SURFACE POTENTIALS OF SOME NON-CHOLERA VIBRIO STRAINS IN RELATION TO THEIR SEROLOGY.

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It will be endeavoured in this part to account for certain discrepant serological reactions of some non-cholera vibrio strains from a consideration of their surface potentials (electrokinetic potentials). The observations to be detailed deal with the following strains:—

1. Kohat original.
2. Kohat current.
3. Metchnikovi original.
4. Metchnikovi passage.

TABLE I.

Direct Agglutinations of Kohat strains.

Antisera.	Organisms.	
	Kohat original.	Kohat current.
Kohat original H & O	6,400	400
,, ,, O	3,200	200
Kohat current H & O	1,600	6,400
,, ,, O	800	3,200

TABLE II.

Absorption tests of Kohat strains.

Sera.	Absorbed by	Kohat original.	Kohat current.
Kohat original H & O	Kohat original H & O	0	100
,, ,, ...	K. original O	1,600	200
,, ,, ...	K. current H & O	1,600	0
,, ,, ...	K. current O	1,600	100
Kohat current H & O	Kohat current H & O	0	0
,, ,, ,, ...	K. current O	400	1,600
,, ,, ,, ...	K. original H & O	0	1,600
,, ,, ,, ...	K. ,, O	0	1,600
Kohat original O	K. original O	0	0
,, ,, ...	K. current O	800	0
,, current O	K. ,, O	0	0
,, ,, ...	K original O	0	1,600

Kohat strains.

The original Kohat strain was isolated in February, 1934, from water in a non-endemic area in Persia which had long been free from epidemic cholera. The original strain was carried in subcultures in ordinary media for a period of eight months and was then found to have developed serological reactivity with a typical cholera vibrio strain 1617, and was in all respects similar to the majority of agglutinable strains found in clinical cholera (Taylor and Ahuja, 1935). The two Kohat strains belonged to chemical Group V.

Serology of the Kohat strains:—

Antisera were formed against the two strains by the injection of living organisms to produce H & O sera and by the injection of organisms heated for two hours at 100°C. to produce O sera. The serology of the strains is given in Tables I and II.

It appears that serologically the two strains are closely allied, excepting for the fact that the cross-absorptions are not complete, although considerable loss on agglutinins does occur.

Table III gives the results on cataphoresis of the Kohat strains. The p.d.'s were determined according to the method described by Linton, Mitra and Seal (1936).

TABLE III.

	P.D. in millivolts. Concentration of NaCl.				
	0·0093*N*	0·187*N*	0·0375*N*	0·0075*N*	0·15*N*
Kohat original in—					
Saline	−29·6	−24·0	−19·0	−14·0	−9·1
Normal serum	−28·5	−23·8	−18·5	−13·6	−8·8
'Original' H & O	−23·6	−18·6	−13·5	−8·8	−3·9
'Current' H & O	−24·0	−19·0	−14·0	−9·0	−4·0
'Original' O	−24·7	−19·5	−14·5	−9·8	−4·4
'Current' O	−25·2	−20·2	−15·0	−9·8	−4·6
Kohat current in—					
Saline	−23·9	−18·2	−13·7	−7·9	−1·2
Normal serum	−22·0	−16·9	−12·2	−6·5	−0·8
'Current' H & O	−18·9	−14·2	−9·5	−5·0	−0·0
'Original' H & O	−19·3	−14·6	−9·8	−5·2	−0·0
'Current' O	−20·0	−15·0	−10·3	−5·5	−0·2
'Original' O	−20·5	−15·6	−10·8	−5·8	−0·4

Kohat original has a higher surface potential than Kohat current in salt solution. Normal serum has a slight but definite effect in lowering the potential. The original H & O and O sera have a marked lowering effect on the 'Original' strain. Current H & O and O sera have a similar effect in lowering the potentials of the 'Original' strain. It apears, therefore, that the sera of both the strains lower the potentials of the 'Current' strain to about the same extent.

It follows that both the sera are almost equally specific, although the depression effect in the two strains differs. The cross-absorption tests also show that the two strains are very nearly alike. The absence of complete absorption in the case of cross-absorptions thus appears to be due to a difference in their surface potentials.

Metchnikovi strains.

V. Metchnikovi was originally isolated from domestic fowl and obtained from the Institute for Tropical Diseases, Hamburg. It has colony appearance, morphology and biochemical reactions similar to those of V. cholerae but was inagglutinable with any

of the standard cholera high-titre sera. It was found non-virulent for mice, and in an attempt to derive a strain which would be virulent, it was passed through mice. At about the 80th passage a highly agglutinable but still a virulent strain was obtained. For the purpose of distinction, the two strains were called V. Metchnikovi original and V. Metchnikovi passage (Taylor and Ahuja, 1935*b*).

