

the gases, but it is clear that gas bubbles are essential in the coagulation of albumin by supersonic waves. The rapidly vibrating gas bubbles on their ascent to the surface cause coagulation of the albumin at the interface much in the same way as albumin is coagulated by shaking which has been shown to be a surface phenomenon.²

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Stimulation of *B. typhosus* Agglutinin in Typhus Fever.

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It has long been recognized that the agglutinin for typhoid bacilli as shown by the Widal reaction is often encountered during typhus fever. In our experience here we observe that the sera obtained from proved cases of typhus may show early Widal test up to a higher titre while the Weil-Felix reaction or the presence of agglutinin for *B. proteus* X19 may appear later or in a much lower titre. Some of these cases may give the history either of previous typhoid infection or prophylactic vaccination, but in others none of these factors could be definitely ascertained. It has been suggested by some observers that during typhus fever the appearance of the Widal reaction is due to non-specific stimulation of latent or low titre antibodies by the fever.¹

In a recent observation, however, it has been found that experimental typhus in animals (guinea pigs, rabbits and monkeys), previously vaccinated with typhoid bacilli, had no influence on the Widal reaction.² This is contradictory to actual findings made in human beings. In view of the value of *B. proteus* X19 as an antigen for the diagnosis of typhus fever, it seems to us of interest to determine whether the introduction of that organism into typhoid inoculated animals would stimulate the production of anti-typhoid agglutinin.

In our present experiment we are not concerned with the action of other microorganisms in this direction, since non-specific stimulation of specific antibodies has been recorded on experimental ani-

² Wu, H., and Ling, S. M., *Chinese J. Physiol.*, 1927, **1**, 407.

¹ Felix, A., *J. Hyg.*, 1929, **28**, 418.

² Reimann, H. A., and Wu, C. J., *J. Immunol.*, 1930, **18**, 159.

mals through the use of certain heterologous bacteria³ that have no relation whatsoever with typhus fever.

For the purpose of our study, a series of 5 rabbits was inoculated with *B. typhosus*. Altogether 4 injections were given at weekly intervals, the first being 1/10 of a heat killed agar slant culture given subcutaneously, and the others in increasing doses of 1/40 to 1/10 of a living culture administered intravenously. Similarly, another series of 5 animals was immunized with *B. proteus* X19. Following this each animal of both series was bled at regular intervals from the 7th to the 45th day after the last injection and the agglutinin titres determined by standard macroscopic method. The agglutinin in titre for the typhoid series ranged from 1:10,240 to 1:20,480 the first week after the last injection and those for the proteus series were practically the same. No cross agglutination was present. Subsequent weekly examinations showed that after 45 days, the anti-typhoid and anti-proteus agglutinins have fallen to a titre of 1:640 or 1:280.

At this period another course of inoculations was given to serve as a non-specific stimulating agent. The animals which were immunized with *B. typhosus* received now injections of *B. proteus* X19 and *vice versa*. Altogether 4 injections were given to each animal at weekly intervals in increasing doses of 1/5,000 to 1/50 of a living agar slant culture. The animals were bled at regular intervals from the 7th to the 60th day after the last injection. It was found that the group of rabbits immunized with *B. typhosus* and subsequently stimulated by the injection of *B. proteus* X19 showed considerable increase in the agglutinin for typhoid bacilli rising from a titre of 1:1,280 to that of 1:81,920, whereas the agglutinin titre for *B. proteus* X19 was not stimulated as shown in the table. At the same time, the animals immunized with *B. proteus* X19 and then stimulated by the inoculation of *B. typhosus* also showed a higher agglutination titre for the latter, but for the former there was no appreciable increase. Control animals injected throughout with either *B. typhosus* or *B. proteus* X19 only showed no cross agglutination in their blood.

As summarized in the table it has been found that in rabbits, inoculation of *B. proteus* X19 results in a considerable stimulation of a low titre or latent anti-typhoid agglutinin, whereas the inoculation of *B. typhosus* fails to stimulate markedly a pre-existing low titre anti-proteus agglutinin. This finding is in agreement with the observations recorded in human cases of typhus fever and like the

³ Dreyer, G., and Walker, E. W. A., *J. Path. and Bact.*, 1909, 14, 28.

TABLE I.
Agglutination titres determined on various days after the last injection following immunization and subsequent stimulation.

Rabbit No.	Immunized with	Agglutination titre						Stimulated with	Titre after stimulation					
		<i>B. typhosus</i>			<i>B. proteus</i> X19				<i>B. typhosus</i>		<i>B. proteus</i> X19			
		7 days	45 days	7 days	45 days	7 days	45 days		7 days	60 days	7 days	60 days		
1	<i>B. typhosus</i>	20,480	1,280	0	0	0	<i>B. proteus</i> X19	40,960	1,280	5,120	160			
2		10,240	1,280	0	0	0		40,960	1,280	10,240	160			
3		10,240	1,280	0	0	0		81,920	640	10,240	160			
4		20,480	1,280	0	0	0		40,960	640	2,560	80			
5		10,240	1,280	0	0	0		81,920	1,280	2,560	80			
6	<i>B. proteus</i> X19	0	0	20,480	640	640	<i>B. typhosus</i>	81,920	640	1,280	80			
7		0	0	10,240	1,280	640		20,480	640	640	80			
8		0	0	20,480	1,280	1,280		40,960	1,280	2,560	160			
9		0	0	10,240	640	640		40,960	1,280	1,280	160			
10		0	0	20,480	640	640		20,480	640	1,280	80			

Titre 0 = a negative result in dilution 1:20.

agglutinins for the Weil-Felix reaction in the blood serum of such patients, the agglutinin for *B. Proteus* X19 does not respond so readily to a non-specific stimulus.