FATAL INFECTION OF IRRADIATED WHITE MICE WITH EUROPEAN TYPHUS BY THE INTRA-ABDOMINAL ROUTE

By P. Y. LIU,* M.D., JOHN C. SNYDER,** M.D., AND JOHN F. ENDERS, PH.D.

(From the Department of Bacteriology and Immunology, The Harvard Medical School, Boston)

PLATE 33

(Received for publication, March 4, 1941)

The study of classical European typhus in the laboratory has long been handicapped by the lack of suitable experimental animals. The guinea pig, which until very recently has been the only convenient species available, usually shows no evidence of infection other than a mild febrile period lasting a few days. To prove that this fever is the result of infection with European typhus, it must be shown that typical brain lesions are present, that bacteria cannot be grown from the blood or tissues of the guinea pig, and that resistance to known homologous passage strains has developed after convalescence. Furthermore, since 2 to 4 per cent of normal guinea pigs fail to exhibit fever following inoculation of fully virulent material, the inclusion of several animals for each test is essential. The interpretation of febrile episodes in guinea pigs without resort to the criteria just noted is hazardous; and it follows, therefore, that rigidly controlled experiments are laborious and time-consuming. Demonstration of rickettsiae by tissue culture method or egg inoculation, though very helpful, does not shorten materially the time required to determine the presence or absence of the specific disease.

In normal mice, European typhus exists as an inapparent infection in which the virus disappears after three passages, when the method of passing brain to the abdominal cavity is employed (1). Inoculation of the murine variety of rickettsiae, on the other hand, according to Wohlrab (2) leads to a fatal outcome in 60 per cent of his strain of mice, whether the inoculum is given intraperitoneally or intranasally. Castaneda has described a lethal pneumonitis in mice and rats resulting from the intranasal inoculation of the murine strain (3).

Recently Durand and Sparrow (4), using lice heavily infected with the

* Research Fellow of the China Foundation for the Promotion of Education and Culture and the China Program of The Rockefeller Foundation.

** Staff Member of the International Health Division of The Rockefeller Foundation.

rickettsiae of European typhus, inoculated mice intranasally and were able to pass the infection thus established in series. The mortality was high among their animals. The mouse obviously possesses advantages in many ways over the guinea pig for the investigation of certain problems, but the intranasal route is dangerous, a fact attested by the occasional human infections in laboratories where this procedure is employed (5). It is especially to be avoided if unvaccinated persons have access to the rooms containing infected animals. Since the data presented by Durand and Sparrow (4) show that only a heavy inoculum is effective and since x-radiation increases the susceptibility of rats to murine typhus (6), it seemed probable that, provided large inocula were employed, the intra-abdominal route might prove successful in irradiated mice with European typhus. This report describes experiments which were undertaken with these considerations in mind.

Methods

X-Radiation.—A metal container 3 cm. deep and 20 cm. in diameter, having a flange about the edge, was fitted with a wire screen (2 or 3 meshes to the inch) which, as a cover, could be clamped tightly to the flange. Twenty mice can be accommodated with ease in a container of this sort. In it they cannot change appreciably either their distance from the target of the x-ray tube by standing up, or their exposure to the rays by crawling on top of one another. Uniform irradiation of each animal is thus obtained. The x-ray machine was operated at 200 kilovolts, 8 milliamperes, 0.5 mm. copper filter, 30 cm. distance. Under these conditions an exposure of 7 minutes and 30 seconds represents 450 Roentgen units. Data relating to the determination of the optimum interval between the time of irradiation and injection and to the effect of irradiation on non-infected mice will be presented after a description has been given of the basic observations concerning the nature of the infection in irradiated animals.

Typhus Strain.—The Breinl strain of European typhus obtained from the National Institute of Health in Washington, D. C., through the courtesy of Dr. R. E. Dyer, in February, 1940, was isolated in tissue culture (7) and maintained for 5 months by serial passages in the yolk sac of developing chick embryos (8) or on agar tissue cultures until the inception of the experiments described below.

Suspensions for injection of mice were prepared from infected chick embryonic tissue incubated at 37°C. for 7 days on beef serum-Tyrode agar in Kolle flasks. Only those flasks were used which showed many rickettsiae on direct smear. The tissue from each Kolle flask was washed down with buffered saline (pH 7.0) and ground to a fine suspension in a special 50 cc. centrifuge tube fitted with a pestle. In one experiment the tissue from one Kolle flask was diluted with 18 cc., in another with 30 cc. The supernatant fluid obtained after the "Griffith tube" had been standing for a few minutes was injected into the mice. The suspension was not centrifuged.

