THE BIOCHEMICAL REACTIONS OF VIBRIOS

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In a recent paper Gardner and Venkatraman (1935) discuss the problem of the grouping of vibrios.

A more extensive investigation into the same subject was published by Heiberg (1935) at about the same time. A comparison of these two papers is, therefore, not without interest, particularly as forty-five strains of well-known origin were examined by Gardner and Venkatraman as well as by Heiberg. In a previous paper Heiberg (1934) has shown that biochemical reactions, provided certain sugars were employed, could quite well be used for grouping vibrios. Gardner and Venkatraman write, however: "The general experience of bacteriologists is strongly against the possibility of an accurate biochemical classification of the group (Nobechi, 1923). Heiberg's (1934) claims in that direction have been denied by Doorenbos (1934), and have found little support in our limited study of the question."

In reply to this statement it may be affirmed that Doorenbos does not deny the utility of biochemical reactions when examining vibrios in general, for this point is not discussed in his paper. He confirms Heiberg's observation that it is not possible to distinguish between most of the El Tor strains and the true cholera strains by means of biochemical reactions, but he gives prominence to the fact that biochemical reactions may be of great value in the examination of strains which are inagglutinable with cholera serum at the time of isolation, whilst Heiberg also points out the practical value of these reactions. To reject the utility of this method because it cannot be used to distinguish between the El Tor vibrios and the true cholera vibrios is not justifiable.

It is not surprising that Gardner and Venkatraman have found little support for Heiberg's claims, since no investigations on grouping based on Heiberg's scheme are to be found in the published paper. The main grouping of vibrios by Gardner and Venkatraman is based on biochemical reactions in such a way that the non-fermenting vibrios form a separate group, while all other vibrios are collected together under their "cholera group". For the biochemical reactions, Gardner and Venkatraman used acid formation in media containing glucose, mannite, maltose, dulcite and saccharose, notwithstanding that Heiberg's investigations showed that of these sugars saccharose is the only one of definite importance. The two other sugars pointed out by Heiberg to be of some importance, mannose and arabinose, are not included in their investigation.

By using mannose, saccharose, and arabinose for biochemical reactions it is possible to distinguish six different types of fermentation in vibrios (see Table I).

Table I. *A grouping of vibrios according to fermentation types*

Type	Mannose	Saccharose	Arabinose
I	+	+	0
II	0	+	0
III	+	+	+
IV	0	+	+
V	+	0	0
VI	0	0	0

Note. + indicates acid formation and no gas; 0 indicates no acid formation.

The distribution into these six types of 384 strains of vibrios collected at random is given in Table II.

Table II. *Distribution of* 384 *strains into the six types of fermentation*

Type	I	II	III	IV	V	VI
Number of strains	287	75	12	3	2	5

Fermentation type I includes, with one exception, all the strains in the serological group I of Gardner and Venkatraman. The only exception is the strain vibrio Dunbar which has a little of this antigen. All the strains fermenting according to type II–VI, except V, Dunbar, differ from the true cholera vibrios both serologically—that is, in not belonging to serological group I of Gardner and Venkatraman (the true cholera vibrios)—and biochemically. *On the basis of this observation it is permitted to conclude that any vibrio not fermenting according to type I has no (or hardly any) O-antigen common to the true classical cholera vibrios (Gardner and Venkatraman O-group I).*

As already mentioned, forty-five strains identical with those found in Gardner and Venkatraman's collection were also examined by Heiberg. Therefore it is quite simple to investigate the biochemical grouping of these strains in relation to the occurrence of O factor I.

Table III shows the scheme of Gardner and Venkatraman, and in addition the fermentation types and serological behaviour according to Heiberg.

In these forty-five strains Gardner and Venkatraman found three biochemically atypical and two non-fermenting strains. All these five strains are not in the serological groups of Gardner and Venkatraman and have therefore no O-antigen common to the classical cholera vibrios.

According to Heiberg's types of fermentation no less than twenty-four of the forty-five strains may be excluded as having O-antigen common to the classical cholera vibrios, namely two non-fermenting strains (Fermentation type VI) and twenty-two strains fermenting like the types II–V. Only in the remaining twenty-one strains is it possible to find O-antigen I.

Vibrios have so few characteristic features that a method of examination which enables the exclusion of a strain having O-antigen common to the classical cholera vibrios should not be rejected.

