	0	5/5
p.m.									6	0	0		5	0	0	
Aug. 19, a.m.									4	2	0	1/2	4	1	0	0/1
p.m.									4	0	0		4	0	0	
Aug. 20, a.m.									2	2		2/2	3	1	0	0/1
p.m.										2	0/2			3	0/3	

\* Column headings as described in the table in Experiment 1.

*Results of Gerbille Inoculations.*—Control lice: ten normal, uninfected lice from the stock colony were smeared: no rickettsiae seen; the segments from each louse were ground in 0.25 cc. saline; the entire suspension from each louse was inoculated intraperitoneally into one *Gerbillus gerbillus*. Eight days later each gerbille was sacrificed; peritoneal smears were made: no rickettsiae seen in any; the brain of each gerbille was ground in saline and inoculated into four normal gerbilles. These were observed for 2 weeks and then given approximately two fatal doses of Breinl strain infected yolk sac suspension intravenously. All of these second passage gerbilles succumbed, either to toxic effect or to fatal infection, indicating absence of immunity and therefore absence of living rickettsiae in the original louse suspensions. Infected lice: after smears were made the segments were placed in 0.25 cc. saline and stored at  $-76^{\circ}\text{C}$ . for several days. Six lice which had shown positive smears were thawed, ground, and inoculated exactly as were the control lice. The gerbilles were sacrificed and brains inoculated into second passage gerbilles which were given the same challenge dose of Breinl strain yolk sac. The second passage gerbilles were immune to challenge as follows: from one louse in group *a* which died Aug. 15, a.m.; from one louse in group *b* which died Aug. 15 a.m.; from one louse in group *c* which died Aug. 20 a.m.; from one louse in group *d* which died Aug. 18 a.m. The gerbilles inoculated with two lice in group *c* were not immune (it is probable that these two louse suspensions were thawed too slowly, and that the rickettsiae were thereby inactivated). Four lice with positive smears, the segments of which were stored at  $-10^{\circ}\text{C}$ . and thawed slowly once during the several days of storage, did not infect gerbilles, indicating that the rickettsiae had been killed by slow thawing in saline suspension.

*Results of Guinea Pig Inoculations.*—A group of guinea pigs was bled before and 25 days after inoculation with lice.

Control lice: 18 normal stock colony lice were smeared and the segments were divided into 3 pools. Guinea pigs 7-06, 7-07, and 7-16 were inoculated intraperitoneally, each pig receiving the segments of 6 normal lice. Nos. 7-06 and 7-16 were afebrile for 25 days and their sera obtained on the 25th day were negative in the complement fixation test against both



Breil and Wilmington antigens. No. 7-07 became febrile on the 13th day (temperature of 40.1°C.). Blood culture was negative. The animal was sacrificed: no exudate on the spleen; liver full of small necrotic lesions 1 to 2 mm. in diameter which showed no organisms in smears. Brain of No. 7-07 was suspended in broth and inoculated into two guinea pigs, neither of which showed any fever for 25 days; one died on the 26th day with liver lesions similar to those found in pig 7-07.

Lice fed on the inoculated rabbit: Individual lice were examined by smears and the segments were stored in 0.1 cc. saline in cork-stoppered tubes at -70°C. The segments of lice which were positive on smear were thawed and inoculated into normal guinea pigs. One positive louse from group *a* and 1 from group *d* produced evidence of infection in the guinea pigs (both febrile reactions and development of positive complement fixation tests). One positive louse did not produce a febrile response or rise in complement fixation titer in the guinea pig, and it is probable that slow thawing was responsible for inactivation of rickettsiae in this instance. Seven lice from groups *c* and *d* which were not called positive on smear did not evoke any evidence of infection in the guinea pigs into which they were inoculated.

*Experiment 6*

Aug. 23, 1944: Rabbit weighed 228 gm. Inoculated with 2.0 cc. of 12 per cent yolk sac suspension (Breil strain) at 5:22 p.m. The yolk sac suspension was centrifuged lightly to permit removal of large tissue particles and some of the yolk. This inoculated rabbit died on Aug. 26; no rickettsiae were seen in smears of liver, lung, spleen, or adrenals. Three groups of lice were allowed to feed once only on the inoculated rabbit: Group *a*, 20 lice fed in first 15 minutes after intravenous inoculation; group *b*, 20 lice fed in interval from 15 to 30 minutes after intravenous inoculation; group *c*, 200 lice fed in interval from 1/2 to 2 1/2 hours after intravenous inoculation. After this single infective meal all the lice in groups *a*, *b*, and *c* were subsequently nourished on an uninoculated rabbit. From Aug. 31 to Sept. 10, a group of 20 normal lice from the stock colony was fed on the same uninoculated rabbit upon which groups *a*, *b*, and *c* were nourished, as control group. Daily notes are tabulated below:—

