

convulsions in this species when injected intracranially. The convulsive factor was absorbed on and eluted from chicken red blood cells as

was the virus. The convulsive factor was inactivated by heating at 56°C for 30 minutes. It was specifically neutralized by antiserum.

14990

Simultaneous Infection in Laboratory Animals with Murine and Classic Typhus. II. Recovery of Strains.*

ROBERTO SILVA AND M. RUIZ CASTANEDA.

From the Department of Medical Research, General Hospital, Mexico, D.F.

In a previous paper¹ we reported on the production of typhus pneumonia in rats and mice with a mixture of murine and classic rickettsiæ. The assumption that both viruses were present was supported by serological and immunological studies. The experiments here reported demonstrate that the mixed infection was still detectable after 10 generations in the lungs of mice and that then both strains were recovered by inoculation in guinea pigs. The means by which we could separate the murine from the classic strain were based on the observation of the behavior of male and female guinea pigs towards the 2 types of typhus infection. Male guinea pigs, when inoculated with murine typhus, develop, as is well known, a characteristic swelling of the scrotum accompanied by a rise of temperature which lasts several days. The same virus inoculated into female guinea pigs produces a considerably milder disease apparent only in a smaller rise of temperature for a shorter period than in male animals. Often the disease is inapparent. The transfer of the strain from female to female is difficult and the strain is often lost after 2 or 3 generations. On the other hand, females react readily to classic typhus infection and the strain can be preserved indefinitely.

Material and Methods. The murine typhus strain used was isolated from a case of human typhus in 1936 in Mexico City. It has been

kept by a considerable number of transfers in guinea pigs and constantly produces scrotal swelling in males. In females inoculated with tunica material the infection is restricted to less than 4 days of moderate febrile reaction. The classic virus is the "Breinl" strain which produces a febrile reaction lasting from 6 to 10 days in male and female guinea pigs when inoculated with 0.1 g of brain of an animal killed on the 5th day of fever.

The material for the inoculation of mice by the intranasal route was prepared according to methods described elsewhere.² Once the virus was adapted to the mouse lung, a mixture of equal amounts of murine and classic material was inoculated into mice by the intranasal route. At 48-hour intervals transfers were made using sulfathiazole³ as a preservative to prevent contamination of the lungs by secondary invaders.

The separation of the 2 typhus rickettsiæ was made from the 10th transfer of the mixed virus from lung to lung. Guinea pigs, male and female, were inoculated by intraperitoneal route with an emulsion of an infected mouse lung. The material was diluted conveniently to reduce the chances of peritonitis by secondary invaders. Subsequent transfers from male to male guinea pigs were made using as inoculum the tunica vaginalis, while in female guinea pigs the inoculum was brain emulsion. Transfers from tunica material to female

* The expenses of this study were defrayed in part by a grant from Eli Lilly and Company.

¹ Castaneda, M. R., and Silva, Roberto, *Proc. Soc. Exp. Biol. and Med.*, 1944, **57**, 80.

² Castaneda, M. R., and Silva, Roberto, *J. Infect. Dis.*, 1944, **75**, 103.

³ Castaneda, M. R., *Revista del Instituto de Salubridad y Enfermedades Tropicales*, 1944, **5**, 1.

TABLE I.

Transfer of Mixed Typhus Virus from the 10th Generation in Mice into a Series of Male Guinea Pigs, Then Through a Series of Female Guinea Pigs and finally Through Males Again. Recovery of the Murine Strain.*

Transfer No.	Guinea pig No.	Material used for transfer	Days of fever	Scrotal swelling	Complement murine	Fixation classic	Day of bleeding
First Group.							
1	1 male	Mouse lung	13	Yes	640	320	22
2	3 "	Tunica	K.f.t.	"	20	20	6
3	4 "	"	9	"	640	160	22
4	6 "	"	K.f.t.	"	5	0	6
5	7 "	"	3	"	320	40	20
6	9 "	"	2	No	320	20	21
7	10 "	"	4	Yes	160	40	20
8	12 "	"	4	"	320	80	30
	13 "	"	4	"	80	40	30
9	15 "	"	4	"	160	40	23
10†	17 "	"	4	"	320	40	23
	18 female	"	K.f.t.	—	10	5	9
Second Group.							
2	19 "	Brain	K.f.t.		160	0	15
	20 "	"	0		0	0	22
3	21 "	"	K.f.t.		0	0	9
	22 "	"	7		320	20	16
4	24 "	"	7		640	160	21
5‡	26 male	"	K.f.t.				
Third Group.							
2	28 "	Tunica	2	Yes	80	5	20
3	30 "	"	7	"	160	5	27

* The animals used for transfer of the strain are not included, except those bled for complement fixation tests.