Table III

Gardner and Venkatraman No.	Heiberg No.	Name	Origin	Gardner and Venkatraman Biochemical characters	Gardner and Venkatraman Antigens (subgroup)	Heiberg Type of fermentation	Heiberg O-antigen (like f, classical cholera)
1	**40**	Kasauli 11	Case of cholera	Atypical (sacch. 0, c.r.† 0)	Individual	V	Atypical
2	33	Kasauli 73	,,	Typical	VI	I	,,
3	**35**	Kasauli 77	,,	Atypical (c.r.† 0)	Individual	III	,,
4	37	Kasauli 1410/1	,,	—	I	I	Typical
5	38	Kasauli 1416/1	,,	—	I	I	,,
6	32	Kasauli 1485	,,	—	I	I	Spontaneous
7	41	Kasauli 1486/2	,,	—	I	I	Typical
8	**39**	Kasauli 3205/2	,,	Typical	II	II	Atypical
9	36	Kasauli 3214/4	,,	,,	I	I	Typical
10	34	Kasauli 3222/1	,,	,,	I	I	,,
11	8	Manila 30/529	,,	,,	I	I	,,
12	**26**	Manila Ha 10	,,	,,	Individual	II	Atypical
13	**27**	Manila Ha 11	,,	—	Rough	II	,,
14	**28**	Manila Ha 19	,,	—	Individual	II	,,
15	58	Nyback	,,	Typical	I	I	Typical
17	215	Shillong X	,,	—	I	I	,,
24	**21**	Pasig O 27/9	,,	—	Individual	II	Atypical
25	**29**	Bulacan 215, 530	,,	—	III	II	,,
32	**382**	Nanking 32/121	,,	Typical	II	II	,,
33	**383**	Nanking 32/123	,,	,,	III	II	,,
34	384	Nanking 32/124	,,	,,	II	I	,,
35	**386**	Nanking 32/126	,,	,,	II	II	,,
36	**387**	Nanking 32/127	,,	,,	Individual	II	,,
37	**388**	Water vibrio 32/101	Water	,,	,,	II	,,
46	**389**	Water vibrio 32/110	,,	,,	IV	II	,,
54	252	El Tor 34–D 16	Healthy pilgrim	,,	I	I	,,
66	101	El Tor Doorenbos 6	Healthy person	,,	I	I	Typical
67	102	El Tor Doorenbos 20	Case of "malaria"	,,	V	I	Atypical
68	103	El Tor Doorenbos 47	Healthy pilgrim	,,	I	I	Spontaneous
69	105	El Tor Doorenbos 67	Bacillary dysentery	,,	V	I	,,
70	106	El Tor Doorenbos 80	Healthy pilgrim	,,	I	I	Typical
79	103	Tor 47–D	,,	,,	I	I	Spontaneous
80	**104**	El Tor Doorenbos 49	Bacillary dysentery	,,	VI	II	Atypical
81	100	Tor A	,,	,,	I	I	Typical
82	69	Paracholera Martin	Choleric diarrhoea	,,	III	I	Atypical
83	70	Paracholera A, Mackie	,,	Atypical (malt. 0)*	Not I–VI	I	,,
84	**68**	Paracholera B, Mackie	,,	Typical	,,	II	,,
90	**75**	Cholera-like vibrio, H 309	Healthy person	,,	VI	II	,,
91	**79**	Cholera-like vibrio, P 57	—	,,	Not I–VI	II	,,
92	**85**	Cholera-like vibrio, W 832/3	Water (India)	,,	,,	II	,,
93	**93**	Cholera-like vibrio, W 835/1	,,	,,	,,	II	,,
94	**73**	Cholera-like vibrio, P 615	—	,,	,,	II	,,
95	**72**	Cholera-like vibrio, W 833/5	Water (India)	,,	? IV	IV	,,
96	**83**	Cholera-like vibrio, Dennet	—	Non-fermenting	0°	VI	,,
97	**78**	El Tor 1908	—	,,	0°	VI	,,

Numbers in heavy type indicate strains not biochemically classical cholera vibrios.

* By Heiberg + malt. — = not tested or unknown (G. and V.). 0° = neither biochemically nor antigenically related to the rest.

† Denotes cholera-red reaction, 0 = negative.

The three biochemical reactions have proved constant, a fact which strengthens their value. Nobechi's (1923) negative results, in trying to find a biochemical grouping of the vibrios, are easily explained; for he only examined twenty-nine strains, out of which twenty-one were true cholera vibrios. On the basis of such a small and homogeneous material the general condemnation of a method of investigation is not justified.

SUMMARY

In reply to Gardner and Venkatraman's rejection of the use of biochemical reactions in the grouping of vibrios according to Heiberg's method, it is shown that in forty-five of the strains examined by Gardner and Venkatraman it is possible to exclude twenty-four as being classical cholera vibrios by means of fermentation reactions with certain sugars, *i.e.* mannose, saccharose and arabinose. The author believes that it is not possible to deny the value of such reactions in attempting to group vibrios. The constancy of the reactions is emphasised.

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