

TWO SEROLOGICALLY DIFFERENT GROUPS AMONG THE TRUE CHOLERA VIBRIOS

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In a paper published in 1933 Scholtens showed that among eight strains of vibrios agglutinable with agglutinating cholera serum there were two types with a different antigenic composition which he called A and AB. The author of the present paper worked on this problem independently at the same time and found, like Scholtens, that among the true cholera vibrios it was possible to differentiate two serologically well-defined groups, the grouping being based on O factors alone.

The technique plays a definite role in agglutination and absorption tests with vibrios. This was shown by Heiberg (1935), and is mentioned by Gardner and Venkatraman (1935). The use of formalised broth cultures and readings at 20 hours give an H or a combined H + O reaction; on the other hand, a suspension of living vibrios from an agar culture in saline and readings after a few hours give an O reaction alone. In absorption tests the use of a suspension of an agar culture in saline as absorbing antigen gives absorption of agglutinins for both the homologous H and O factors, the H-agglutinins being absorbed by the antigens even if they cannot agglutinate them.

To investigate whether two strains agglutinable with sera produced by the other strain are identical or not, it is necessary to do a cross absorption test: a simple cross-agglutination test is not sufficient. An example of a complete cross absorption test is given below: If we call the two bacteria, the antigenic composition of which we wish to investigate, A and B, an absorption reaction may be carried out in the following manner:

Sera are prepared from both strains, and absorption and agglutination between these sera and the two strains carried out according to the following scheme:

Serum	Antigen	Dilutions of serum
A/O	A	
A/O	B	
A/A	A	
A/B	B	
A/B	A	
B/O	B	
B/O	A	
B/B	B	
B/A	A	
B/A	B	

In the first column is shown the serum used for agglutination; A/B indicates that serum A has been absorbed by strain B, the letter above the line

giving the serum used for absorption and the letter below the strain used for absorbing the serum.

The figure 0 below the line indicates that the serum has not been absorbed in the experiment in question.

The second column gives the antigen used for the agglutination reactions.

Of the ten agglutination tests carried out altogether, one-half are to find whether all the antigen of A is present in B and the other whether all the antigen of B is present in A.

The two first agglutination tests with non-absorbed serum A and the antigens A and B show how highly the serum can agglutinate the strains. The third agglutination test shows whether all agglutinin present in serum A is removed by absorption with A (with the technique used here). The next two agglutination tests show the agglutinogenic power of serum A after the agglutinins fixed by B have been removed. The agglutination test with B controls whether the absorption of the B agglutinating factors was complete. The agglutination test with A shows whether serum A possessed agglutinins other than those removed by B. Agglutination taking place here means that the strain A contains one or several antigenic factors not present in B; no agglutination means that all the antigenic factors of strains A are found in strain B.

Similarly the next five agglutination tests show whether all the antigens of strain B are present in strain A.

Only by carrying out such a cross absorption test, *i.e.* the examination of both strains with both sera, is it possible to determine whether two strains are identical or not.

By carrying out a series of absorption tests between true cholera vibrios and some El Tor strains it was found, as shown below, that all the strains agglutinable with ordinary cholera serum (using a technique giving O reaction only) contained a common antigenic factor A, and that a few El Tor strains and some true cholera vibrios contained two antigenic factors: A and B. This terminology agrees with that of Scholtens, since two of his strains are included in my investigation.

By carrying out cross absorption tests between El Tor Doorenbos 47 (here called 94) and *Vibrio cholerae* Manila 30/529 (here called 8) it was found (see Table I) that while all the antigenic factors of the cholera strain were present in the El Tor strain—the El Tor strain could exhaust the cholera serum of agglutinins for the cholera strains—the El Tor strain contained one more antigenic group; for the cholera strain could not exhaust the El Tor serum of all the agglutinin for the El Tor strain.

The common group of antigen for the two strains was, as already mentioned, called A, and the group which was present in strain 94 alone was called B; thus strain 94 contains AB, strain 8 A alone.

As strain 94 was found to be constantly haemolytic, the relationship between serum 94 and some strains that were both agglutinable with cholera

Table I. *Cross absorption between the strains V. cholerae Manila 30/529 ("8") and V. El Tor Doorenbos 47 ("94")*

Serum	Antigen	Serum dilutions					Saline
		100	200	400	800	1600	
8/0	8	3	3	3	2	1	0
8/0	94	3	3	3	2	0	0
8/8	8	0	0	0	0	0	0
8/94	94	0	0	0	0	0	0
8/94	8	2 (0)*	0	0	0	0	0

Serum	Antigen	Serum dilutions				
		200	800	1600	3200	6400
94/0	94	3	3	3	3	2
94/0	8	3	2	1	0	0
94/94	94	0	0	0	0	0
94/8	8	0	0	0	0	0
94/8	94	3	3	3	2	0

3 indicates strong agglutination.

2 and 1 indicates lower degrees of agglutination.

0 indicates no agglutination.

* By repeating the test no agglutination appeared in this tube.

serum and haemolytic was investigated, bearing in mind the possibility that the factor B in particular might be found in the strains of this serological group, which had at some time or other appeared to be haemolytic.

Absorption of serum 94 with these strains gave the results indicated in Table II.

It will be seen that strains 101, 103, 106 and 113 completely exhaust serum 94; they must therefore contain the factors A and B. Strain 258 causes nearly complete exhaustion; as it is agglutinated by serum 8 it contains factor A and, as seen from this experiment, very probably B also; but it has not caused total absorption.

In contrast to this strains 44, 45, 49, 54, 71, 100 and 369 can influence the agglutinogenic power of serum 94 towards strain 94 only slightly. As all these strains are agglutinable with serum 8, and therefore contain factor A, this probably means that they do not contain B. Whether or not the strains contain all the factors of group A may be gathered after absorption of serum 8 with the strains.