Establishment of Rickettsial Infection in Irradiated Mice

Injection intra-abdominally of 1 cc. of a tissue culture suspension of rickettsiae produced no evidence of sickness in sixteen normal mice. This

was confirmed by a second experiment. Rickettsiae could not be demonstrated in smears of fluid obtained by aspiration from the peritoneal cavities of such non-irradiated mice 2 to 10 days after injection. No titration of the infectivity of the inoculum for guinea pigs was carried out. Mice weighing from 10 to 20 gm. given 450 to 600 Roentgen units and injected with 1 cc. of the inoculum which had been shown to produce no recognizable disease in normal mice, either died or presented evidence of illness (anorexia, ruffled fur, conjunctivitis). Numerous rickettsiae (demonstrated by Macchiavello stain) were visible inside the cells of the peritoneal exudate and in scrapings of the peritoneal surface from the mice which died, as well as in the mouse sacrificed for passage. Tissue culture suspensions were inoculated into a total of twenty-four irradiated mice; two survived, one was sacrificed in a moribund condition for passage, and twenty-one died.

Serial Passage in Irradiated Mice.—From one set of irradiated mice infected with material from tissue cultures, three series of transfers in mice were made. In two of them material for transfer was taken after the death of the animals, and bacterial infection occurred. The third remained free of bacteria and has been carried through twenty-two passages by successive intra-abdominal inoculation. The material for passage was obtained either by bleeding from the heart just before death, or by sacrificing a moribund mouse and washing out the peritoneal cavity with 5 to 10 cc. of saline, nutrient broth, or phosphate buffer at pH 7.0. At present broth is employed as routine. In other experiments successful transfers were made not only with blood and peritoneal washings but also with brain, lung, pleural exudate, and spleen. Rickettsiae were demonstrated by Macchiavello stain in the peritoneal exudate and also in blood, suprarenal gland, tunica vaginalis, kidney, spleen, liver, and pleural fluid, but not in brain. Occasionally the lungs of a moribund mouse exhibited hemorrhagic areas varying in size from 1 to 5 or 6 mm. in diameter. On direct smear rickettsiae could be found in such lesions, chiefly inside the phagocytic cells.

Fig. 1 shows the appearance of the peritoneal smear on the third and eleventh mouse passages. In the latter the rickettsiae are smaller, and many more are present both inside and outside the cells.

In a titration of the infectivity of blood after sixteen passages, in one small experiment 0.00017 cc. of heart's blood (diluted in nutrient broth) was given intra-abdominally to irradiated mice. They died 10 to 12 days later, and a few rickettsiae were demonstrable in peritoneal smears at death. Brain was found to be infectious only in a dilution of one to five hundred. Further titration experiments after the strain has been passed through several more generations will be needed before conclusions can be drawn as to the infectivity of blood and other tissues. It is clear, however, that

blood from one moribund mouse was sufficient to infect fatally a considerable number of irradiated mice. In routine passage experiments with mice given 450 R, the mortality following injection of 0.5 cc. to 1.0 cc. of peritoneal washing was regularly 95 to 100 per cent in 4 to 9 days. It is especially important to select a moribund animal for passage rather than to use one which has been dead even for a few hours. Bacterial contamination in the latter instance was not an infrequent finding in our experiments.

Results Obtained in Normal Mice with Passage Material from Irradiated Mice.—Normal mice failed to show any evidence of infection when injected with passage material from irradiated infected mice. Eighty-six animals were inoculated with peritoneal washing, blood, or brain obtained from the first to the fifteenth passages, inclusive, and from the eighteenth passage. Attempts were made to infect very young mice (1 day to 3 weeks old) on five occasions. In two trials rickettsiae were demonstrable at death of the baby mice, but three additional experiments resulted entirely in failure. It can be stated at present that as a result of eighteen passages in irradiated mice the strain had not acquired virulence for non-radiated mice with the possible exception of newborn animals.

Behavior of the Mouse Passage Strain in Guinea Pigs.—To show whether the organisms observed in the irradiated mice after serial passage were identical with the typhus rickettsiae originally introduced, guinea pigs were inoculated intra-abdominally with material from the first, second, third, fourth, and eleventh mouse passages, allowed to recover, and then tested for immunity to the Breinl strain which had been maintained continuously in the guinea pig. The daily temperatures of guinea pigs after injection with mouse passage material are recorded in Table I*a* and Table II*b* (Nos. 7-84, 7-29, 7-31, 7-89). Table I*b* represents the temperatures of some of those animals following the test dose with the Breinl strain (0.1 gm. of guinea pig brain removed on the 3rd or 4th day of fever), along with the temperatures of ten normal guinea pigs injected with the same quantity of the same suspension of brain. It should be stated that for the purpose of an experiment unrelated to this work forty-two normal guinea pigs were injected with portions of the same pooled inoculum. The ten described here in detail are representative. Of the forty-two normals, forty showed fever curves characteristic of European typhus. Temperatures over 104°F. (40°C.) are set in bold-faced type and are taken to indicate fever in our stock of guinea pigs. It is evident from Table I*a* and *b* that the guinea pigs given material from the first few passages in irradiated mice exhibited an irregular, atypical febrile response, but all were immune to a dose of infective guinea pig blood and brain which caused fever in forty of forty-two normal controls.

