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Stability of Vi Antigen of *Salmonella typhi.**

C. A. PELUFFO.† (Introduced by P. R. Edwards.)

From the Department of Animal Pathology, Kentucky Agricultural Experiment Station, Lexington, Kentucky.

The lability of the Vi antigen of the typhoid bacillus has been emphasized as its most marked characteristic. Felix and Pitt[1, 2] found that Vi antigen was destroyed in 90 minutes at 58°C and in an hour at 100°C and disappeared when treated with alcohol, chloroform or toluol. Exposure to ether resulted in a partial loss of the substance. Kauffmann[3] suggested the use of formalized suspensions for agglutination tests. Felix and Bhatnagar[4] reported that formalized suspensions retained their agglutinability by Vi antiserum and their agglutinogenic properties. Serums prepared with formalized suspensions were, however, inferior in protective antibodies to serums prepared with living organisms. They also found that phenolized suspensions progressively lost their agglutinability by Vi antiserum and serums produced with such suspensions contained no Vi agglutinins and were devoid of protective antibodies. Felix and Pitt[5] found that alcohol-treated suspensions retained their agglutinogenic and agglutinin-binding properties but that their agglutinability by Vi serums was "almost annulled or at least very much reduced." Felix and Petrie[6] also stated that alcohol treatment almost annulled the Vi agglutinability. All the workers cited above observed that as the agglutinability of the bacilli by Vi antiserums disappeared they progressively became more agglutinable by O antiserums.

Since the observations of the writer were not in full accord with the descriptions of the characteristics of Vi antigen, particularly as regards its susceptibility to heat and alcohol, a systematic study of

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1 Felix, A., and Pitt, R. M., *J. Path. and Bact.*, 1934, **38**, 409.

2 Felix, A., and Pitt, R. M., *Lancet*, 1934, **2**, 186.

3 Kauffmann, F., *Z. Hy.*, 1935, **116**, 617.

4 Felix, A., and Bhatnagar, S. S., *Brit. J. Exp. Path.*, 1935, **16**, 422.

5 Felix, A., and Pitt, R. M., *Brit. J. Exp. Path.*, 1936, **17**, 81.

6 Felix, A., and Petrie, G. F., *J. Hyg.*, 1938, **38**, 673.

TABLE I.
Effect of Temperature on Vi Antigen.

	5°C		25°C		37°C		50°C	
	O+ Vi++	O++ Vi—	O+ Vi++	O++ Vi—	O+ Vi++	O++ Vi—	O+ Vi++	O++ Vi—
0.85% saline	1 mo	6 mo	5 da	10 da	4 hr	3 da	20 min	4 hr
Saline + 0.3% formalin	17 da	>7 "	4 "	2 mo	2 "	12 hr	10 "	2 "
Saline + 0.5% phenol	7 "	17 da	2 hr	2 da	20 min	2 "	4 "	15 min
Saline saturated with chloroform	2 hr	7 hr	30 min	5 hr	10 "	30 min	2 "	4 "

influence of temperature and certain chemicals on the substance was undertaken. The Watson strain of *S. typhi* was used because of the constancy of its content of Vi antigen. Additional experiments with other strains of *S. typhi* gave comparable results. In the preparation of Vi suspensions the culture was plated and Vi colonies selected as recommended by Craigie[7] and Giovanardi.[8] This selection was continued until cultures isolated from Vi colonies and maintained in the refrigerator yielded apparently pure Vi growths when transferred. The suspensions used in the tests consisted of organisms scraped from 20 hour agar plate cultures and suspended directly in the vehicle to be tested. All these suspensions were actively agglutinated by pure Vi serum but were not affected by O antiserum. The Vi antiserum used in the tests was prepared with a formalized suspension of a Vi culture of *S. typhi* (Watson). It was freed of H and O agglutinins by absorption with *S. typhi* (H901) before use. In slide agglutination tests the serum had a titer of 1 to 50. It was used in a dilution of 1 to 10. The O serum was prepared with *S. gallinarum* and had a titer of 1 to 150 for the W form of the Watson strain in slide agglutination tests. It was used in a dilution of 1 to 20. While the work reported here is based on slide agglutination tests, these were adequately controlled by expanded tube agglutination tests. The only discrepancy between the slide and tube tests was the absence of O agglutination on the slide with suspensions which in the tube gave traces of O agglutination. These traces of O agglutination occurring in Vi suspensions were commented upon by Felix.[9] The use of the slide test not only allowed the performance of a large number of tests but, in addition, it permitted the immediate determination of the antigenic properties

[7] Craigie, J., and Brandon, K. F., *J. Path. and Bact.*, 1936, **43**, 233.