Thus it has been shown that the factor B is not connected with all haemolytic strains of this group. To find more strains containing the factor B, and whether the latter was present in non-haemolytic strains, 236 strains agglutinable with a cholera serum—serum 8—Manila 30/529, *i.e.* a serum containing factor A or at least a part of A—were agglutinated with a serum that contained agglutinin for the factor B and not for the factor A.

This serum was produced by absorbing serum 94 (El Tor Doorenbos 47), which contains agglutinin for both factors A and B, with strain 8, which can only absorb agglutinin for the factor A.

This test showed that a total number of thirty-four strains gave strong agglutination with the serum containing agglutinin for the factor B alone, namely the strains mentioned in Table IV.

Table IV. *List of the strains containing both antigenic factors*

Name	When received	Received from	Isolated from
Siam B-1	1932	East. Bur. Health Org.	
Siam B-2	1932	" "	
V. chol. 1155	1932	Saigon Pasteur Inst.	Case of cholera
V. chol. 1155	1934	" "	"
V. chol. K. 1473	1932	" "	"
V. chol. K. 1473	1934	" "	"
Kommavibr. Bed No. 10	1928	Calcutta	
Chol. Jenkins N.C. 1633	1926	Nat. Coll. Type Cult., London	Case of cholera
El Tor D 47	1933	" " "	
El Tor D 6	1933	Lab. v. Gezondhl., Amsterdam	
El Tor D 47	1933	" "	
Cholera 51	1933	School Trop. Med., Calcutta	
Cholera 58	1933	" " "	
Cholera 63	1933	" " "	
Cholera 618	1933	" " "	
Cholera 836	1933	" " "	
Cholera 1528	1933	" " "	
Cholera 1543	1933	" " "	
Chol. 1609/1	1933	" " "	Case of cholera
Chol. 1609/2	1933	" " "	"
Chol. 1610	1933	" " "	"
Chol. 1621	1933	" " "	"
Chol. 1624	1933	" " "	"
Chol. 1633	1933	" " "	"
Chol. 1651/2	1933	" " "	"
Chol. 1653	1933	" " "	"
Chol. 1655	1933	" " "	"
Chol. 1659	1933	" " "	"
Chol. 1662	1933	" " "	"
Chol. 1668	1933	" " "	"
Chol. 1676	1933	" " "	"
Chol. 1683	1933	" " "	"
Mekka-Medow	1933	Lab. v. Gezondhl., Amsterdam	
Shanghai 30/32	1933	Nat. Health Adm., Nanking	

Some strains containing the antigenic factor A alone—the most common of *V. cholerae*—are: *V. cholerae* Kedah (N.C.T.C. 22); *V. cholerae* 7812f, Kasauli; Manila 30/529; *V. El Tor* 4 (N.C.T.C. 307); and *V. El Tor* D 80/31.

The strains containing the two antigenic factors, as well as the strains containing a single factor only, have been isolated in many different places geographically far apart.

In regard to factor B, the question arises as to whether it is constantly present in these strains. It might well be a labile factor, lost readily and appearing readily. In order to get some answer to this question, fresh cultures of two of the strains, included in my collection of 1932, were again received in 1934 from the institute at the place of isolation—l'Institut Pasteur at Saigon. Examination of the cultures subcultured at Saigon and the cultures subcultured in Copenhagen showed the presence of both factors A and B in all of them. Further, the reactions of two of the other cultures showed the same

phenomenon, since they really represent the same strain, having been sent to me from Amsterdam as well as from London.

By agglutination of 384 strains of vibrios with a serum A and a serum AB it was found that no strain was agglutinable with serum AB without being agglutinable with serum A. This indicates that none of these strains contained the antigenic factor B alone, and only with factor A. This may explain why so many previous examinations proved negative.

DISCUSSION

Until 1933 several fruitless attempts were made to find different serological groups among the true cholera vibrios (Gotschlich and Kolle, 1903; Neufeld and Haendel, 1907; Douglas, 1921). In 1933 Scholtens published a small investigation relating to this subject. He found that among the true cholera vibrios there were two groups, one containing a single factor A, the other containing two factors AB.

Independently of Scholtens the author obtained the same results in an investigation on a larger scale.

The reason why investigations have hitherto proved negative is that the absorption test has not been carried out completely, *i.e.* no cross absorption tests were made. If the latter is not carried out the examination does not give full information about the possible identity of the strains examined. The findings, in this paper, of two groups among true cholera vibrios, namely a large group with the common factor A alone and a smaller group with one factor more (B) in addition to the common factor A may be of interest in epidemiological investigations. It may also be of importance for the preparation of vaccines should any pathogenic characters be combined with the antigenic factor B. The two antigens A and B are thermostable, *i.e.* they are somatic antigens.

The strains examined here were isolated from all parts of the world where the existence of cholera is or has been of any importance. It was found that strains belonging to both serological groups have been isolated from many different places. There is nothing to indicate that any one of the groups predominates in any special place. Strains belonging to group A as well as group AB have been isolated in Siam, British India, Northern China and Shanghai; moreover some of the strains isolated at El Tor belong to group A and some to group AB.

The finding of these two groups is of no importance in bacteriological diagnosis, because factor A is common to both groups.

SUMMARY

By careful investigation of the somatic antigens of the true cholera vibrios—the strains containing O factor I according to Gardner's grouping (1935)—it was found, as did Scholtens (1933), that the cholera vibrios may be divided

into two serological groups: one containing the antigenic factor A and the other the antigenic factors AB.

This finding may be of importance in epidemiological investigations and in the preparation of vaccines.

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