THE IMPORTANCE OF RECEPTOR ANALYSIS FOR THE STUDY OF PHYSICO-CHEMICAL PROPERTIES OF TYPHOID BACILLI

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In an article (Scholtens, 1937) published elsewhere, I have shown that the external smooth properties of typhoid bacilli (stability in physiological salt solution and colony form) depend on the body antigens, not only on the O antigen but also on the Vi antigen.

The stability in salt solution varies with the body antigens, viz. strains containing either body antigen, O or Vi antigen, or both, are stable in salt solution (0·9% NaCl). All strains devoid of either of these two antigens are auto-agglutinable.

The occurrence of these antigens on the surface of typhoid bacilli influences the stability in salt solution. As this is pre-eminently a physico-chemical feature, I decided to examine if other physico-chemical features (viz. acid agglutination) depend on the specific antigens.

Michaelis (1911) indicated that typhoid bacilli flocculate at a special *p*H. For examination purposes he used a series of six acetate buffer solutions, the preparation of which is given below. He added to 1 c.c. of these buffers 2 c.c. of a suspension in distilled water of the strain under examination and found typhoid bacilli to flocculate first in the third tube of his series (*p*H = ± 4·3). Frequently a further agglutination appeared in some tubes containing stronger acid. The flocculation intensity ran parallel with the agglutinability by specific serum.

Arkwright (1914) discovered, in addition to the optimum found by Michaelis, a further optimum at ± *p*H 3. He pointed out the close relation of the substance agglutinated by specific serum to the substance agglutinated by acid of *p*H 4·6.

Jaffé (1912) confirmed the results obtained by Michaelis, but he did not find the optimum in the third tube (as in the case of Michaelis) but in the second (*p*H 4·6), although his acid regulator mixtures were of identical composition. Also Jaffé and others pointed to the parallelism existing between acid agglutination and the flocculation by specific sera. The present paper is concerned with the relation between acid agglutination and the bacterial antigens.

I. Technique

Bacterial suspensions. With a platinum loop a suspension was prepared from the same agar culture, both in distilled water and in physiological salt solution. The suspension in distilled water was used for the acid agglutination; the suspension in physiological salt solution for the serum agglutinations.

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Acetate buffer solutions. According to Michaelis' prescription increasing quantities, *N* acetic acid, 7½, 10, 15, 25, 45 and 85 c.c. respectively, were added to 5 c.c. *N* NaOH. All mixtures were made up to 100 c.c. In this way buffers were obtained, which, controlled electrometrically, appeared to have *p*H of ± 4·9, 4·6, 4·3, 4·0, 3·7 and 3·4 respectively.

Citrate buffer solutions. Increasing quantities, *N* citric acid, 20, 30, 45, 65 and 95 c.c. respectively, were added to 5 c.c. *N* NaOH. These gave buffer solutions of *p*H ± 3·35, ± 3·0, 2·75, ± 2·45 and ± 2·25 respectively.

Technique of acid agglutination. 1 c.c. bacterial suspension in distilled water was added to ½ c.c. acid regulator mixture. Readings were made after 24 hr. at room temperature.

H serum. From a serum prepared with a typhoid strain containing O, Vi and H antigen the H, O and Vi agglutinins were eliminated by two subsequent absorptions with the strain O 901 and a further absorption with a suspension heated to 100°, prepared with a mixture of four Ty strains, containing O, Vi and H antigens.

O serum. A Gaertner serum served for this purpose.

Vi serum. From a serum prepared with the strain Ty 4516 (containing Vi and H antigens), the H agglutinins were absorbed by means of the strain H 901. The serum agglutinations were read after 2 hr. in the incubator at 37° C. and 24 hr. at room temperature. The strains 6 S 441 II and O 901 were obtained from Dr Felix, the other strains were isolated at the Rijks Instituut.

II. Acid agglutination and H agglutination

Various strains were examined as to their agglutinability with the buffers of Michaelis and the results compared with the agglutinability by O, Vi and H agglutinins. The acid agglutination occurring with these buffers, almost without exception, appeared first and strongest in the second tube, of *p*H 4·6, which corresponds with the results obtained by Jaffé but not with those by Michaelis. In most cases a further agglutination arose in one or more of the tubes containing stronger acid. If the flocculation was restricted to only one tube it was almost invariably to the second. On comparing the acid agglutination with the agglutination caused by various agglutinin fractions it appeared that three strains, which were H inagglutinable, did not show this zone at about *p*H 4·6. The fifteen strains examined, which were H agglutinable, had a more or less extensive zone of acid agglutination at about *p*H 4·6.