Date	Group <i>a</i>				Group <i>b</i>				Date	Control group			
	F*	D*	S*	R*	F	D	S	R		F	D	S	R
Aug. 23, p.m.	20				20				Aug. 31, a.m.	20			
Aug. 24, a.m.	20	0	0		20	0	0		p.m.	20	0	0	
p.m.	20	0	0		20	0	0		Sept. 1, a.m.	20	0	0	
Aug. 25, a.m.	19	0	0		20	0	0		p.m.	20	0	0	
p.m.	20	0	0		20	0	0		Sept. 2, a.m.	20	0	0	
Aug. 26, a.m.	20	0	0		20	0	0		p.m.	20	0	0	
p.m.	20	0	0		20	0	0		Sept. 3, a.m.	20	0	0	
Aug. 27, a.m.	17	1	0	1/1	20	0	0		p.m.	20	0	0	
p.m.	19	0	0		19	0	0		Sept. 4, a.m.	20	0	0	
Aug. 28, a.m.	15	1	0	1/1	15	4	0 4/4		p.m.	20	0	0	
p.m.	14	3	0	3/3	14	1	0 1/1		Sept. 5, a.m.	20	0	0	
Aug. 29, a.m.	9	1	0	1/1	9	6	0 6/6		p.m.	20	0	0	
p.m.	9	2	0	2/2	9	1	0 1/1		Sept. 6, a.m.	20	0	0	
Aug. 30, a.m.				12			8		p.m.	20	0	0	
p.m.									Sept. 7, a.m.	18	2	0 0/2	
									p.m.	18	0	0	
									Sept. 8, a.m.	16	2	0 0/2	
									p.m.	16	0	0	
									Sept. 9, a.m.	16	0	0	
									p.m.	8	0	0	
									Sept. 10, a.m.	10	3	0 0/3	
									p.m.	7	0	6 0/6	
									Sept. 11, a.m.	7	0	7	

\* Column headings as described in the table in Experiment 1.

Daily counts of lice which had fed and died were not made in group *c*. A high percentage of ruptured intestines occurred in this group, without obvious cause. On Aug. 29, 48 of the lice were still alive, and they were sacrificed for animal inoculations.

*Results of Animal Inoculations.*—On Aug. 30 a pool was made of twelve surviving lice from group *a* and eight from group *b*. This pool was then divided into four lots of five lice each. Two lots were crushed between glass slides and rubbed at once over freshly scarified skin of guinea pigs. Two lots were ground in saline and inoculated intraperitoneally into guinea pigs. Evidence was obtained that each lot contained viable typhus rickettsiae (febrile reactions, immunity tests, or demonstration of intracellular rickettsiae in exudate over spleen of guinea pigs at autopsy). Two lots of normal stock lice were treated similarly for controls; negative results were obtained with each lot.

On Aug. 29, the 48 surviving lice of group *c* were divided into three lots and inoculated intraperitoneally into gerbilles. Two of the lots were sprayed with mixtures of lousicidal compounds as part of a separate study; one gerbille inoculated with sprayed lice died on the 5th day with numerous intracellular rickettsiae in the peritoneal exudate. All three of the gerbilles inoculated with the unsprayed lot of lice showed typical intracellular rickettsiae in the peritoneal exudates when sacrificed on the 5th day for information. Two lots of sixteen normal stock colony lice were inoculated into six gerbilles as controls. No rickettsiae were found in any of the six; brains of these were inoculated into second passage gerbilles which were subsequently challenged with approximately two fatal doses of Breinl yolk sac and found to be entirely non-immune.

The twenty normal lice which were fed on the rabbit from Aug. 31 to Sept. 10 were smeared, and the segments stored at  $-76^{\circ}\text{C}$ . until Sept. 15 when all were pooled, ground in 2.0 cc. of 1 per cent dextrose broth, and inoculated intraperitoneally into two guinea pigs. One pig died as a result of perforation of the intestine at the time of inoculation. The other pig was observed for 30 days and was afebrile during that period.

### *Experiment 7*

*Sept. 2, 1944:* Rabbit weighed 800 gm. Twenty normal stock lice were fed on this rabbit before any rickettsiae were inoculated; these lice are referred to as "group *a* controls;" they were fed only on a normal rabbit thereafter. The rabbit was then given pentobarbital intravenously. At 12:10 o'clock, twelve lice fed into a bleb produced on the tip of the rabbit's ear by the inoculation of 0.5 cc. of a mixture composed of 1.5 cc. human serum plus 0.5 cc. of  $33\frac{1}{3}$  per cent yolk sac suspension (Breinl strain). The tip of the ear was clamped off with a long hemostat which prevented loss of fluid from the bleb or the diffusion into it of blood or lymph. The twelve lice which fed into the bleb were observed to have obtained a meal with no trace of blood. These lice are referred to as "group *b* (bleb);" they fed only on a normal rabbit after their single meal from the bleb. At 12:33 to 12:35 o'clock the rabbit was given 8.0 cc. intravenously of 11 per cent Breinl strain yolk sac suspension. Groups of lice were fed at various intervals thereafter, as follows: group *c*, 165 lice, 0 to  $\frac{1}{2}$  hours; group *d*, 20 lice,  $8\frac{1}{2}$  hours; group *e*, 180 lice, 21 hours; group *f*, 200 lice, 45 hours; group *g*, 200 lice, 69 hours; group *h*, 150 lice, 93 hours; group *i*, 50 lice, 117 hours. All of the groups *c* to *i* inclusive, fed twice daily on the *inoculated* rabbit for the duration of the experiment. The inoculated rabbit was sick for a few days but it did not die as had the rabbits in Experiments 4, 5, and 6. Daily notes are tabulated below:—