Blood was taken on the 3rd day of fever from one of the animals infected with mouse material of the fourth passage (guinea pig 30) for culture and injection intra-abdominally into a normal guinea pig (No. 38). No bacteria

TABLE I*a*

Temperatures of Guinea Pigs after Injection of Brain, Pleural Fluid, or Peritoneal Washings of Irradiated Infected Mice, First to Fourth Passages Inclusive

(Temperatures in degrees above 100°F.)

Guinea pig No.	Inoculum	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22
9	Per. wash. mouse 1st gen.	3.0	4.1	2.2	2.3	3.2	3.4	3.4	4.1	3.5	3.6	2.1	2.0	1.8	2.6	4.6	2.2	1.8	2.1	1.8	2.2	2.4	
13	Brain mouse 1st gen.	4.0	3.0	1.7	4.2	5.6	4.5	3.9	2.7	2.2	1.8	2.4	3.4	4.5	4.7	3.9	4.6	5.0	5.6	4.2	3.3	2.8	
12	Pleural fl. mouse 1st gen.	3.8	3.2	3.0	2.8	2.9	3.1	3.0	2.4	1.9	2.2	3.2	4.6	4.7	4.5	4.0	3.0	4.2	4.1	3.5	3.2	2.0	
7-99	Per. wash. mouse 2nd gen.		3.0	1.7	2.9	3.9	4.5	4.8	5.4	4.3	4.6	4.7	5.0	3.9	4.7	2.4	2.5	2.0	2.4	2.7	3.1	2.6	
92	Brain mouse 1st gen.	2.8	4.2	2.7	3.8	3.7	3.8	3.2	3.0	4.9	4.1	3.6	3.8	2.3	3.0	2.8	3.0	3.6	2.4	3.5	2.4	2.6	
22	Per. wash. mouse 3rd gen.	3.5	2.5	1.0	2.5	4.1	4.4	4.7	4.6	3.9	5.2	4.6	4.5	3.7	4.4	2.2	2.0	2.0					
30	" " " 4th "	3.6	3.5	4.5	5.6	5.9	4.9	5.0	2.3	4.3	4.8	4.7	4.5	4.0	3.2	2.0	1.8	2.6	2.7	2.3			
16	" " " 4th "	2.9	3.5	3.8	3.9	4.4	3.9	5.2	5.3	5.4	4.0	3.8	3.4	3.0	2.4	2.4	2.5	2.1	2.6				
38	Blood, pig 30	2.0	1.3	1.8	4.9	2.4	2.2	3.8	3.8	4.6	4.6	5.6	4.7	5.3	4.8	4.7	4.0	3.0	2.9	2.5	2.7		

TABLE I*b*

Temperatures of Guinea Pigs Recovered from Infection with Mouse Material Given Subsequent Inoculum of Breinl Passage

(Temperatures in degrees above 100°F.)