Table I shows various examples. Strain 4516 (Table I), which is agglutinated by H agglutinins, shows a small zone of agglutination about *p*H 4·6 and, moreover, part of another zone (detached therefrom) which manifests itself in the sixth tube. This other zone will be discussed under another heading. Other strains, Ty 155, Ty 379 and Ty 288, showed complete agglutination in all tubes except in the first (*p*H 4·9). This parallelism between H agglutination and acid agglutination at about *p*H 4·6 was confirmed in further experiments.

The strains Ty 2P, Ty 8P (not tabulated) and Ty 469, which showed a small zone of acid agglutination at about *p*H 4·6, were plated out and subcultures were obtained from single colonies. Some of these did not possess the zone of acid agglutination at about *p*H 4·6. These subcultures appeared to be H inagglutinable, whereas those which actually had the zone of acid agglutination at about *p*H 4·6 were H agglutinable. The results obtained with strain Ty 469 are indicated in Table I.

Table I. *Acid agglutination with Michaelis buffer solutions compared to the serum agglutinations dependent on various antigens*

	Flocculation with Michaelis buffers with indicated *p*H						Serum agglutinations		
Strains	4·90	4·65	4·33	3·98	3·70	3·39	H	O	Vi
4516 N	−	+ + +	+ +	−	−	+ + +	+	−	+
6 S Ty 441 II	−	−	−	−	−	sp.	−	−	+
O 901	−	−	−	−	−	−	−	+	−
155	−	+ + +	+ + +	+ + +	+ + +	+ + +	+	−	+
469 H (3 subcultures)	−	+ + +	+ ±	−	−	−	+	−	+
469 O (3 subcultures)	−	−	−	−	−	−	−	−	+
P_3	−	+ + +	+ + +	−	−	−	+	−	+
P_3 carbolic	−	−	−	−	+ ±	+ + +	−	+	+
379 sec.	−	+ + +	+ + +	+ + +	+ + +	+ + +	+	−	+
379 sec. carbolic	−	−	−	−	−	−	−	±	+
288	−	+ + +	+ + +	+ + +	+ + +	+ + +	+	−	+
288 carbolic	−	−	−	−	−	+ + +	−	+	+

+ + + = complete clarification.
+ + / + = less complete clarification.
− = no agglutination.

The serum agglutinations were indicated positively or negatively without any distinction as to quantity. With the strains P_3, 379 sec. and 288 the addition "carbolic" indicates the strain on 0·1 % carbolic agar.

Further evidence of the dependency of acid agglutination on the H antigen was obtained as follows. It is known that freshly isolated typhoid strains at times do not show H agglutination. In examining strains freshly isolated by our bacteriological department I found two H inagglutinable strains. These two strains did not possess a zone of acid agglutination at about *p*H 4·6. After a single broth passage they became H agglutinable and acquired at the same time the propensity of being flocculated by the buffers of Michaelis at about *p*H 4·6. Resuming these results, a total of eight strains H inagglutinable forms were found. These did not have the zone of acid agglutination at about *p*H 4·6. H agglutinable forms of five of these strains and fifteen other H agglutinable strains actually possessed this zone of acid agglutination.

H inagglutinable forms can also be obtained more artificially from typhoid strains grown on 0·1 % carbolic agar. In this way it was found with ten strains that the zone of acid agglutination at about *p*H 4·6 disappeared together with the H agglutinability. Table I shows the results obtained with three strains (viz. P_3, 379 sec. and 288). We see from the acid agglutination of strain

P_3 that the culture developed on 0·1% carbolic agar flocculated in the sixth tube (*p*H 3·4). The culture grown on normal agar did not show this agglutination. I shall revert to this point in the following section.

As any other unforeseen changes may occur in the bacteria during growth on carbolic agar, the fact that under these circumstances the loss of H agglutination and of the zone of acid agglutination at about *p*H 4·6 coincide is no conclusive proof as to the relation between these two. However, it supports the result obtained by the experiments mentioned previously. The H agglutination and the zone of acid agglutination at about *p*H 4·6 thus depend on identical factors.

III. Acid agglutination and Vi antigen

In the introduction and in the preceding section it has been stated that a second zone of acid agglutination exists at a lower *p*H area than covered by the buffers of Michaelis. This zone was studied with the citrate buffers mentioned in section I. H inagglutinable strains were first chosen since the zone of acid agglutination dependent on H antigen might extend to this lower *p*H area and perhaps cause difficulties in the examination of other centres of acid agglutination. Strain 6S441 II and also the forms without H antigen, described in section I, which were obtained from strains Ty2P, Ty8P and Ty469, each of these four containing Vi antigen, were flocculated in this series of buffers, in the tubes at a lower *p*H (see Table II, strain 6S441 II). Strain O 901 and the secondary cultures, obtained from strains Ty2P, Ty8P and Ty469 by means of Vi bacteriophages (these four strains were devoid of Vi antigen), did not show any acid agglutination. The presence of Vi antigen in the strain seems necessary to produce the zone of acid agglutination in this *p*H area. Analogous results were obtained with strains actually containing H antigen, though in these experiments acid agglutination depending on this antigen complicated results.