[8] Giovanardi, A., *Zbl. f. Bakt., I, Orig.*, 1938, **141**, 341.

[9] Felix, A., *J. Hyg.*, 1938, **38**, 750.

of the suspension, and eliminated further changes during the period of incubation in tube agglutination tests.

In Table I are summarized the results of a number of experiments on the thermostability of Vi antigen in saline suspension and in saline suspension with the addition of formalin, phenol and chloroform. Under each temperature are given the average length of time that elapsed before agglutination of the suspensions by O serum appeared (O+,Vi++) and that necessary for the complete disappearance of the agglutinability of the suspensions by Vi antiserum (O++, Vi—). The disappearance of Vi agglutinability is accelerated with increasing temperature. Agglutination by Vi serums no longer occurs after the suspensions are heated at 60°C for 30 minutes or at 100°C for 5 minutes. The addition of formalin to the suspensions hastened the appearance of O agglutinability with a corresponding decrease in the reaction with Vi serum. However, a peculiar characteristic of formalized suspensions is that at low temperatures they retain traces of Vi agglutinability for a longer period than suspensions in saline alone. At higher temperatures this retention of traces of Vi agglutinability is not apparent. The addition of phenol to the suspensions hastens the disappearance of Vi agglutinability and the addition of chloroform has an even more marked effect.

While the results given above agree with the general concept of the lability of Vi antigen, those obtained by treatment with alcohol, glycerin and acetone and by simple dehydration of the bacilli are at complete variance with previous reports. Organisms suspended in glycerin retained their agglutinability by Vi serums and their resistance to O agglutination after 8 months at 25°C. Bacilli suspended in acetone and heated at 56°C for 3 hours, or suspended in absolute alcohol and heated at 75°C for 3 hours, were still strongly agglutinable by Vi serum and inagglutinable by O serum. Bacilli suspended in absolute alcohol or in acetone and dehydrated *in vacuo* over sulfuric acid retained their Vi agglutinability and their O inagglutinability for at least 8 months at 25°C, 2 hours at 100°C and 1 hour at 150°C. Bacilli removed from agar plates and simply dehydrated *in vacuo* behaved in the same way. On the contrary, organisms suspended in 50% alcohol were no longer agglutinable by Vi serum after heating at 50°C for 2 hours. Apparently, resistance of Vi antigen to heat is dependent upon dehydration.

Organisms taken directly from agar plates, suspended in absolute alcohol, heated at 75°C for 3 hours and dehydrated *in vacuo* not only retained their Vi agglutinability, but completely absorbed ag-

glutinins from Vi antiserum prepared with living bacilli. Further, organisms treated in this way upon injection into rabbits gave rise to a serum having a Vi titer of 1 to 2000 in tube agglutination tests. The O and H titers of the serum were each 1 to 1000. Absorption of this serum with living bacilli removed all Vi, H and O agglutinins, thus demonstrating the serological identity of Vi antigen before and after treatment with heat and alcohol. The serum prepared with organisms which had been treated with heat and alcohol was used in mouse protection tests. Its protective power was the same as that of a serum of comparable titer which was prepared with living bacilli.‡

The results show that the Vi antigen of the typhoid bacillus is not affected by absolute alcohol and that its resistance to heat is much greater than was previously supposed. This stability to heat and alcohol is also shared by the Vi antigen of *S. paratyphi* C, *S. ballerup* and certain strains of *E. coli* in which Kauffmann[10] described V-W variation. The thermolability described by other investigators is accounted for by the fact that, in previous work, aqueous suspensions were used in the study of Vi antigen.

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Melanin Formation by Deplanted Fragments of Thalamus in Amphibian Larvae.

PAUL WEISS.*

From the Department of Zoology, University of Chicago.

In the course of experiments on the functional deplantation of brain fragments into the dorsal fin of urodele amphibian larvae described in earlier communications,[1] a striking local pigmentation effect was observed, whenever thalamic grafts were used.

Donors and hosts were larvae of *Amblystoma tigrinum,* between 3 and 4 cm in length. The brain part which produced positive effects is shown as shaded area in Fig. 1. One-half of this portion, either

‡ The writer is indebted to Lieut. Geo. F. Luippold of the Army Medical School for performing the mouse protection tests.

10 Kauffmann, F., *Acta Path. Microb. Scan.*, 1941, **18**, 225.

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1 Weiss, Paul, PROC. SOC. EXP. BIOL. AND MED., 1940, **44**, 350; 1941, **46**, 14.