The Incidence and Character of Vibrios in British Waters

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SUMMARY: Forty-nine strains of vibrio were isolated from 32 of 43 samples of fresh water collected in various parts of England and Wales during the period October 1945 to July 1946. More than one kind of vibrio were found in 14 of the samples.

The types of vibrio isolated were heterogeneous in their fermentation reactions. Only three gave a cholera-red reaction. Twenty-five produced haemolysin for goat erythrocytes and all the haemolysins tested were filterable through gradacol membranes of 75 m μ pore diameter; an antiserum prepared against the haemolysin of one strain neutralized those of the other haemolytic water strains and of an El Tor vibrio.

Only one serological group of five strains related in 'O' and 'H' antigens was found; a few other strains showed H relationship and one (or two) possessed the H antigen of *Vibrio cholerae*; otherwise the organisms isolated were serologically heterogeneous with little antigenic similarity to one another or to known Asiatic vibrio types.

Compared with the extensive studies of the organisms of the *Vibrio* group which have been undertaken in India and the Far East, there is little information concerning their distribution and characters in western countries where cholera no longer occurs. In Britain, Houston (1913) examined a large number of London water samples, mainly from the Thames, for the presence of 'water microbes culturally resembling vibrios', but after finding 1885 strains atypical in the cholera-red test and in their behaviour towards gelatin he rejected them without further study.

The present investigation was therefore started with the following objects: (1) to determine the incidence of vibrios in open waters in this country; (2) to investigate their biochemical and serological characters; (3) to compare them with the true cholera vibrio and with water vibrios isolated in other parts of the world.

Method of isolation

Specimens were taken in ordinary unsterilized glass beakers, washed out several times in the water under examination. Each sample (180 ml.) was transferred to a sterile screw-capped bottle containing 20 ml. of an enrichment medium composed of 10% peptone and 9% NaCl in distilled water, with the pH adjusted to 9.2 and buffered with a boric acid solution, a few drops of thymol blue being added as indicator. The samples were usually collected during the afternoon and on returning to the laboratory the bottles were left on the bench at room temperature overnight. They were transferred to the 37° incubator at 10 a.m. the following day for 6 hr., after which a few drops of the surface scum was plated on Aronson agar. The bottles were again left on the bench overnight and a second plating was made the following morning.

Incidence of vibrios in water

During the period October 1945 to July 1946, 48 water samples from various parts of England and Wales were examined and vibrios were isolated from 82 of them. From 14 of the samples more than one type of vibrio was obtained (Table 1).

Although the media used were basically similar to those used in India by Taylor & Ahuja (1937), and found by them highly selective in their investigation of waters in Northern India, considerable difficulty was experienced in suppressing other water bacteria which tended to overgrow the more delicate vibrio. The procedure of isolation was adjusted from time to time. It was found that vibrios could often be picked up off plates, negative at first examination, which had been lying on the bench for several days, and it became the practice to examine all plates daily for a week before rejecting them as negative. This method was eventually adopted as giving the greatest number of positive results. It is probable that earlier failures to isolate vibrios from some of the samples were due to inadequacies of technique and that the incidence is actually higher than the 75 % found in this small survey.

At the beginning of the investigation, a period during which results were variable, the weather was mild and damp, but the samples from sources 22, 23 and 24, which failed to yield vibrios, were collected during a cold spell when there was snow on the ground, although no ice on the surface of the water. From March 1946 onwards all collections were made during warm weather and there were no vibrio-free samples. The series is, however, too small to allow any conclusions to be drawn about the influence of weather on incidence. Other observers (Houston, 1909; Taylor & Ahuja, 1937) were of the opinion that it did not play any definite part.

It is obvious, however, that organisms of the vibrio group are very widely distributed in the waters of this country and are to be found in such diverse sources as hill streams, rain-water collections and stagnant ponds.

Biochemical characters of the strains isolated

Fermentation reactions. The fermentation reactions of all the strains with mannose, sucrose and arabinose were studied and the vibrios classified according to the six types of Heiberg (1935). Organisms of type I formed by far the largest group, comprising 64 % of the whole series; 12 % only were of type II, and 16 % of type VI. There were none of types IV and V and only two organisms of type III. The non-fermenting group of type VI was subdivided into two subtypes according to the reactions with mannitol and glucose. Only one, no. 8b, fermented these sugars, thus falling into subtype 2, and the others remained non-fermenters of subtype 1. The overwhelming predominance of type I, to which *Vibrio cholerae* belongs, is of interest. Taylor, Read & Pandit (1936), in a study of the fermentation reactions of recently isolated vibrios from patients with cholera, carriers and water sources in the Calcutta area, found that only 11.4 % of vibrios isolated from waters belonged

to type I, while 87 % were of type II. In a later study of water vibrios in Northern India, Taylor & Ahuja (1938) found that during the cold weather strains of type II formed only 6 % and type I 28 %. The incidence of type VI

