THE Q PROTEINS AND NON-SPECIFIC O-ANTIGENS OF THE CHOLERA VIBRIO

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In a short note (White, 1934) I reported the isolation from the cholera vibrio of alcohol-soluble protein antigens. These I called Q proteins, on analogy with the similar substances earlier obtained by myself from *Salmonella* and related bacteria, and I described their fractionation into two parts, Q_1 and Q_2. In the interim Linton and Mitra (1934), Linton, Mitra and Seal (1935) and Linton, Singh and Seal (1935), using a very similar method of extraction, have obtained from *Vibrio cholerae* an "acid-soluble A substance", corresponding in all probability to my total Q fraction, and have subjected it to chemical and other examination.

In the course of their recent survey of cholera and other vibrios, Gardner and Venkatraman (1935) noted the existence of a widely non-specific O (heat-stable) antigen, patent in agglutination tests where vibrio suspensions heated at 100° C. were exposed to the antisera of similarly heated vibrios, though inevident where suspensions killed with 0·2 per cent. formalin or the antisera of such suspensions were employed.

In the present note some further information regarding the serological properties of the Q proteins is summarised, and evidence is presented to show that these substances represent or contribute to the non-specific O-antigen of Gardner and Venkatraman.

The Q substances used in the present studies were isolated by the original method: extraction of mass cultures of agar-grown vibrios with alcohol containing 5 per cent. of $N/1$ HCl solution at 45–50° C.; removal of the vibrios by filtration; precipitation of the total Q fraction by neutralisation of the filtrate with NaOH; treatment of the total Q fraction with alkalised water to extract the Q_1 substance and precipitation of the Q_2 substance from an aqueous suspension of the residue with HCl, so separating it from the salts which hitherto rendered it insoluble in neutral or slightly alkaline water.

The Q_1 substance has been purified by precipitation with acid at the isoelectric point (about *p*H 5·5) and by removal of material insoluble on further acidification. The Q_2 substance has been twice or three times reprecipitated by HCl at a *p*H below the precipitation range of Q_1. Solutions of the Q_1 and Q_2 proteins of S and R races of a typical strain of *V. cholerae* (Kasauli 92/1, type Ogawa) were prepared and intravenously injected into rabbits. Sera of high precipitating potency were in all cases readily obtained:

https://doi.org/10.1017/S0022172400032514 Published online by Cambridge University Press

as in tests mentioned in the earlier note the sera showed relatively little selectivity for the homologous antigen, precipitating vigorously with all the other antigens of the series.

The results of agglutination and absorption tests performed are indicated in the accompanying tables. These tests were made by the usual macroscopic technique. The tubes were incubated in the water-bath at 50° C. for at least 4 hours, and the recorded readings were made after the racks had stood thereafter at room temperature overnight. Smooth strains, living or heated at 100° C. for 15–30 min., were tested in a medium containing 0·85 per cent. of NaCl; rough strains in one containing 0·42 per cent. of NaCl. Young agar-grown cultures were used throughout. The strains studied were selected from those employed by Gardner and Venkatraman (1935) and White (1935): to the papers of these authors the reader is referred for such information as is available regarding the nature and origin of the cultures. Throughout this communication Gardner and Venkatraman's classification of vibrios on the basis of their O-antigens is adopted.

TESTS WITH Q_1 ANTISERA

Table I (*a*) and (*b*), relates to anti-Q_1 sera: the observations may be summarised as follows:

(1) The three Q_1S and three Q_1R cholera antisera prepared appeared to be qualitatively identical in all agglutination and absorption tests and it is concluded that the Q_1 proteins of S and R vibrios are serologically the same.

(2) These anti-Q_1 sera have caused slow clumping, up to a dilution of 1 : 50 or 1 : 100 of most S vibrios (O groups I–VI and "individual" strains) tested in the living state; R vibrios have proved rather more sensitive: protracted immunisation of rabbits did not materially increase the titre of this reaction.