9	Recovered from infection		2.7	1.7	2.3	2.7	1.8	2.0	2.0	1.9	2.0	1.4	2.6	1.8	1.8	1.9	1.1	1.2	1.8	2.1			
13	with mouse material		1.8	1.3	2.6	3.2	2.0	2.6	2.4	1.7	2.3	2.2	1.8	1.9	2.2	2.8	2.4	3.4	2.9	2.2			
12			1.9	2.2	2.1	2.1	2.0	2.9	2.0	1.6	2.4	1.7	2.0	2.4	1.8	1.8	1.9	1.9	1.4				
7-99			2.5	3.5	2.7	3.2	3.0	2.8	2.6	2.9	2.4	2.0	2.8	2.3	3.0	2.2	2.2	1.8	2.4	2.0			
92			3.0	2.1	2.3	3.2	2.7	2.9	2.2		2.3	2.2	2.1	2.7	3.7	2.8	4.0	2.7	3.2	2.7			
22			2.6	2.8	2.9	3.5	3.1	2.2	2.6	2.0	2.2	1.6	2.8	2.0	2.6	2.5	1.8	2.0	1.7	2.2			
30			2.8	2.2	2.5	2.6	1.8	2.0	2.8	2.6	1.8	1.6	2.7	2.0	2.8	2.5	1.8	2.2	1.9	2.0			
16			2.8	1.7	2.5	3.4	2.4	2.2	2.0	2.0	2.3	2.0	2.6	1.9	2.3	2.1	2.3	2.6	2.6	2.5			
38			2.6	3.0	2.5	2.6	2.2	3.0	2.6	3.0	2.8	3.0	3.6	2.4	2.1	2.6	2.8	1.7	2.8	2.6	2.4		
7-64	Normal controls	3.0	3.8	3.2	2.7	2.7	2.8	2.2	2.2	3.0	5.3	4.7	5.0	5.2	5.6	3.9	4.1	3.6	2.3	2.2			
7-53		3.0	3.6	3.3	3.7	2.8	2.7	2.7	3.3	3.5	2.8	5.4	4.7	5.8	5.6	5.6	5.3	4.8	4.0	3.2	4.0	2.5	2.1
7-80		3.5	2.8	3.1	2.9	2.9	2.6	2.7	3.0	3.4	3.6	5.4	4.7	4.5	4.8	4.9	4.9	4.5	3.0	2.8	2.5		
7-56		3.3	2.8	3.0	2.8	3.0	2.6	2.8	3.2	3.6	3.6	3.8	4.6	4.6	5.5	5.4	4.5	3.5	3.4	2.8			
7-66		3.5	1.2	2.0	3.4	3.4	3.5	3.0	3.3	3.5	4.8	5.0	4.3	4.4	5.8	4.7	4.2	3.5	2.8	2.4			
7-50		3.8	2.6	2.7	2.7	2.2	3.3	2.7	2.9	3.7	3.6	3.7	4.7	5.2	5.2	5.4	5.0	4.8	4.2	4.3	3.3	3.6	2.9
7-61		3.5	3.2	2.5	3.0	2.9	2.9	1.9	3.6	3.5	4.7	3.3	3.8	5.8	5.1	5.2	5.0	4.1	3.8	3.4	3.4		
7-55		2.3	3.6	3.2	2.6	3.3	3.2	3.5	2.5	2.7	4.0	4.6	4.5	4.4	5.4	4.8	4.0	3.8	3.0	2.5			
7-38		3.8	2.5	2.5	2.9	2.1	3.0	3.0	2.0	2.5	3.2	4.6	4.6	4.8	4.8	4.5	4.3	3.3	2.8	2.8			
7-45		2.8	3.2	3.3	3.2	2.8	3.0	2.5	3.1	3.0	3.3	4.5	4.0	3.8	3.8	3.6	2.6	3.0	2.8				

were found in the blood culture (3 cc. blood, 50 cc. broth, 2 weeks' incubation). The temperatures of guinea pig 38 are recorded in Table I*a* and are typical of European typhus in the guinea pig. (In a later experiment, three or four guinea pig passages sometimes were made before the temperature curve became consistent and typical of European typhus.) On subse-

quent immunity tests with the guinea pig passage strain, the second generation pig 38 showed no fever (Table I*b*, No. 38). The controls for guinea pig 38 are not included in Table I*b*; twenty normal guinea pigs received 0.1 gm. of 10 per cent brain suspension of Breinl passage animals and nineteen of the twenty showed characteristic temperature curves.

TABLE II *a*

Temperatures of Normal Guinea Pigs Given Routine Inoculum of Breinl Guinea Pig Brain

(Temperatures in degrees above 100°F.)

Guinea pig No.	Previous infection	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
7-04	None	**4.2**	2.6	3.2	2.9	3.7	3.3	**4.5**	**5.0**	**4.7**	**4.7**	**5.2**	**5.1**	**4.5**	**4.0**	3.3	2.7	1.7			
7-16	"	3.6	3.3	3.0	2.7	3.6	3.7	**4.6**	**4.8**	3.8	**4.6**	3.8	**4.5**	3.8	2.8	3.5	3.4				
7-23	"	**4.6**	3.0	**4.3**	2.9	3.8	2.0	**4.5**	**5.2**	**5.1**	**5.8**	**5.0**	**5.3**	**5.0**	**4.6**	**4.4**	**4.3**	3.7	3.4	3.2	
7-24	"	2.8	2.0	2.6	2.5	2.8	2.3	2.5	2.5	2.7	2.9	**5.0**	3.6	**4.5**	**4.7**	**5.0**	**4.6**	3.1	2.7	2.2	
7-46	"	2.6	2.9	2.8	2.8	2.9	2.9	3.5	3.4	**4.7**	**4.0**	3.7	**5.8**	**5.7**	**5.7**	**5.3**	**5.8**	**5.0**	**4.0**	3.2	1.8

TABLE II *b*

Temperatures of Recovered Breinl Guinea Pigs and Normal Controls after Injection of Peritoneal Washing of Irradiated Infected Mice, Eleventh Passage

(Temperatures in degrees above 100.0°F.)

