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Simultaneous Infection of Laboratory Animals with Murine and Classic Typhus. Immunological Results.

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The intranasal inoculation of mice with murine or classic typhus produces pneumonia which may be 100% fatal provided the inoculum is rich enough in rickettsiæ. Rats similarly treated are affected only by inoculation with murine virus. However, an inoculum containing large numbers of classic rickettsiæ may kill the animals although microscopic examination of smears from the lungs shows so few organisms that it would be worthless to attempt to use such material to obtain rickettsial suspensions.

It was considered of interest to study the effect of the inoculation of mice and rats with mixtures of murine and classic viruses, hoping to find evidence of a simultaneous growth of the 2 rickettsiæ and perhaps improve the antigenic value of vaccines prepared in these rodents.

Mice inoculated intranasally with mixtures of murine and classic viruses showed generalized pneumonia which killed the animals in about 48 hours. Transfers from mouse lung to other mice were successful for 13 generations, then were lost because of secondary infection. According to experiments to be discussed in a future paper, both viruses were present in the lungs at the 10th generation. The inoculation of rats with mixtures of murine and classic typhus prepared from the lungs of mice infected separately or with the lungs of mice treated with mixtures of both viruses, produced pneumonia which killed the animals in 72 to 96 hours. Transfers from rat to rat were successful through 2 generations only because of considerable contamination by secondary invaders. The possibility of a simultaneous growth of both types of rickettsiæ was investigated as follows:

From the lungs of rats killed when very ill, we prepared saline suspensions with which guinea pigs were inoculated by intradermal route. The animals developed typical skin lesions and were bled 20 days after the inoculation for complement fixation tests.

From the lungs of rats killed 72 hours after the infection, we prepared suspensions of rickettsiæ according to a method described elsewhere.[1] The final product was a rich suspension of rickettsia bodies practically free

TABLE I.
Complement Fixation with Monovalent and Bivalent Guinea Pig Sera and Monovalent and Bivalent Rickettsial Antigens.

Sera	Dilution of serum 1/10	1/20	1/40	1/80	1/160	1/320	1/640	Antigens
Classic	4	4	4	4	4	2	0	Classic
"	4	2	0	0	0	0	0	Murine
"	4	4	2	2	0	0	0	Bivalent
Murine	4	4	2	0	0	0	0	Classic
"	4	4	4	4	4	2	0	Murine
"	4	4	4	4	2	0	0	Bivalent
Bivalent	4	4	4	4	2	1	0	Classic
"	4	4	4	4	3	1	0	Murine
"	4	4	4	4	1	0	0	Bivalent

0 indicates that no complement fixation was observed.
From 1 to 4, various degrees of complement fixation.

[1] Castaneda, M. R., *Brit. J. Exp. Path.*, 1941, **22**, 167.

TABLE II.
Monovalent and Bivalent Vaccines Prepared from the Lungs of Rats, Tested Against Classic Typhus.

Guinea pig No.	Vaccinated with 2 doses, 1 cc each	Daily temperature in degrees C after inoculation with classic typhus														
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
1	Monovalent murine diluted to 1:20	.2	.4	40.0	.9	.5	40.1	40.3	40.4	41.0	40.7	40.6	40.3	40.0	.6	.8
2		.4	.5	.7	.6	.3	40.3	40.1	.7	.4	.8	.9	40.0	40.0	.5	.7
3	" " " " 1:30	.4	.8	.9	.8	40.1	40.7	40.1	40.3	40.7	40.9	40.8	40.7	40.6	40.1	.9
4		.7	.7	.9	.6	.4	.8	.9	41.0	40.6	40.7	40.9	40.8	40.3	.8	.7
5	" " " " 1:40	.6	.6	.8	.9	.9	.8	.6	40.9	41.7	41.0	41.2	40.9	40.5	40.0	.9
6		.7	.5	40.0	.9	.3	.7	.9	40.2	40.1	40.0	40.3	40.2	40.1	40.0	.4
7	" " " " 1:50	.3	.6	.9	.3	40.0	.8	40.4	40.9	40.8	41.1	40.5	40.3	.7	.8	.9
8		.4	.3	.8	.5	40.3	.8	40.6	41.0	41.2	40.6	40.7	40.2	.9	40.0	40.0
9	Bivalent diluted to 1:20	.4	.3	.9	.9	.0	.6	.7	40.0	.6	.7	.6	.8	.7	.6	.6
10		.4	.6	40.0	.8	.9	.4	.4	.4	40.3	.8	.8	.7	.5	.4	.5
11	" " " 1:30	.3	.5	40.0	.7	.4	.7	.3	40.0	.7	.7	.6	.7	.8	.3	.6
12		.6	.6	40.5	.9	.5	.6	.2	.6	40.9	40.1	40.0	.4	.5	.4	.8
13	" " " 1:40	.4	.4	.9	.8	.2	.4	.6	.8	40.9	40.1	40.0	.4	.5	.4	.5
14		.6	.6	.7	.7	.4	.6	.1	41.4	.8	.8	.7	.7	.8	.7	.9
15	" " " 1:50	.6	.4	.9	.8	.8	.2	.7	40.0	.9	.9	.7	.5	.8	.9	.6
16		.6	.3	.4	.6	40.1	.0	40.2	40.3	.9	.8	.7	.5	.6	40.0	.8
17	—	.6	.5	.8	.9	40.0	40.3	40.8	40.2	40.0	40.4	40.6	40.1	40.0	40.0	.6
18	—	.5	.6	.9	.8	40.0	40.1	40.2	40.7	40.6	40.0	40.7	40.5	40.3	40.5	.8
19	—	.6	.5	.5	.6	40.3	40.0	40.6	40.3	40.5	40.5	40.8	40.7	40.0	.6	.6