(3) To the rule stated in (2) the vibrio Manila Ha 11 R provided a notable exception: in the living state it was frequently clumped by all the Q_1 antisera available to a high titre (1 : 1000–1 : 5000). The suspensions prepared on different occasions varied enormously in sensitiveness.

(4) When agglutination tests were made with vibrios previously heated in saline suspension at 100° C. for 15–30 min., the majority became highly agglutinable with all Q_1 antisera, simulating living cultures of Manila Ha 11 R. The relative and absolute sensitiveness of the heated cultures varied from one series of preparations to another; zone phenomena were frequently observed.

(5) Strong evidence was obtained by means of absorption tests to show that the very general reaction of heated vibrios to Q_1 antisera was of the same nature as that of living Manila Ha 11 R and it was found that living cultures could fix the Q_1 agglutinins which clump the heated vibrios. It is a sound conclusion that the Q_1 antigen is not a serological artefact but that its

Table I (*a*)

Vibrio tested: O group of Gardner and Venkatraman	Strain	Tests with living vibrios: Anti-Q_1 S (cholera) serum	Anti-Q_1 R (cholera) serum	Normal rabbit serum	Tests with vibrios heated at 100° C. — Anti-Q_1 S (cholera) serum: Untreated	Absorbed c Q_1 R	Absorbed c Q_2 R	Absorbed c S cholera carbohydrate	Absorbed c vibrio Ha 11 R (100° C.)	Absorbed c *V. cholerae* Kasauli 92/1 living	Anti-Q_1 R (cholera) serum: Untreated	Absorbed c Q_1 S	Absorbed c Q_2 S	Absorbed c R cholera carbohydrate	Absorbed c *V. cholerae* Kasauli 92/1 living	Absorbed c vibrio Ha 11 R living	Absorbed c vibrio Ha 11 R (100° C.)	Normal rabbit serum
I	Kasauli 92/1	50	100	<50	1000	<100	<100	1000	<100	<100	>2000	<100	<100	>2000	<100	500	<100	<50
	Shillong 610	50	100	,,	1000	,,	,,	—	,,	,,	>2000	,,	,,	>2000	—	—	—	,,
	Inaba	50	100	,,	1000	,,	,,	1000	,,	,,	>2000	,,	,,	>2000	100	500	<100	,,
II	Nanking 32–74	50	100	,,	200	—	—	—	—	—	200	—	—	—	—	—	—	,,
	,, 32–121	50	100	,,	500	—	—	—	—	—	500	—	—	—	—	—	—	,,
	,, 32–126	50	100	,,	200	—	—	—	—	—	500	—	—	—	—	—	—	,,
III	Bulacan	50	50	,,	500	<100	<100	1000	<100	<100	1000	<100	<100	—	—	—	—	,,
	Nanking 32–123	<50	50	,,	200	—	—	—	—	—	500	—	—	—	—	—	—	,,
IV	Nanking 32–102	50	50	,,	500	—	—	—	—	—	1000	<100	<100	—	—	—	—	,,
	,, 32–106	50	50	,,	500	—	—	—	—	—	500	—	—	—	—	—	—	,,
	,, 32–110	50	50	,,	100	—	—	—	—	—	200	—	—	—	—	—	—	,,
VI	Kasauli 73	50	100	,,	2000	<100	<100	1000	<100	<100	>2000	<100	<100	>2000	100	500	<100	,,
"Individual" strains	Kasauli 11	50	100	,,	2000	<100	<100	2000	<100	<100	>2000	<100	<100	>2000	<100	500	<100	,,
	,, 77	<50	50	,,	1000	,,	,,	—	,,	,,	>2000	—	,,	—	—	—	—	,,
	Nanking 32–101	50	100	,,	2000	,,	,,	2000	,,	,,	>2000	<100	,,	>2000	—	—	—	,,
	,, 32–127	<50	<50	,,	100	—	—	—	—	—	100	—	—	—	—	—	—	,,
	Manila Ha 10	50	50	,,	500	—	—	—	—	—	2000	—	—	—	—	—	—	,,
Rough races (see White, 1935)	Kasauli 92/1 R	100	200	,,	—	—	—	—	—	—	—	—	—	—	—	—	—	—
	Shillong 1077 R	100	200	,,	—	—	—	—	—	—	—	—	—	—	—	—	—	—
	Manila Ha 10 R	100	200	,,	—	—	—	—	—	—	—	—	—	—	—	—	—	—
	,, Ha 11 R	1000	2500	,,	—	—	—	—	—	—	—	—	—	—	—	—	—	—
	Kasauli 11 R	100	200	,,	—	—	—	—	—	—	—	—	—	—	—	—	—	—
	,, 77 R	<50	100	,,	—	—	—	—	—	—	—	—	—	—	—	—	—	—
	Nanking 32–101 R	50	200	,,	—	—	—	—	—	—	—	—	—	—	—	—	—	—
	Bulacan R	200	200	,,	—	—	—	—	—	—	—	—	—	—	—	—	—	—