Date	(a) controls F*D*S*R*	(b) bleb F D S R	c† D S R	d F D S R	e† D S R	f† D S R	g† D S R	h† D S R	i† D S R
Sept. 2, p.m....	20	12	165 fed	20					
3, a.m....	20 0 0	10 1 0 0/1		20 0 0	180 fed				
p.m....	19 0 0	10 0 0		19 0 0					
4, a.m....	20 0 0	9 1 0 0/1		19 1 0 0/1		200 fed			
p.m....	20 0 0	10 0 0		19 0 0					
5, a.m....	20 0 0	10 0 0		18 0 0			200 fed		
p.m....	20 0 0	9 0 0	1 0 1/1	19 0 0					
6, a.m....	20 0 0	9 1 0 0/1	5 0 5/5	17 1 0 0/1	5 0 0/5			150 fed	
p.m....	20 0 0	9 0 0		17 1 0 0/1					
7, a.m....	20 0 0	9 0 0	21 0 4/4	15 2 0 0/2	33 0 3/5	36 0 0/5			50 fed
p.m....	15 0 0	8 0 0	19 0	14 0 0	18 0	12 0			
8, a.m....	16 0 0	9 0 0	11 0	11 3 1 4/4	11 0	20 0 0/3	20 0 0/3		
p.m....	20 0 0	8 0 0	11 0	9 2 0 1/2	7 0	14 0	12 0		
9, a.m....	19 0 0	9 0 0	10 0	7 2 0 2/2	14 0 5/5	19 0 3/4	35 0 0/5	17 0 0/5	
p.m....	19 0 0	9 0 0		4 2 0 1/2					
10, a.m....	19 1 0 0/1	7 1 0 0/1	5 0	1 3 0 3/3	16 28 2/5	16 0 3/6	24 0 1/5	13 0 0/5	5 0 0/5
p.m....	18 0 0	7 0 0	18	0 2 0 2/2	10 0	8 0	12 0	6 0	4 0
11, a.m....	19 1 0 0/1	8 0 0				8 0	19 0 4/5	16 0 1/5	15 0 0/5
p.m....	18 0 0	8 6/8				10 0	10 0	15 0	6 0
12, a.m....	11 0 0					6 0	5 0 4/5	8 0 0/5	2 0 0/2
p.m....						6 13	4 26	6 0	3 0
13, a.m....	18 0/18							20 0	10 0 0/5
15.....								51	15

\* Column headings as described in the table in Experiment 1.

† Accurate counts were not made of the numbers of lice which fed twice daily and the numbers which died in the interval between first infective meal and the first notes in the table.

*Results of Animal Inoculations.*—Group a. Normal controls: Segments of nineteen lice stored in CO<sub>2</sub> box after being smeared. On Sept. 15 they were thawed rapidly and ground in 2 cc. broth. The suspension was injected as follows: 0.5 cc. into two guinea pigs which succumbed to intercurrent infection without yielding any information; 0.25 cc. intraperitoneally into four gerbilles. These remained well; two were sacrificed on the 10th day and brains pooled in saline to make a 10 per cent suspension which was inoculated into four normal gerbilles. These and the two gerbilles remaining from the original lot were immunity tested on Oct. 10 by the intravenous injection of approximately two fatal doses of yolk sac suspension (Breinl strain); all six succumbed either to toxic effects or to the infection.

Group b. Bleb lice: One louse which died on Sept. 10 was stored in the CO<sub>2</sub> box until Sept. 11 and then added to the lot of eight lice which were sacrificed at that time. After smears had been made the segments were pooled, and ground in 2.0 cc. saline. Two guinea pigs were inoculated intraperitoneally with 1.0 cc. each (Nos. 762 and 766). Both developed characteristic febrile reactions; No. 762 was sacrificed and in the smears made from the fibrinous exudate over the spleen, numerous cells were found which contained many typical rickettsiae in their cytoplasm. Serum was obtained from guinea pig 7-66 on the 7th day after the febrile period had ended; titer of 1/320 against epidemic antigen was obtained in the complement fixation test.

Group c. Eighteen live lice were sacrificed on Sept. 10. They were divided into two lots, one of which was sprayed with lousicidal chemicals for the purpose of another experiment. The unsprayed lot was ground in broth and inoculations were made intraperitoneally into two gerbilles. These gerbilles were sacrificed on Sept. 16, smears of peritoneal exudate were negative; brains pooled in 10 cc. broth and inoculated into two guinea pigs. Both pigs succumbed to intercurrent infection, but in one of the two, typical intracellular rickettsiae were demonstrated in abundance in smears from spleen exudate and from tunica vaginalis.

Group *d*. No animal inoculations were made.