7-84	None	**4.0**	3.7	**5.2**	**4.2**	3.9	**5.3**	**5.3**	**4.7**	**4.6**	**4.2**	3.6	2.6	2.6		2.2	2.0				
7-29	"	3.5	3.6	**5.0**	**4.6**	3.5	**5.5**	**4.2**	2.9	3.6	**4.0**	3.0	1.9	2.2		1.6	1.6				
7-31	"	3.0	2.6	**4.5**	3.6	3.5	**4.0**	**4.5**	**4.0**	**4.0**	3.0	3.5	3.4	2.8		2.7	1.9				
7-89	"	3.8	3.6	**4.8**	**5.3**	2.5	**5.0**	**5.3**	**5.0**	**4.9**	*										
7-04	Breinl	2.5	2.8	3.3	2.6	1.7	2.7	2.3	2.5	3.2	2.5	3.3	1.8	2.3	2.4	1.8	1.6				
7-16	passage	**4.8**	2.0	3.0	1.6	1.8	2.8	2.6	2.0	2.5	2.5	3.7	2.0	2.3	2.6	2.3	1.0				
7-23	" "	3.5	2.8	3.1	2.9	2.0	3.0	2.9	2.9	2.8	3.6	3.2	2.6	2.2	2.8	2.5	2.5				
7-24	" "	**4.5**	1.7	1.8	2.5	3.0	2.5	2.0	1.5	2.5	2.1	2.1	2.0	2.1	1.7	1.6	1.3				
7-46	" "	3.5	2.6	2.6	2.6	2.3	2.9	2.9	2.6	2.3	3.3	2.7	2.3	2.5	2.4	1.4	1.7				

* Sacrificed for passage.

To test for complete cross immunity with the Breinl strain, five guinea pigs which had received guinea pig passage material and which had exhibited typical typhus temperature responses (Table II*a*) were given a rest period of 3 weeks or more and then injected intra-abdominally with 1 cc. of mouse peritoneal washing of the eleventh passage. At the same time four normal guinea pigs were injected with the same inoculum (Table II*b*). The normal guinea pigs developed an early fever and two exhibited very slight scrotal swelling on the 4th day, whereas in the recovered Breinl pigs neither of these morbid manifestations was observed. One of the pre-

viously uninfected pigs (No. 7-89) was sacrificed on the 10th day to initiate a new guinea pig strain which will be referred to as the M XI strain since it derives from the eleventh mouse passage. It was maintained by routine

TABLE III *a*

Temperatures of Guinea Pigs Given Routine Inoculum of Breinl Guinea Pig Brain

(Temperatures in degrees above 100°F.)

Guinea pig No.	Previous infection	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21
7-09	None	3.2		2.8	3.0	2.9	3.8	3.9	4.6	4.3	4.7	4.6	5.0	4.6	4.2	3.5	3.0	4.0	3.3	2.9	3.4	
7-20	"	2.0	2.0	1.0	1.8	1.6	4.6	4.8	4.6	5.5	5.1	5.0	5.0	4.8	3.5	2.3	2.9	1.8				
7-33	"	3.6		2.7	2.7	2.6	4.7	3.9	4.0	4.2	4.1	5.1	5.4	4.7	3.7	3.5	3.0					
7-34	"	2.3	2.4	3.0	2.5	3.3	3.7	4.7	5.5	4.0	4.5	4.5	4.4	4.3	3.3	3.4	2.5					
7-37	"	3.8		2.3	3.8	3.5	4.3	4.0	4.7	4.0	4.6	4.6	5.4	4.7	5.2	3.9	3.2	3.0				
7-62	"	3.8	1.5	2.8	2.5	3.0	2.5	3.5	2.6	3.0	3.8	4.0	4.5	5.2	4.7	5.7	4.5	4.0	4.6	2.5	2.4	2.4
7-69	"	3.3		1.7	3.5	2.1	2.6	2.9	2.6	2.8	3.3	2.7	5.1	4.4	4.5	4.0	4.5	4.5	4.8	4.7	3.6	2.6
7-48	"	2.5		1.5	5.5	3.1	3.8	4.0	3.2	4.3	4.6	4.1	5.4	5.4	4.8	3.8	4.9	3.8	3.0	3.0		
7-72	"	2.8		2.0	2.1	2.6	2.5	4.2	4.5	4.0	4.7	4.6	4.7	4.6	4.2	3.0	2.4	2.0				
7-75	"	3.5		3.2	2.8	2.7	3.0	3.0	4.7	4.0	4.5	4.0	4.8	4.6	4.6	3.8	3.1	2.6				
7-81	"	2.4		2.6	2.5	3.6	3.7	3.2	5.0	3.4	4.5	4.0	4.8	4.6	4.0	4.1	3.3	2.6	2.2			
7-94	"	3.0		2.2	3.1	4.0	3.8	3.8	4.4	3.6	5.0	5.0	4.4	4.4	3.5	3.4	2.4					

TABLE III *b*

Temperatures of Recovered Breinl Guinea Pigs and Normal Controls Injected with Brain of M XI Strain, 6th Generation

(Temperatures in degrees above 100°F.)