.0 to .9 are abbreviations for 39.0 to 39.9°C.
40.0°C and over are not abbreviated in order to show the intensity of the febrile reaction.

from cellular detritus. This suspension was prepared in such concentration that a 1:10 dilution corresponded to No. 2 of the McFarland scale, thus having an equal rickettsial content to our monovalent concentrated murine vaccine.

The theoretically "bivalent vaccine" was tested for specificity and potency by means of selective complement fixation tests[2] and by protection experiments in guinea pigs comparing the vaccine with a monovalent murine vaccine.

For complement fixation tests the "bivalent vaccine" was titrated with sera from guinea pigs bled after recovering from murine and classic typhus. The titration was made comparing the antigen with known monovalent murine and classic antigens prepared from the lungs of mice. Table I shows the results of tests made with monovalent and bivalent guinea pig sera and monovalent and bivalent antigens. It is apparent that the bivalent antigen acts as a mixture of murine and classic rickettsiæ.

According to previous experiments, the monovalent murine vaccine protected 3 out of 4 guinea pigs against classic typhus when the animals were vaccinated with a 1:10 dilution of the stock rickettsial suspension. For the comparative protection experiment we vaccinated guinea pigs with dilutions of the stock vaccines ranging from 1:20 to 1:50. Care was taken to have equivalent suspensions of monovalent and bivalent vaccines. Eight guinea pigs were vaccinated with monovalent vaccine and 8 with the bivalent rickettsial suspensions. Two animals were treated with each dilution, administering subcutaneously 2 doses of 1 cc each at weekly intervals. The vaccinated animals were inoculated with 0.1 g of the brain of a guinea pig infected with the "Breinl" strain of typhus, 18 days after the last dose of vaccine. Table II shows the results of this experiment, including 3 controls.

It may be seen in Table II that the temperatures of the animals vaccinated with "bivalent vaccine" show many more figures below 40°C than were found in the group of animals vaccinated with the monovalent murine vaccine. It is evident, therefore, that vaccines prepared in rats with mixtures of murine and classic viruses produce a better protection against classic typhus than the material obtained after the inoculation with murine virus alone.

The serologic and protection tests here described seem to indicate that the inoculation of mixtures of murine and classic viruses produce a mixed infection in which the classic virus seems to have an important part.

Summary. Typhus rickettsiæ of the classic type which have no tendency to produce massive infection in rats, seem to multiply more readily when associated with murine rickettsiæ. The animals inoculated by intranasal route produce "lung vaccines" of higher immunizing value against classic typhus than monovalent murine vaccines.

[2] Plotz, H., *Science*, 1943, **97**, 20.

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Distribution of Alkaline Phosphatase in Kidney Following the Use of Histochemical Azo Dye Test.

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The technic of a histochemical azo dye test for the demonstration of alkaline phosphatase in kidney has recently been described in detail by us.[1] The test consists of a direct coupling of calcium β-naphthol, following its release from calcium β-naphthol phosphate by renal

[1] Menten, M. L., Junge, J., and Green, M. H., *J. Biol. Chem.*, 1944, **153**, 471.