Group *e*. Twenty-eight live lice were used on Sept. 10 for a spray experiment. Nine of the lice, unsprayed, were ground in 1 cc. broth and the resulting suspension was inoculated intraperitoneally into two gerbilles. One of these died on Sept. 14; at autopsy a sticky peritoneal exudate was present, and many cells packed full of rickettsiae were seen in the smear. The second gerbille became sick, was sacrificed on Sept. 16 for passage of brain to four gerbilles (0.25 cc. of brain in 5 cc. broth). The latter were tested on Oct. 10 and were immune to approximately two fatal doses of Breinl yolk sac, intravenously.

Group *f*. On Sept. 12, six live lice were ground in saline and inoculated intraperitoneally into two gerbilles. The gerbilles were sacrificed on Sept. 22; brains were pooled in 10 cc. saline, and 0.25 cc. of the resulting suspension was inoculated into four second passage gerbilles. Immunity test Oct. 10 showed them to be immune.

Group *g*. On Sept. 10, eight live lice were worked up exactly as were the lice in group *f*, with identical results.

Group *h*. In this group, twenty lice were smeared shortly after death, and the segments were stored in the CO<sub>2</sub> box until Sept. 15 when 51 live lice were added to the segments and the entire lot ground in 2.0 cc. broth. The resulting suspension was inoculated intraperitoneally into four gerbilles. All four died in less than 24 hours without evidence of bacterial infection; death was presumed to be due to toxic effects of rickettsiae.

Group *i*. The segments from ten dead lice after smears were made, were added to fifteen live lice on Sept. 15. The entire lot was ground in 1.0 cc. broth. Two gerbilles were inoculated intraperitoneally with 0.5 cc. each. One of these died on Sept. 19 but the organs were so badly decomposed that no information was obtained from smear. The other gerbille was sacrificed on Sept. 25 and brain suspension inoculated intraperitoneally into four second passage gerbilles. The latter were tested on Oct. 10 and were immune to approximately 2 fatal doses of Breinl yolk sac intravenously.

### *Experiment 8*

*Oct. 2, 1944:* 900 gm. rabbit. Anesthetized with pentobarbital intravenously. 8 cc. of 11 per cent yolk sac suspension (Breinl strain) injected intravenously between 11:02 and 11:04 a.m. Approximately 2000 lice were fed on the rabbit's abdomen between 11:30 and 12:30 o'clock. A second meal on the inoculated rabbit was permitted at 5:00 p.m. on Oct. 2. This rabbit died a few hours later. The lice were fed thereafter on a normal rabbit, twice daily, with one exception, in the afternoon of Oct. 5 when no feeding was done. The last meal was given on Oct. 8 in the morning. The lice were starved for 24 hours, then harvested and stored in the CO<sub>2</sub> box. All the lice which died between Oct. 2 and Oct. 5 were discarded. Those which died between Oct. 5 and Oct. 8 were stored in the CO<sub>2</sub> box. The feces deposited between Oct. 2 and Oct. 5 were discarded; collections were made daily between Oct. 5 and Oct. 9, and stored in the CO<sub>2</sub> box. On Oct. 9 all the surviving lice were combined with those which had been stored. The feces were similarly prepared. No fluid was added either to the tubes of lice or feces before they were placed in the CO<sub>2</sub> box.

*Results of Cotton Rat (22) Inoculations.*—One tube of infected louse feces was thawed rapidly, and approximately 50 mg. of dry feces were ground in 1.5 cc. sterile milk. Two cotton rats inoculated intraperitoneally with 0.5 cc. each. One rat died of bacterial infection. The brain of the surviving rat was removed 10 days after inoculation and six cotton rats were inoculated intraperitoneally with 0.25 cc. of a 10 per cent suspension. These rats were immunity tested by intracardial inoculation of infected yolk sac and all six resisted a dose of rickettsiae which killed all the control cotton rats.

The frozen lice were rapidly thawed. Fifty lice were ground in 2.0 cc. of saline. Another group of 50 lice was ground in 2.0 cc. of milk. Nine cotton rats were inoculated intracardially

with these suspensions, 0.25 cc. for each rat. Three rats died of bacterial infection in less than 48 hours. Six rats became sick on the 6th day and died or were sacrificed for information on the 7th day. Abundant rickettsiae were observed in each of the six. Passages and immunity tests showed the presence of a typical louse-borne strain of typhus in the lice.

Smears at random showed definitely positive rickettsiae in eighteen of twenty lice and in the saline suspension of the infected feces.

*Results Obtained with the "Bleb Technique" of Infecting Lice.*—In the bleb experiments lice ingested a variety of mixtures: (1) human defibrinated blood

TABLE I  
*Lice Infected by "Bleb Technique"*  
*Summary of Experiments 1, 2, 3, and 7*

Experiment	Initial No. of lice	Ratio and per cent of lice which survived 3 days*		Ratio and per cent of lice which survived 10 days*		Ratio and per cent smears showing definite rickettsiae to total No. of smears examined			Presence of typhus in lice shown by results of animal inoculations
						Lice dead or sacrificed between			
						0 to 2 days	3 to 10 days		
		<i>per cent</i>	<i>per cent</i>		<i>per cent</i>	<i>per cent</i>	<i>per cent</i>		
1	53	40/44	91	7/24	29	0/5	16/19	84	Yes
a	6	6/6	100	2/5	40	0/1			Yes
2 b	6	6/6	100	4/6	67	0/1			Yes
c	9	9/9	100	5/8	63	0/1			Yes
3	7	7/7	100	4/7	57				Yes
7	12	10/12	83	8/12†	67	0/2	6/10	60	Yes
Total of all four experiments.....	93	78/84	93	30/62	48	0/10	22/29	76	

\* When living lice were removed for examination, the number thus sacrificed was subtracted on the proper day from the total number originally present for the calculation of the percentage survival.