7-09	Breinl passage	2.8	2.7	3.0	2.8	3.3	2.8	3.3	2.7	3.5	1.9	1.8	3.0	2.5	2.6	3.4	2.8	2.8	2.7	2.7	2.7	2.9
7-20	" "	3.5	3.0	3.5	3.0	2.3	3.0	3.3	2.9	2.8	2.6	2.8	2.6	2.5	2.3	3.3	3.5	3.1	3.0	2.7	2.1	3.0
7-33	" "	2.6	2.8	2.9	3.0	2.8	2.5	2.7	2.3	3.3	2.7	2.6	2.6	2.5	2.4	3.3	2.8	2.9	2.7	2.1	2.1	2.6
7-34	" "	2.7	2.9	2.7	2.6	3.6	3.1	3.5	2.8	3.3	2.9	3.1	3.7	3.0	2.5	3.5	3.5	2.7	2.5	3.4	2.9	2.9
7-37	" "	2.6	3.3	2.5	3.3	2.5	3.5	2.7	2.1	3.0	2.3	2.7	2.7	2.6	1.8	3.7	3.2	2.5	2.0	2.3	1.8	3.5
7-62	" "	3.2	2.8	2.9	3.6	4.3	3.6	2.8	2.7	2.8	2.8	2.8	3.5	2.8	2.6	3.0	3.9	2.5	2.8	3.3	2.5	2.5
7-69	" "	2.7	2.9	2.8	3.5	3.4	2.7	3.2	2.7	3.0	2.6	2.5	3.3	3.0	2.3	3.1	2.8	2.4	3.1	3.0	3.0	3.0
7-48	" "	2.8	2.4	3.6	2.7	3.6	4.0	3.5	2.8	3.6	3.3	3.4	3.5	3.6	2.9	4.2	2.5	2.6	3.0	2.5	3.0	3.2
7-72	" "	3.1	3.1	3.5	3.2	3.1	2.0	3.1	3.0	3.1	2.3	3.1	2.9	3.1	2.3	3.2	3.3	3.1	2.7	3.0	2.7	3.0
7-75	" "	2.8	2.9	3.5	3.1	3.5	3.5	3.3	2.9	3.5	2.7	2.5	3.0	2.2	2.5	3.5	2.4	2.4	2.0	2.8	1.8	2.6
7-81	" "	2.9	3.5	3.5	2.8	3.0	2.1	3.0	2.7	3.5	3.4	2.7	3.0	3.2	2.1	3.3	3.1	3.0	2.8	2.8	3.1	3.3
7-94	" "	2.9	2.6	3.0	3.1	3.5	3.9	4.1	4.1	3.5	2.9	3.1	3.3	2.9	2.5	3.2	3.2	2.9	2.9	3.0	3.0	3.1
17-02	None	3.5	4.0	3.1	2.9	4.8	4.1	3.9	3.9	4.5	3.5	4.5	4.8	4.8	4.8	4.9	4.1	4.1	3.7	3.5		
17-03	"	2.2	3.7	4.4	4.4	3.4	3.7	3.9	4.1	4.5	5.0	4.8	4.6	5.8	5.5	5.0	4.8	4.4	3.7	3.3	3.4	
17-10	"	2.8	2.9	2.4	2.5	3.4	4.5	3.6	4.6	4.4	4.7	5.8	*									
17-11	"	3.5	4.1	3.6	3.0	3.7	4.5	4.6	4.9	5.3	4.9	5.9	6.0	5.9	5.2	4.9	4.6	3.9	3.5	3.2		
17-16	"	2.5	2.0	2.0	1.9	3.1	3.3	3.5	4.0	4.6	4.6	4.9	5.1	5.8	5.8	6.2	6.4	5.8	2.0	†		

* Sacrificed for passage.

† Died of intercurrent bacterial infection.

brain passage through seven generations of guinea pigs. Twelve recovered Breinl guinea pigs, whose original infection is recorded in Table III*a*, were given 0.1 gm. of the brain and 0.2 cc. defibrinated blood of the fifth guinea pig passage of M XI; six normal control animals received identical

inocula. The course of the infection in these animals is found in Table III*b* from which it can be clearly seen that residence of the strain in mice had not altered appreciably its capacity to persist in serial guinea pig passage or led to obvious changes in immunologic properties. The absence of scrotal swelling as well as the finding of rickettsiae in the tunica cells during early stage of infection after long search in the M XI strain, and the similarity of the temperature curve to the Breinl strain maintained constantly in guinea pigs indicate that no permanent change in its characteristics had taken place as a consequence of eleven mouse passages.