† Series carried to the 19th transfer, then discontinued.

‡ Series carried to the 7th transfer.

K.f.t. means killed for transfer.

The figures in the Complement Fixation column indicate the maximum dilution of the serum producing a reaction with murine and classic typhus rickettsiæ.

guinea pigs were made whenever it was desired to study the reaction of females to the virus carried in males. Also brain material from the female group was transferred to males at various stages of the experiment to find out if the murine virus persisted in such a group.

Results. *Series of male guinea pigs.* Guinea pig No. 1 (Table I) showed a rather severe infection with 13 days of fever and swelling of the scrotum. Serial transfers to male guinea pigs starting from a mate of guinea pig No. 1 produced infection of less severity. The transfers were made usually to 2 animals one of which was used to obtain the tunica vaginalis for inoculation, the other animals being saved to be bled for serological typing of the infection. The virus produced scrotal swelling in all but 2 male guinea pigs. The experiment was discontinued after 19 transfers. At the 10th transfer the virus was inoculated to 1 female guinea pig and carried on through 8 generations in females. The

infection was mild at the beginning and even failed to infect 1 animal. Subsequent transfers showed a certain increase in virulence through 5 generations. However, the infection became inapparent and was discontinued. Transfers from female guinea pigs carrying the "male strain" into the male guinea pigs produced scrotal swelling.

The serological study of this group of animals, including the females, showed a constant tendency to fix complement to a higher titer with the murine antigen. The diagnosis of murine type was corroborated by agglutination tests made with suitable antigens.

According to the clinical and serological results in this group of male guinea pigs it is apparent that after a few generations the murine virus remained alone. It is interesting to note that in early transfers, though complement fixation was higher in titer with the murine antigen, there was also a cross-fixation with classic rickettsiæ which was of greater

TABLE II.
Transfer of Mixed Typhus Virus from the 10th Generation in Mice into a Series of Female Guinea Pigs,
Then through a Series of Males. Recovery of the Classic Strain.*

Transfer No.	Guinea pig No.	Material used for transfer	Days of fever	Scrotal swelling	Complement fixation		Day of bleeding
					murine	classic	
First Group.							
1	1 female	Mouse lung	K.f.t.				
2	2 "	Brain	K.f.t.		0	5	9
3	3 "	"	K.f.t.		0	0	9
4	4 "	"	3		0	160	18
5	5 "	"	7		5	160	21
6†	7 "	"	8		0	320	20
	8 male	"	K.f.t.	no	0	80	13
Second Group.							
2	24 "	"	K.f.t.	"	0	5	13
3	25 "	"	K.f.t.	"	0	0	10
4	26 "	"	K.f.t.	"	0	0	19
5	28 "	"	8	"	0	40	19
6	30 "	"	5	"	0	40	10
7	32 "	"	9	"	0	80	18

* Most of the animals killed for transfer of the strain not used for complement fixation tests are not included in this table.

† From the 7th to the 13th generation in female guinea pigs the bleedings made about the 20th day after the inoculation produced complement fixation tests ranging from 1:40 to 1:320 with the classic antigen, being lower or negative with murine rickettsiae.

intensity than in subsequent transfers.

Female series. Female guinea pig 1 inoculated with lung material showed a rather moderate infection. Serial transfers were made with the brains of the animals killed usually from the 9th to the 10th day after the inoculation. Bleedings for complement fixation tests were made from 9 to 21 days after the inoculation. After the 5th generation the series was subdivided into female and male groups which were carried on for 7 to 8 transfers and then discontinued.

It is interesting to note that none of the 10 male guinea pigs carrying the "female strain" developed scrotal swelling. Serological studies in the female groups and the male subgroup showed that the type of the infection was classic typhus.

From these experiments it is apparent that

the mixture of murine and classic typhus can be kept through an indefinite number of transfers in mice by intranasal inoculation of "lung material" and that the individual viruses can be recovered by means of inoculation into groups of male and female guinea pigs.

Summary. When mice are inoculated by the intranasal route with mixtures of murine and classic typhus the strains are carried on for an indefinite number of transfers and then both viruses can be isolated by inoculation into series of male and female guinea pigs. Serial transfers in both sexes seem to favor the prevalence of one or the other virus. Males favor the isolation of the murine type while females favor the preservation of classic typhus.