† Sacrificed on the 9th day.

and saline peritoneal washings from an infected gerbille; (2) human serum and gerbille brain; (3) human serum and gerbille tunica washings; (4) human serum and infected yolk sac suspension. The results are summarized in Table I.

In spite of the diversity of composition of the mixtures which the lice ingested, 93 per cent survived 3 days or more and 48 per cent were alive at the end of 10 days. Approximately three-fourths of the smears made of lice which died or were sacrificed from 3 to 10 days after the infective meal showed definite rickettsiae. Animal inoculations gave positive results from each lot of bleb-infected lice. Both murine and epidemic louse-borne strains were successfully employed in these tests.

TABLE II  
*Lice Infected by the I. V. Technique*  
 Summary of Experiments 4, 5, 6, and 7

Interval between inoculation of rickettsiae into the rabbit and the first feeding of each group of lice on that rabbit	Initial No. of lice	Ratio and per cent of lice which survived at least 3 days*		Ratio and per cent of lice which survived at least 7 days*		Ratio and per cent of smears showing definite rickettsiae to total No. of smears examined; lice which died or were sacrificed		Presence of typhus in the lice shown by the results of animal inoculations		
			per cent		per cent	In the interval from 0 to 48 hrs. after first infective meal	60 or more hrs. after first infective meal			
hrs.			per cent		per cent	per cent	per cent			
0-1/4	229	51/64	80	44/228	19	4/9	44	48/49	98	Yes
1/4-1/2	64	43/64	67	19/62	31	2/6	33	37/40	93	Yes
8 1/2	20	19/20	95	5/19	26	0/1	0	13/19	68	Not attempted
16	20	10/20	50	5/18	28	0/6	0	4/11	36	Not attempted
21	180			38/180	21			10/20	50	Yes
22 1/2	20	20/20	100	10/20	50			8/20	40	Yes
40	21	14/21	67	9/21	43	0/4	0	8/17	47	Not attempted
45	200			35/200	18			6/18	33	Yes
46	20	16/19	84	5/19	26			8/20	40	Yes
69	200			30/200	15			9/23	39	Yes
93	150			51/150	34			1/20	5	Inconclusive
117	50			15/50	30			0/17	0	Yes
Total of all lice fed on inoculated rabbits regardless of interval between I.V. injection and first feeding.....	1174	173/228	76	266/1167	23	6/26	23	152/274	56	
Normal lice fed only on uninoculated rabbits.....	40	40/40	100	38/40	95			0/40	0	No

\* When living lice were removed for examination, the number thus sacrificed was subtracted on the proper day from the total number originally present for the calculation of the percentage survival. Complete daily counts were not made on all groups of lice.

*Results Obtained with the "I.V. Technique" of Infecting Lice.*—The data from Experiments 4, 5, 6, and 7 are summarized in Table II. Various aspects of this summary are discussed in the following paragraphs.

Rickettsiae could not be found in smears of normal control lice from the stock colony which were nourished on uninoculated rabbits, nor did such lice contain *R. prowazeki* as judged by results of animal inoculations.

By contrast, of the lice which fed in the first 15 minutes after the intravenous injection of the rabbit, rickettsiae were found in smears of four of nine lice examined less than 48 hours after the first infective meal, and in 48 of 49 lice which died or were sacrificed more than 60 hours after their first infective meal.

The percentage of positive smears decreased as the interval between inoculation of the rabbit and the first infective meal for the lice became longer: the 8½ hour group had 68 per cent positive; the 46 hour group, 40 per cent; the 69 hour group, 39 per cent; the 117 hour group, 0 per cent. (These figures refer only to smears made from lice which survived for 3 days or more after their first infective meal.)

After feeding on an inoculated rabbit the lice suffered a high mortality: of 1174 lice, approximately three-fourths were alive at the end of 3 days (incomplete counts), and only 23 per cent at the end of 7 days (complete count). In contrast, the control groups suffered almost no mortality: of 40 lice which fed only on uninoculated rabbits, all were alive at the end of 3 days, and 95 per cent at the end of 7 days.

The low percentage of survivors 7 days after the first infective meal is entirely consistent with the course of typhus infection of lice as described in the literature. The high percentage of survival of two different groups of normal lice fed on uninoculated rabbits in a similar fashion is taken as an indication that the high mortality of the infected groups was not ascribable to the feeding technique employed.

The results of guinea pig, gerbille, and cotton rat inoculations clearly showed that the lice which ingested an infective meal developed rickettsial infections and that the normal stock lice contained no typhus rickettsiae after comparable periods of feeding on uninoculated rabbits.

