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Immunization of Guinea Pigs Against Typhus Exanthematicus.

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Although many attempts have been made to discover a method of immunizing animals against *Typhus exanthematicus*, only a few isolated partially successful results have been reported.

Immunization has been accomplished with live virus mixed with immune serum[1] as well as with repeated injections of minute doses of live virus.[2] Immunization with heated tissues from infected animals has yielded negative results. Positive or partially positive results have, however, been reported with carbolized and formolized suspensions of Rickettsia. Breinl[3] has partially immunized guinea pigs with carbolized suspensions of infected lice stomachs containing large numbers of Rickettsia, while Zinsser and Castaneda[4] have recently reported successful results with formolized suspensions of Rickettsia from tunica material from guinea pigs infected with Mexican Typhus.

Our experiments were conducted with the European virus and we were concerned with the possibility of producing immunity with dead or attenuated virus in tissue suspensions. Suspensions of brain material from infected guinea pigs treated in a variety of ways with antiseptics, metallic sols and colloidal adsorbents yielded negative results. Positive immunization has, however, been obtained by 2 different procedures. These results, interesting in themselves, are significant also because of the theoretical problem involved.

We confirmed the work of Breinl[3] and succeeded in immunizing guinea pigs with formolized Rickettsia suspensions obtained from infected lice. The degree of immunity seems to depend on the quantity of suspension injected. No animals showed temperature above 39°C.

In other experiments brain suspensions were used. In connection with the colloidal adsorption experiments it was found that distilled water inactivated typhus virus after a relatively brief exposure. Virulent brain virus suspended in water becomes inactive in 6 to

[1] Noguchi, H., *J. Exp. Med.*, 1923, **38**, 605.

[2] Nicolle, C., Sparrow, H., and Conseil, E., *Arch. Inst. Past. de Tunis*, 1927, **16**, 1.

[3] Breinl, F., *Z. für Immunitäts.*, 1924, **41**, 97.

[4] Zinsser, H., and Castaneda, Ruiz M., *J. Exp. Med.*, 1931, **53**, 325.

TABLE I.

G. P.	Material injected*	Date of inoculation	Date of infection	Material infected	Reaction	Lice infected with	Date
No. 913	Emulsion 5 infected lice intestines in			Brain material from		Brain	5/17/31
W. 405 gm.	¼ cc. saline + ¼ cc. 0.2% formalin, 24 hours in icebox. Intraperitoneal.	5/27/31	6/15/31	infected guinea pig. 1 cc.	Typical	G. P. blood	5/20/31
No. 944	½ cc. suspension of 7 infected intes-				Incubation 8 days, du-	Infected lice intestines	
W. 400 gm.	tines + ½cc. of 0.2% formalin, kept over night in icebox. Intraperitoneal.	6/23/31	7/19/31	2 cc.	ration of fever 4 days (modified course)	plus G. P. brain	6/15/31
	4 intestines in ½ cc. 0.1% formalin, kept over night in ice box. Intraperitoneal.	7/7/31				Lice rickettsia	6/30/31
	5 injections:						
No. 895	17 intestines emulsified in 1 cc. 0.1%				No fever; all 3 ani-		
W. 440 gm.	formalin, kept over night in icebox. Intraperitoneal, 0.3 cc. each.	7/20/31	8/5/31	½ cc. suspension	mals immune	Brain	7/10/31
No. 893	4 intestines in 1 cc. 0.1% formalin.						
W. 400 gm.	Intraperitoneal, 0.3 cc. each.	7/21/31				Brain	7/19/31
No. 967	5 intestines in 1 cc. 0.1% formalin.						
W. 540 gm.	Intraperitoneal, 0.3 cc. each.	7/22/31					
	5 intestines in 1 cc. 0.1% formalin. Intraperitoneal, 0.3 cc. each.	7/27/31					
	5 intestines in 1 cc. 0.1% formalin. Intraperitoneal, 0.3 cc. each.	7/30/31					
10 Control W. 400 gm.							
11 Control W. 675 gm.			8/5/31	½ cc.	Typical course		

*The suspensions contained large numbers of Rickettsia.

8 hours and no longer produces an apparent infection in guinea pigs. Four to 5 successive injections of 0.5 to 1.0 cc. of a 4% brain suspension into guinea pigs immunized them against a reinfection with a dose of typhus virus which produced 100% infections in controls. A summary of these experiments is tabulated in Table II.

TABLE II.

No. of injections	No. of animals	Amount injected	Interval between infections	Duration of inactivation	Reaction	Result* of infection
		cc.	days	hours		
5	1	0.25-1.0	4-6	6	4 days' fever, max. 39.8°	Immune
5	1	0.5	4-6	3 times 6 2 " 5	No fever	"
4	1	0.5	5-6	2 " 6 2 " 4½	2 days' fever, max. 39.9°, after 3rd injection	" (intercurrent infection)
5	2	0.5	3-7	8, 7, 6, 5, 7	No fever	Immune
5	4†	0.5	4-7	7, 6, 5, 5, 4½	3 no fever; 1 small rise in temperature for 2 days; after 3rd injection	3 immune 1 typical infection

* In all cases controls infected at the same time with the same dose developed a typical infection after an incubation period of 6-7 days.

† 7 cc. mixed serum from these animals taken just before the infection protected a guinea pig against an infection with typhus virus.

Thus far carbolized or formolized brain material has failed to produce the same results, and it would appear, therefore, that in the case of the water suspension we are dealing with an attenuated virus. The interesting aspect of these 2 series of experiments is that the concentrated Rickettsia suspension behaves like a bacterial antigen, whereas the organ suspension reacts in the manner of a filterable virus; in the one case immunization can be effected by a presumably dead suspension of the infectious agent, and in the other it can be produced only by a live attenuated virus. This problem is now being studied more comprehensively, but the idea naturally suggests itself that the probable reason for the failure thus far to immunize with dead tissue virus, is the relatively small quantity of antigen present. If, as it appears, the degree of immunity produced is dependent upon the quantity of antigen injected, then there may be a simple explanation for the failure to immunize with dead virus suspensions.