TABLE IV.

Serological reaction of the two Metchnikovi strains.

Antisera prepared against vibrio No.	V. Metchnikovi passage.		V. Metchnikovi original.	
	Living.	Heated.	Living.	Heated.
V. Metchnikovi original	0	0	5,000	2,500
V. Metchnikovi passage	2,500	2,500	0	250
1617 H & O	2,500	2,500	0	0
1617 'O'	1,000	500	0	0

The two Metchnikovi strains were serologically different in their reactivity with an antiserum against 1617, a standard cholera strain. Both the strains belonged to chemical Group V. The only difference in the constitution of the two strains was in the polysaccharide content, the original having 4·1% and the passage 6·5%. As in Kohat strains, the two Metchnikovis were accordingly studied in respect of cataphoresis, and the results obtained are given in Table V.

TABLE V.

Cataphoresis of the Metchnikovi strains.

	P.D. in millivolts. Concentration of Saline.				
	0·0093*N*	0·0187*N*	0·0375*N*	0·0075*N*	0·15*N*
Metchnikovi original in—					
Saline	−36·4	−29·1	−22·5	−15·6	−8·1
Normal serum	−33·8	−27·3	−20·1	−13·4	−7·2
Rangoon smooth [1] serum—					
(i) 1: 6,400	−32·2	−25·5	−18·8	−11·7	−6·3
(ii) 1: 25,600	−31·7	−24·7	−16·9	−11·7	−5·2
1617 O	−33·0	−26·1	−18·5	−12·4	−7·1
Metchnikovi passage in—					
Saline	−26·0	−19·5	−13·0	−6·6	−1·8
Normal serum	−24·0	−18·2	−11·7	−5·4	−0·8
Rangoon smooth [1] serum—					
(i) 1: 6,400	−19·2	−14·8	−10·0	−4·9	−1·0
(ii) 1: 25,600	−17·2	−13·0	−9·1	−4·2	−0·0
1617 O	−20·1	−15·6	−10·4	−5·2	−0·8

The above table shows that the two Metchnikovi strains have different surface potentials. Under all conditions, both with and without normal or immune sera, Metchnikovi

[1] Rangoon smooth serum is used to show the reaction of these strains with a typical cholera vibrio.

original shows a higher potential than the passage strain. Normal serum has a slight reducing effect on both, and Rangoon smooth serum of homologous titre of 1: 25,600 has a marked effect on both. 1617 'O' antiserum affects the passage strain, indicating a specific reaction between the two, but does not cause any more change than normal serum in the original strain. The difference in the constitution of the two strains, therefore, lies mainly in their O antigenic structure. The effect of Rangoon smooth serum at 1: 6,400 has comparatively low reducing effect than at 1: 25,600 which is in agreement with the work of Shibley (1924), that the reducing effect is also dependent on the agglutinin content of the sera.

Discussion and Summary.

It is known that the phenomenon of agglutination of bacteria is due in the first place to the sensitisation of the organisms by the antiserum, which is the specific portion of the reaction, and secondly to the aggregation of the serum-organism complex by salt. (Marrack, 1934). The first reaction is determined by the 'fit' between the determinant sites and the receptors in the serum globulin, whereas the second is solely a colloidal phenomenon depending on two factors, e.g. (1) strong mutual attraction between the particles, and (2) opposite electric charges. It is thus evident that a specific reaction between an antigen and antibody can take place without actual flocculation, whereas cataphoresis gives a direct measure of the amount of reactivity in view of the fact that the surface potential of the antigen is lowered in proportion to the extent to which the reaction takes place between the antigen and the antibody. This fact has been remarkably borne out in the studies reported above.

The two Kohat strains, Original and Current, belonged to chemical Group V. The original strain is a water-vibrio and was inagglutinable, whereas the current strain was derived from the original one by subcultures and was serologically indistinguishable from a clinical cholera vibrio. This difference in the behaviour of the two strains is accounted for by difference in their surface potentials, Kohat original having a higher surface potential than Kohat current in all concentrations of salt. The serology of the strains has been fully discussed.

The two Metchnikovi strains, Original and Passage, are not true cholera vibrios. The original strain was obtained from a domestic fowl and was inagglutinable. The passage strain was derived from it by animal passage and was serologically identical with a cholera organism. They were, so far as the nature of protein and polysaccharide is concerned, chemically the same, both belonging to Group V, with the exception that the original strain had a smaller amount of carbohydrate than the passage. The agglutinability of the passage has been attributed to a comparatively lower potential difference than the original one.

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