There was close correlation between the demonstration of definite rickettsiae in smears and the results of inoculation of the remnants of the smeared lice into animals, although the animal tests were positive in Experiment 7, group *i*, when the smears had not shown any definite rickettsiae.

In this regard, Weigl stated that in his experience it was sometimes necessary to wait 8 to 12 days, or even as long as 25 days, before the invasion of the louse intestinal cells was completed. For this reason he cautioned against concluding from smears or even from fixed sections made after 8 to 12 days that a given louse was not infected with *R. prowazeki* (6). The interval covered by our experiments was only 7 to 10 days and we have no data on the late development of *R. prowazeki* in rabbit-fed lice. It is probable that the lice which had negative smears in Experiment 7, group *i*, which nevertheless infected animals with typhus, would have shown rickettsiae if the experiment had been conducted for a longer period.

In Experiment 8 a large number of lice (approximately 2000) were infected in less than an hour by one technician who was assisted by another person only while the rickettsial suspension was injected into the rabbit.

## DISCUSSION

The experiments demonstrate the ease and rapidity with which lice can be infected with typhus rickettsiae, either murine or epidemic louse-borne strains. No elaborate apparatus is required and no special skill is involved.

At the outset it was anticipated that the rickettsiae which were injected intravenously into the rabbits would be rather promptly cleared from the blood. Contrary to expectations it was found that a considerable number of lice (at least 39 per cent) became infected although their first meal on the inoculated rabbit occurred as long as 69 hours after the intravenous inoculation. This fact indicates that rickettsiae were present in the blood stream of the rabbit for at least 3 days. The animal inoculations and the negative smears of control lice fed on uninoculated rabbits in a similar fashion are taken to indicate that the rickettsiae in smears of the lice which had fed on inoculated rabbits were *R. prowazeki* and not *R. pediculi* or other species. It is probable that some of the lice which were called negative on the basis of the examination of a single smear may have had typhus rickettsiae in numbers too few for detection by such a rapid method of making smears.

The long period during which rickettsiae continue to circulate in the blood of rabbits permits the infection of very many more lice on a single rabbit than would be possible if all rickettsiae were eliminated from the blood within an hour or two.

An interesting question arises in connection with the uninoculated rabbits on which many typhus-infected lice were permitted to feed. Do these rabbits become infected with typhus? The shaved skin of the abdomen was carefully cleaned with ethyl alcohol after each feeding. A group of normal lice was fed for 10 days on one such rabbit beginning several days after its first exposure to several groups of infected lice, and no trace of infection with *R. prowazeki* was observed in this control group, which strongly suggests that there were no circulating rickettsiae in the rabbit's blood during that interval. Unfortunately, the rabbits were discarded without serologic studies which might have thrown more light on this question.

On two occasions we observed that naturally infected human lice (taken from the clothes of typhus patients) could be successfully transferred to rabbits for several days and that, as in Nöller's experiments, the animal blood did not interfere with the development of heavy infection of the lice. Those observations, combined with the data of the eight experiments, have convinced us that, with attention to certain important details, rabbits can be substituted for human hosts in the study of typhus-infected lice. It is our belief that for some types of laboratory studies of typhus, the louse would be more suitable than are various experimental animals, such as guinea pigs, mice, cotton rats, and gerbilles. Certainly the use of lice instead of animals would be less expensive than the usual procedures for the detection of viable *R. prowazeki*.



From the purely biologic and physiologic points of view, the "bleb technique" herein described would make possible a study of the nutritional requirements of the louse. Weigl demonstrated that human lice could be nourished successfully by intrarectal injections of defibrinated human blood twice daily. Whether all of the components of defibrinated blood are actually necessary to sustain life in the louse is a question that could be readily studied with intradermal blebs of varying compositions.

The bleb technique is entirely suitable for the study of the very smallest lice, newly hatched larvae and nymphs, whereas the intrarectal injection of such small lice by Weigl's method is particularly difficult.

On the basis of these experiments one can speculate that it might be simple and practical to study murine strains after specified numbers of serial passages in lice, or the behavior of other rickettsiae and other pathogenic organisms in lice. Certainly the production of Weigl-type typhus vaccine could be greatly simplified by the use of the techniques described herein.

If ordinary precautions are taken in the handling of infected lice and their excreta, *i.e.* wearing of gloves and face masks, it is no longer necessary to regard the study of infected lice as more hazardous than any other laboratory procedure involving *R. prowazeki*. It is worth noting that the technician who performed Experiment 8 had not had typhus previously; he carefully observed the usual precautions and did not contract typhus although he placed the 2000 infected lice twice daily on the rabbit for 7 days. Two other workers who manipulated infected lice *without* wearing rubber gloves and gauze face masks did contract mild typhus in the course of their work, however. All three workers had received typhus vaccine at regular intervals for approximately 2 years before this study began.

The risk of infection of laboratory personnel could be greatly reduced by the use of feeding boxes, as described by Wolbach, Todd, and Palfrey (5).<sup>5</sup> For the study of pathogenic agents against which the laboratory personnel has not been vaccinated the careful technique employed by Wolbach *et al.* should be meticulously followed. A somewhat higher mortality of the lice occurs when they are confined to feeding boxes since they do not feed as readily on rabbits through the meshes of bolting silk as they do on human beings, despite the use of saliva or perspiration to enhance the attractiveness of the rabbit.