TABLE IV

Effect of Irradiation of Mice at Different Intervals before Injection of Infectious Material

	No. mice	Date of x-ray (500 R)	Date of infection	Deaths, time after injection	Average day of death	Survival at 14th day
				days		*per cent*
Lot A	4	Oct. 28	Oct. 31	4, 6, 7, 7	6.0	0.0
Lot B	4	29	31	4, 5, 6, 6	5.2	0.0
Lot C	4	30	31	3, 5, 6, 8	5.5	0.0
Lot D	4	31	31	10, 10, 10, 10	10	0.0
	No. mice	**Date of x-ray (500 R)**		**Deaths, time after irradiation**		**Survival at 14th day**
				days		*per cent*
Lot A	6	Oct. 28	Controls	12		83
Lot B	6	29		12, 13		66
Lot C	6	30		14		83
Lot D	6	31				100

Microscopic sections of brains of first and sixth generation M XI guinea pigs were made, and lesions typical of typhus were demonstrated in each.

Optimum Interval between Irradiation and Injection.—The effect of varying intervals of time between irradiation and infection is illustrated in Table IV. Four lots of mice of approximately the same weight were given 500 R units as follows: lot A, Oct. 28; lot B, Oct. 29; lot C, Oct. 30; lot D, Oct. 31. An inoculum of peritoneal washing of an irradiated, infected moribund mouse (ninth passage) was prepared Oct. 31 and 0.5 cc. injected intra-abdominally into four mice of each lot about 5 hours after the irradiation of lot D. The remaining six mice of each lot served as controls and received 0.5 cc. of saline washing of the peritoneum of an *uninfected*, irradiated mouse. In lot A, x-rayed 3 days before injection, death occurred 6 days after infection on the average; lot B, x-rayed 2 days before injection, 5.2 days after infection; lot C, x-rayed the day before injection, 5.5 days after infection; lot D, x-rayed and injected on the same day, 10 days after infection. These

results suggest that an interval of 1 to 3 days between irradiation and infection is more suitable than an interval of only a few hours, in that the animals succumb much more quickly after inoculation. These results have been confirmed in another experiment similar to that shown in Table IV.

Effect of Irradiation on Mice Not Injected with Infective Material.—In these experiments an arbitrary period of observation has been set at 14 days. The percentage of uninfected irradiated mice dying within this interval

TABLE V

Effect of Irradiation on Uninfected Control Mice

Date	Dose of x-ray	No. of mice	No. dead end of 14 days	Per cent dead end of 14 days	Average day of death
				per cent	
Oct. 17	500 R	5	2	40	9.5
19	"	6	0	0	—
22	"	6	1	17	13
26	"	5	2	40	10
28	"	6	1	17	12
29	"	6	2	33	12.5
30	"	6	1	17	14
31	"	6	0	0	—
Nov. 4	"	6	1	17	8
23	"	6	0	0	—
Dec. 17	"	5	4	80	11
Nov. 9	450 R	10	0	0	—
21	"	5	0	0	—
27	"	6	0	0	—
30	"	6	0	0	—
Dec. 4	"	5	0	0	—
9	"	6	0	0	—
13	"	5	0	0	—

is shown in Table V which presents data for 63 uninfected control mice given 500 R units and 43 given 450 R units. Of the 500 R group, 23 per cent were dead at the end of 14 days, in the 450 R group all animals survived the entire period. The survivors frequently show ruffled fur and may not eat for several days. The injection intra-abdominally of saline 2 or 3 days after irradiation may possibly lessen the severity of postradiation sickness, but no attempt was made to determine this point accurately. The irradiated control mice for the experiments here reported received intra-abdominally as routine either 0.5 cc. normal saline or 0.5 cc. of the peritoneal washing of a previously irradiated but *uninfected* mouse so that the control injection would correspond as closely as possible to that used in the passage

experiments. Under these circumstances organisms other than rickettsiae but which resembled them might have become apparent in our strain of mice (especially if irradiation enhanced the conditions for the appearance of mouse pathogens). Although bacterial contamination of the peritoneal cavity occurred quickly in irradiated mice following death, no organisms which could easily be mistaken for rickettsiae were demonstrated during the course of these experiments.

DISCUSSION

In comparison with the intranasal route used by Castaneda (3) with murine typhus and by Durand and Sparrow (4) with European typhus, the intra-abdominal inoculation of irradiated mice provides a method which we believe is safer for the study of European typhus in the laboratory. Moreover, the results reported here suggest that it may well be possible to devise convenient and accurate techniques for testing the protective capacity of vaccines and of antisera obtained from experimental animals and convalescent or vaccinated human beings. The intra-abdominal route permits a more accurate measure of the quantities of infectious material or protective serum administered than does the intranasal route. Titrations of the infectivity of various materials may also prove practicable and inexpensive through the use of the irradiated mouse. Experiments with these objectives in mind are now in progress.