Table I (*b*)

Vibrio tested	Anti-Q_1 R (cholera) serum: Untreated	Absorbed c *V. cholerae* Kasauli 92/1 living	Absorbed c *V. cholerae* Kasauli 92/1 (100° C.)	Absorbed c vibrio Manila Ha 11 R (100° C.)
Manila Ha 11 R living	>2000	<100	<100	<100
Kasauli 92/1 (100° C.)	>2000	<100	<100	<100

The figures indicate the highest dilution of serum in which agglutination, visible to the unaided eye by artificial light, was observed.

— indicates that no test was made.

"Kasauli 92/1 (100° C.)" signifies that the suspension used for agglutination, absorption or as a vaccine had been heated at 100° C.

characteristic receptors are present and effective in the living vibrio. It seems probable that traces of the Q substance are present in the reacting surface of the intact vibrio, traces which involve the living organism in a "precipitin reaction" with Q antibodies when the serum is not too greatly diluted, and that when the vibrios are heated this Q substance, becoming more fully exposed, can occasion clumping with much diluted serum.

TESTS WITH Q_2 ANTISERA

Table II (*a*) exemplifies the results of numerous agglutination tests made with Q_2S (cholera) antiserum. To summarise the findings:

Table II

(*a*) Anti-Q_2S, type Ogawa (Kasauli 92/1), serum

Vibrio tested	Tests with living vibrios (1) Untreated	(2) Absorbed c *V. cholerae* type Inaba (Inaba)	(3) Absorbed c specific carbohydrate of *V. cholerae*, Kasauli 92/1	Tests with vibrios heated at 100° C. (4) Untreated	(5) Absorbed c specific carbohydrate of *V. cholerae*, Kasauli 92/1
V. cholerae (O group I):					
Type Ogawa, Kasauli 92/1	1000	<100	<100	2,500	2500
Type Inaba, Inaba	1000	<100	<100	5,000	5000
Vibrio Nanking 32–101 (Individual O)	50	—	—	10,000	5000
,, Kasauli 11 (Individual O)	100	—	—	2,500	5000
,, Kasauli 73 (O group VI)	50	—		5,000	5000
,, Manila Ha 11 R	5000	200	2500	—	—

(*b*) O antiserum, type Ogawa (Kasauli 92/1, 100° C.)

Vibrio tested	Tests with living vibrios (1) Untreated	(2) Absorbed c *V. cholerae* type Inaba (Inaba)	(3) Absorbed c specific carbohydrate of *V. cholerae* Kasauli 92/1	Tests with vibrios heated at 100° C. (4) Untreated	(5) Absorbed c Q_1S of *V. cholerae* Kasauli 92/1	(6) Absorbed c Q_2S of *V. cholerae* Kasauli 92/1	(7) Absorbed c specific carbohydrate of *V. cholerae* Kasauli 92/1
V. cholerae (O group I):							
Type Ogawa, Kasauli 92/1	2500	2500	100	2500	2500	2500	250
Type Inaba, Inaba	500	<50	<50	1000	1000	500	100
Vibrio Nanking 32–101 (Individual O)	<100	<50	<50	250	100	50	100
,, Kasauli 11 (Individual O)	<100	<50	<50	500	100	50	100
,, Kasauli 73 (O group VI)	<100	<50	<50	500	50	<50	250

Symbols as in Table I. Salt controls negative.