All active stages of lice were found capable of acquiring the infection when allowed to ingest one or more infectious blood meals. The relatively high mortality of the 1st nymphal instar, even under normal conditions, makes the use of this instar impractical for work of this nature. The optimum size of

<sup>5</sup> Although the members of the Typhus Research Commission of the League of Red Cross Societies who worked in Warsaw in 1920 were not vaccinated, and although they had intimate exposure to infected lice and louse feces, their technique was successful in preventing any cases of typhus in their group (23).

lice for use in such tests appeared to be the 3rd instar nymphs. In general, the mortality among lice of this instar is low, and life expectancy high. The capacity of the ventriculus is large so that a greater number of rickettsiae in the infectious blood meal may be ingested, thus enhancing the possibility of the infection becoming established in the gut tissues of the lice.

#### SUMMARY AND CONCLUSIONS

Experiments are described which demonstrate that human body lice (*Pediculus humanus corporis*), were infected experimentally with murine and epidemic louse-borne strains of typhus fever by feeding on suitably prepared rabbits. Details of the two methods of infection, the "bleb technique" and the "I.V. technique," are presented.

It is concluded that the experimental infection of human lice with typhus can be accomplished very easily and rapidly with these methods. The possible applications of the method are discussed.

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EXPLANATION OF PLATES

PLATE 1

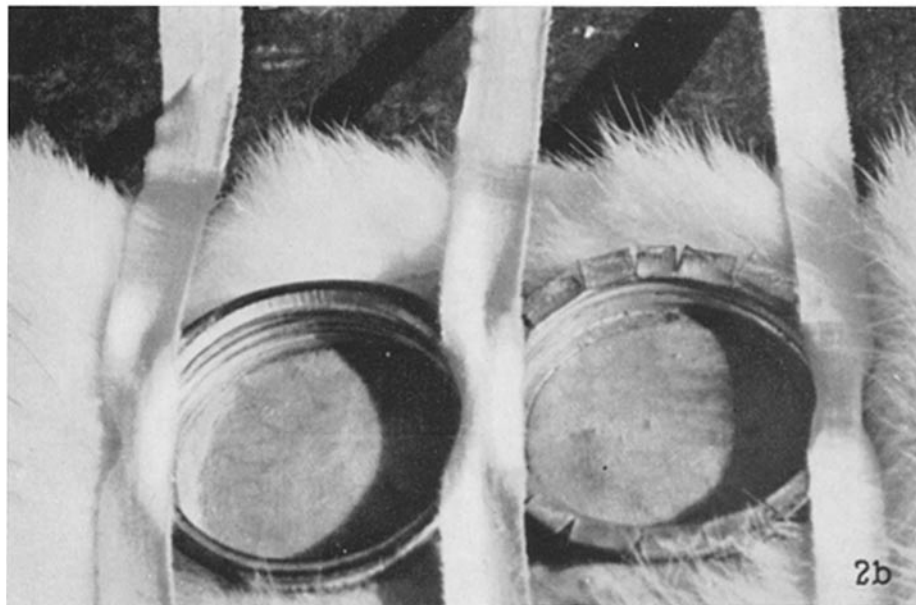
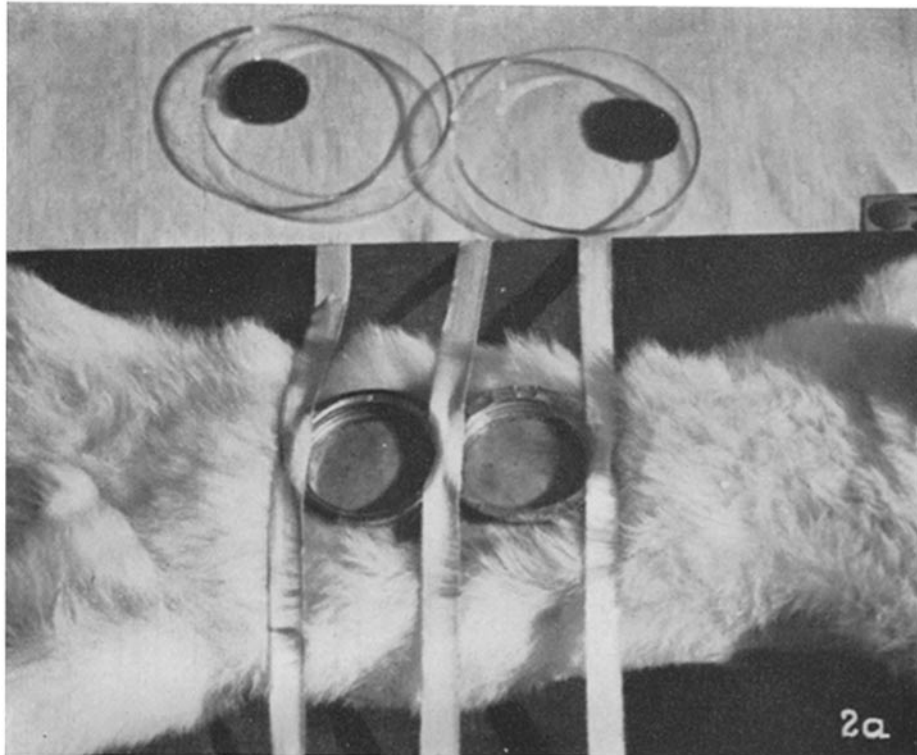
FIG 1. Nourishment of the normal colony of lice