Five strains containing O and H antigen, but no Vi antigen, were flocculated by the citrate buffers. The agglutination was strongest, however, at a higher *p*H, and became gradually weaker as the *p*H was lower. It was nearly always absent in the last tube (*p*H 2·25). This flocculation may be considered as part of the zone depending on the H antigen, for strains not containing this antigen did not show this agglutination.

Ten strains containing O and Vi antigen were either flocculated in the entire series of buffers or agglutinated more completely with buffers at a lower *p*H (Table II, strain 3047). Thorough flocculation occurred, without exception, in the tube containing the strongest acid buffer solution (*p*H 2·25). Under the influence of the Vi bacteriophage from these ten strains containing O, Vi and H antigen secondary cultures were obtained which had only O and H antigen. With regard to the agglutination by acid these cultures behaved exactly like the other strains having only O and H antigen.

The behaviour of cultures grown on carbolic agar (0·1%) at this *p*H area

was determined. After the first passage on this medium, in addition to the H agglutinability, the Vi agglutinability also had disappeared, as well as both zones of acid agglutination. After further passages the Vi agglutinability and the zone of acid agglutination at *p*H 2·25 had come back. The zone thereupon occasionally reached a higher *p*H than the corresponding zone obtained with the

Table II. *Acid agglutination at* pH 3·4–2·2 *compared with serum agglutinations*

	Flocculation with citrate buffers with indicated *p*H					Flocculation with agglutinin fractions		
Strains	3·35	2·95	2·75	2·45	2·25	H	O	Vi
4516 (Vi + H)	+	+ + +	+ + +	+ + +	+ + +	+	−	+
6S441 II (Vi)	−	−	+ + +	+ + +	+ + +	−	−	+
O 901 (O)	−	−	−	−	−	−	+	−
3047 (O + Vi + H)	±	±	+ +	+ + +	+ + +	+	−	+
3047 sec. (O + H)	+ + +	+ + ±	+ +	+	±	+	+	−
430 (O + Vi + H)	+ + +	+ + +	+ + +	+ + +	+ + +	+	−	+
430 sec. (O + H)	+ + +	+ + ±	+ +	±	sp.	+	+	−
430 carbolic	sp.	±	+ +	+ + ±	+ + ±	−	+	+
430 sec. carbolic	−	−	−	−	−	−	+	−

For explanation of signs see Table I.

Strain 430 = original strain, grown on normal agar.

Strain 430 sec. = secondary culture after lysis by Vi bacteriophage, grown on normal agar.

Strain 430 carbolic = original strain, grown on carbolic agar (0·1 %).

Strain 430 sec. carbolic = secondary culture after lysis by Vi bacteriophage grown on carbolic agar (0·1 %).

strain grown on normal agar. This provides an explanation of the fact mentioned in section I, that the strain P_3 grown on carbolic agar gives an agglutinable culture in the last tube of the Michaelis series, while the original strain is not flocculated by this buffer (Table I). In Table II, I have quoted the results obtained with cultures derived from strain 430, the original strain of which was completely flocculated by all citrate buffer solutions. On comparing in Table II the acid agglutination of the original strain 430N (O + Vi + H) with that of culture 430 sec. (O + H) obtained with a Vi bacteriophage, both grown on normal agar, it will be seen that part of the acid agglutination of this strain coincides with the occurrence of Vi antigen. After growing the normal and secondary culture on carbolic agar a large part of the acid agglutination also disappeared. This may chiefly be ascribed to the fact that part of the flocculation of strain 430 in the citrate buffers depends on H antigen.

From these experiments the conclusion may be drawn that the zone of acid agglutination at about *p*H 2·25 depends on the presence of Vi antigen.

Arkwright (1914) has further observed the acid agglutination at *p*H 4·6 to have a floccular appearance, while in the second zone the agglutination was granular. In this respect acid and serum agglutination run parallel and show that these phenomena have many points in common with each other.

The antigens, therefore, play an important part in determining the physico-chemical properties of bacteria.

The question may be put now whether or not the fact of these bacterial substances having both antigenic properties and being physico-chemically active is purely a matter of coincidence. In my opinion it must be the physico-chemical properties of these substances which cause them to be antigens.

Conclusions

I. The acid agglutination may depend on the antigens.

II. In the case of typhoid bacilli one zone of acid agglutination depends on the H antigen. This lies at *p*H 4·6.

III. A second zone of acid agglutination depends on the Vi antigen. This lies at *p*H 2·2 or lower.

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