(1) *In tests with living suspensions* of vibrios anti-Q_2S serum had a strong and selective agglutinating action on all smooth vibrios of O group I, clumping all *V. cholerae* and El Tor strains to approximately the same titre. Neither in agglutination nor in absorption tests were the type distinctions, so marked in the case of ordinary O anti-cholera sera, discernible. (Compare Table II (*a*) cols. 1 and 2 with Table II (*b*) cols. 1 and 2.) The agglutinins concerned were completely fixed by the protein free carbohydrates of *V. cholerae*: it is to be concluded that the active receptors, common to the Ogawa and Inaba types, are "included in" the carbohydrate fraction of the vibrios. On other living

vibrios Q_2S antisera exerted a feeble agglutinating action save in the case of Vibrio Manila Ha 11 R which was clumped, as by Q_1 antisera, to a high titre: the agglutinins concerned in this reaction were not bound by the carbohydrates of *V. cholerae*.

(2) *When vibrios heated at* 100° C. were employed for agglutination tests a generalised agglutination of very high titre (up to a 1 : 10,000 dilution) and resembling that given by Q_1 antisera was superimposed on the reactions of living cultures stated in (1). The antibodies involved were not inactivated by saturating the serum with specific carbohydrate.

(3) Q_2R antisera behaved in agglutination tests with living and heated vibrios very much as did Q_1 antisera: there was no selective action on O group I.

The "non-specific O agglutination" of Gardner and Venkatraman

No difficulty was experienced in confirming the statement of Gardner and Venkatraman that "boiled" vibrios of all the recognised O groups and types cross agglutinate in a non-specific manner with the antisera of "boiled" vibrio vaccines. Results obtained in one experiment are given in Table II (*b*). It was an obvious possibility that this non-specific O agglutination might correspond with the Q agglutination of heated vibrios described above, and steps were taken to compare the two phenomena.

(1) It was found that there was a fair correlation between the behaviour of any series of heated vibrio suspensions with Q_1 antiserum and with O antisera containing the non-specific antibodies; that certain living suspensions of Manila Ha 11 R agglutinated appreciably with the same antibodies, a fact of which there is an indication in Gardner and Venkatraman's paper; that the Q_1 protein gave stronger precipitation with the sera of heated, than with those of unheated vaccines;

(2) That the Q proteins could greatly reduce the titre of the non-specific O agglutinins though always leaving some measure of activity; that saturation of the sera with the specific carbohydrates of *V. cholerae* also effected a reduction, usually less marked, of the non-specific titre. It was concluded that the Q proteins, while probably the most important contributors to the non-specific O agglutination of heated vibrios, are not the sole factors concerned.

Summary

Vibrios heated at 100° C. in saline suspension agglutinate in a generalised manner and often to a high titre with the antisera of the Q proteins of the cholera vibrio. The antibodies concerned are not inactivated by the carbohydrate fraction of *V. cholerae*. Occasional strains of vibrio react similarly in the living state with these Q (cholera) agglutinins. The antiserum of the Q_2 substance of S *V. cholerae* seems to possess agglutinating properties additional to those of anti-Q_1 and anti-Q_2 R sera, rather more specific and possibly

related to "carbohydrate" receptors. There is reason to believe that the Q proteins are true constituents of the living vibrio and are not serological artefacts due to reagents and heat. It seems that these substances and their antibodies are important contributors to the "non-specific O agglutination" of vibrios recently discussed by Gardner and Venkatraman.

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(*MS. received for publication* 19. VIII. 1935.—Ed.)