(Snyder and Wheeler: Experimental infection of human body louse)

PLATE 2

FIG. 2*a*. Feeding boxes in place. Lice on pads in Petri plates beside rabbit.  
FIG. 2*b*. Closeup view of Fig. 2*a*.



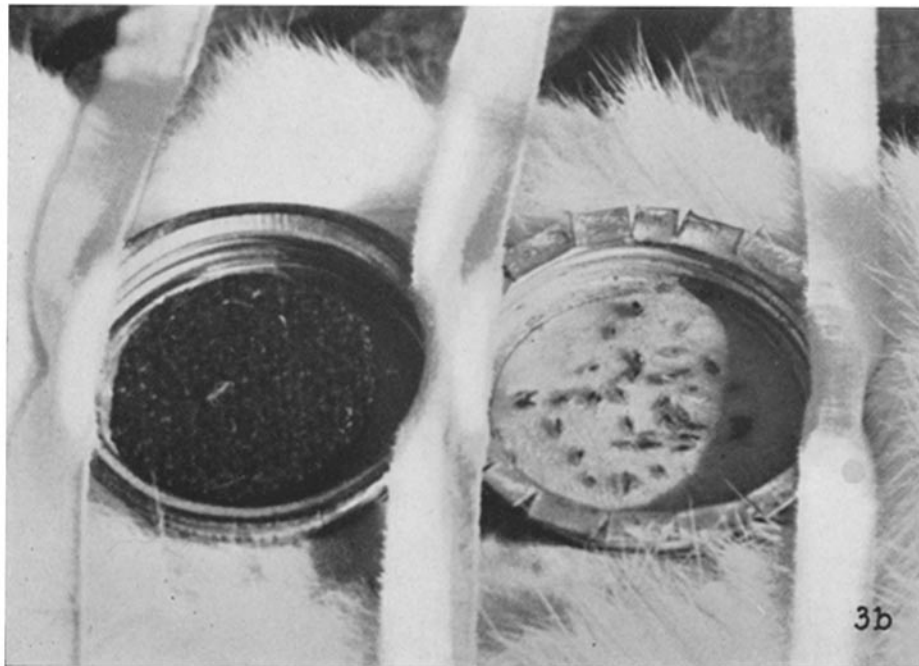
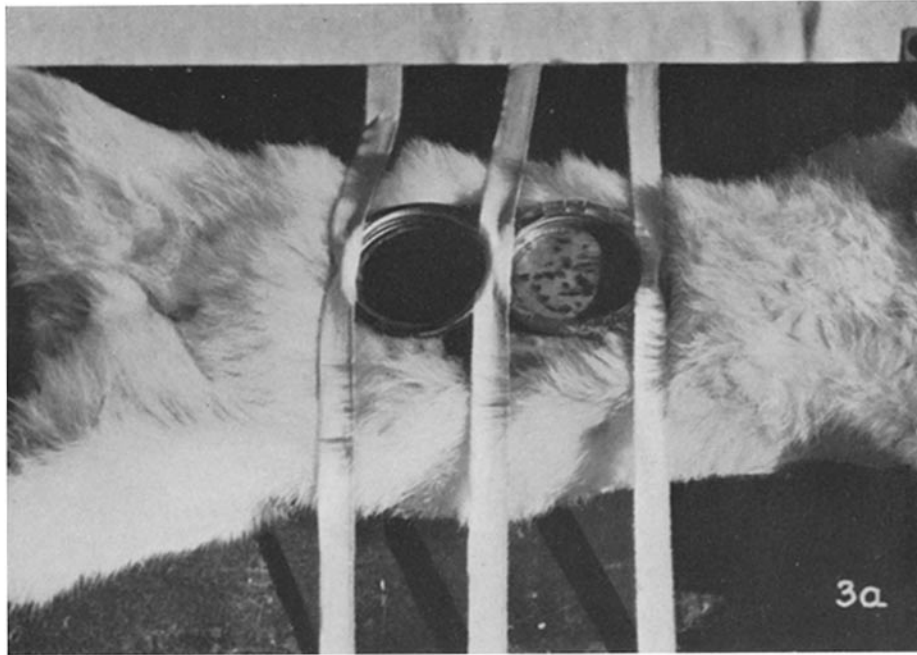
(Snyder and Wheeler: Experimental infection of human body louse)

PLATE 3

FIG. 3*a*. Lice in feeding boxes on the rabbit. At the left, the cloth pad covers the lice. At the right, the lice can be seen at various stages in the feeding.

FIG. 3*b*. Closeup of Fig. 3*a*.





(Snyder and Wheeler: Experimental infection of human body louse)