The high mortality of the infection, its relatively short duration (as opposed to guinea pig experiments), the ease with which rickettsiae may be demonstrated in the dead or dying mice, its simplicity as an experimental method when contrasted with the taking of guinea pig temperatures daily for several weeks, represent distinct advantages of this method.

The chief disadvantage of the technique lies in the necessity for employing irradiation. Obviously it would be much more satisfactory if the strain of typhus rickettsia could be adapted to normal mice, thereby reducing the hazard of apparent or latent infection with other organisms which the use of the x-ray may increase.

In regions where fertile eggs are expensive or cannot be secured, this method may prove practical for the production of vaccine on a moderately large scale. Tests for the efficacy of vaccines prepared from peritoneal washings of irradiated mice are now in progress.

SUMMARY AND CONCLUSIONS

A fatal infection of irradiated white mice with the Breinl strain of European typhus has been established and passed serially for 22 passages by the

intra-abdominal route. Rickettsiae were abundant and easily demonstrable in the moribund or dead mice.

The mortality of irradiated mice infected with passage material (peritoneal washings or blood) was nearly 100 per cent as contrasted to no mortality in the control mice given the same dose of x-ray (450 R) and the same volume of fluid intra-abdominally. (The observation period of control mice was arbitrarily limited to 14 days.)

After eighteen passages in irradiated mice no increase in virulence for non-irradiated adult mice was detected.

After passage in guinea pigs, the rickettsial infection deriving from the mouse passage material was identical with the Breinl strain as judged by fever, cross immunity tests, and brain lesions in sections.

We wish to thank the x-ray staff of the Huntington Memorial Hospital of Boston for their advice and cooperation.

BIBLIOGRAPHY

1. Savoor, S. R., and Velasco, R., *J. Exp. Med.*, 1934, **60**, 317.
2. Wohlrab, R., *Zentr. Bakt., 1. Abt., Orig.*, 1937, **140**, 193.
3. Castaneda, M. R., *Am. J. Path.*, 1939, **15**, 467.
4. Durand, P., and Sparrow, H., *Arch. Inst. Pasteur Tunis*, 1940, **29**, 1.
5. Castaneda, M. R., personal communication.
6. Zinsser, H., and Castaneda, M. R., *Proc. Soc. Exp. Biol. and Med.*, 1932, **29**, 840.
7. Zinsser, H., FitzPatrick, F., and Wei, H., *J. Exp. Med.*, 1939, **69**, 179.
8. Cox, H. R., *Pub. Health Rep., U. S. P. H. S.*, 1938, **53**, 2241.

EXPLANATION OF PLATE 33

FIG. 1. Camera lucida drawings of the cellular peritoneal exudates of irradiated mice infected with European typhus rickettsiae.

(*a*) The third passage in mouse infected with blood.

(*b*) The eleventh mouse passage infected with blood.

Note the comparative richness of intracellular and extracellular rickettsiae in earlier and later mouse passages. Stained by Macchiavello stain. × 1300.

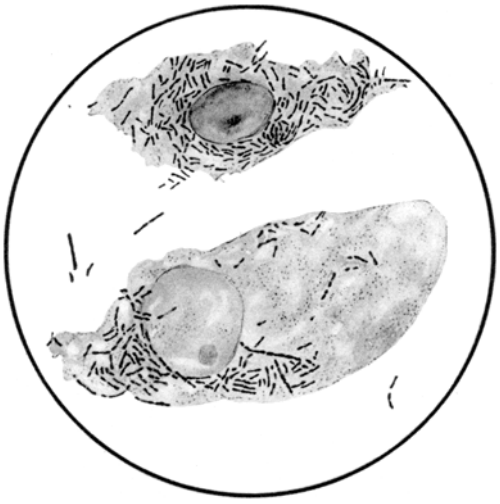

FIG. 1*a*

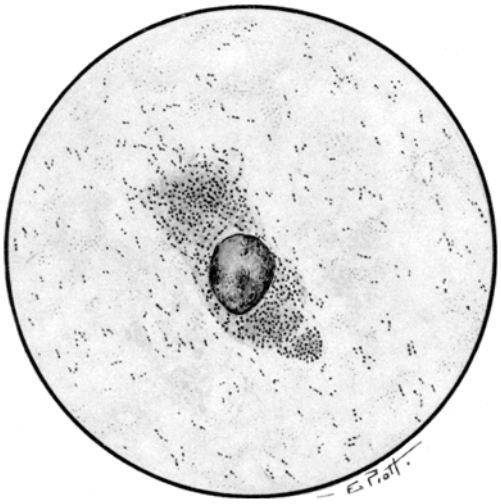

FIG. 1*b*

(Liu *et al.*: Infection of irradiated mice with typhus)