Table 1. *Waters investigated for the presence of vibrios*

No. of source	Source of water	Date of sampling	Vibrio strains isolated
1	Hampstead Heath duck pond (a), London	10 x. 45	1
2	Hampstead Heath swimming pond, London	10. x. 45	2
3	Hampstead Heath duck pond (b), London	10. x. 45	3a, 3b
4	Leg o' Mutton pond, Hampstead, London	16. x. 45	—
5	Stream, Golders Hill Park, London	16. x. 45	—
6	Pond, Barnet-bypass, Middlesex	17. x. 45	—
7	Pond, Mote Mount Golf Course, Barnet	17. x. 45	—
8	Serpentine lake, Hyde Park, London	23. x. 45	4a, 4b
9	Round Pond, Kensington Gardens, London	23. x. 45	5a, 5b
10	River Thames at Westminster Bridge, London	23. x. 45	6a, 6b
11	Fountain pool, Trafalgar Square, London	23. x. 45	—
12	Horse pond, Putney Heath, London	30. x. 45	—
13	Beverly Brook, Ranelagh Club	30. x. 45	7
14	Pond 1, Barn Elms Park	30. x. 45	—
15	Pond 2, Barn Elms Park	30. x. 45	8
16	Pond, Convent grounds, Roehampton	30. x. 45	—
17	River Thames at Hammersmith Bridge, London	30. x. 45	9
18	Canal, Regent's Park, London	5. xi. 45	10
19	Boating lake, Regent's Park, London	5. xi. 45	—
20	New River, Palmer's Green, London	20. xi. 45	11a, 11b
21	Mill stream, Bowcombe, S. Devon	9. xii. 45	12
22	Hill spring, Bowcombe, S. Devon	9. xii. 45	—
23	Cattle trough, Bowcombe, S. Devon	9. xii. 45	—
24	Spring, Bowcombe, S. Devon	9. xii. 45	—
25	Rain water from butt, Kingsbridge, Devon	21. i. 46	13a, 13b, 13c, 13d
26	Pond, Kingsbury, Middlesex	8. ii. 46	14a, 14b
27	Swift stream, slate quarry, N. Wales	24. iii. 46	15, 15b, 15c
28	Stream, Gate of Clwyd, N. Wales	24. iii. 46	22
29	Drinking trough, Llanbedr, N. Wales	24. iii. 46	16
30	North Lake, Kew Gardens, London	29. iii. 46	17
31	Stream, Rock Garden, Kew, London	29. iii. 46	18
32	South Lake, Kew Gardens, London	29. iii. 46	19a, 19b
33	Lily pond in hot-house, Kew, London	29. iii. 46	20a, 20b
34	River Thames at Kew, London	29. iii. 46	21a, 21b
35	Roadside stream, Totland, Isle of Wight	23. iv. 46	23
36	Ditch, Freshwater, Isle of Wight	23. iv. 46	24
37	Duck pond, Compton, Isle of Wight	23. iv. 46	25a, 25b
38	Horse pond, Yarmouth, Isle of Wight	23. iv. 46	26
39	River Thames at Tower Bridge, London	22. v. 46	27a, 27b
40	River Thames at Greenwich, London	22. v. 46	28
41	Stream, Worcester Park, Surrey	25. v. 46	29
42	River Thames at Richmond, London	11. vii. 46	30a, 30b
43	River Thames at Hampton Court, Surrey	11. vii. 46	31a, 31b

was also greater, forming another 28 % of the total, as against none in the Calcutta series and 13 % in Northern Indian waters examined during the hot weather and monsoon periods. In the present series six out of the eight type VI vibrio strains were isolated during the coldest part of the year, between the end of November and the beginning of February.

Cholera-red (C.R.) and indole reactions. The cholera-red reaction was carried out on 24 and 48 hr. cultures, in the nitrate peptone broth recommended by Bleisch (1898). In the whole series there were only three positive reactions, and all of these appeared at 24 hr. Two of the reacting strains were of Heiberg type I, and the other of type II. In the Indian series, during the hot weather and monsoon periods the incidence of positives was about 40 %, but in the cold-weather series no positive cholera-red reactors were found among the thirty-two strains examined.

Indole was produced by 32 % of the strains, including the three positive in the C.R. test.

Voges-Proskauer (V.P.) test. Barritt's (1936) modification was used as being more sensitive than the ordinary test, and the incidence of positives was 50 %. C.R. + strains also reacted positively in the V.P. test, and no Heiberg type I strain was C.R. + V.P. —, a biochemical combination which is considered by Taylor, Pandit & Read (1937) to provide a presumptive diagnosis of the serological O group I. On the other hand, the close parallelism found by these authors between the C.R. and V.P. tests has not been borne out in this series. Similar reactions were obtained with 27 of the vibrios and dissimilar reactions with 23 and, unlike the inagglutinable vibrios examined by them, a number of the C.R. — V.P. — group are of Heiberg type I.

Other biochemical tests. All strains were tested for their ability to liquefy gelatin, and to produce hydrogen sulphide. Only 22 % failed to liquefy gelatin, and 21 % of the strains were found to produce hydrogen sulphide after 3 days incubation at 37°.

The haemolysins of the strains isolated

All strains were tested for haemolysis at 2, 4, 24, 48 and 72 hr. The method was similar to that described by Goyle (1939), 1 ml. of a 5 % suspension of washed goat cells (packed centrifuged deposit v/v) being added to 1 ml. of peptone water culture. After 2 hr. at 37° the tubes were removed to the refrigerator, and the readings taken the following morning.

Approximately 50 % of the strains proved to be haemolytic, and of these all except two gave a positive reading at 24 hr. Greig (1914) and Goyle (1939), using Indian and Far Eastern strains, both found maximum haemolysin production in 72 hr. cultures, but Goyle also found that some of his weakly haemolytic strains had lost their haemolysin during the 3-day period of incubation, and therefore recommended that tests should be read at successive 24 hr. intervals. Taylor *et al.* (1937), in their study of the cholera-like vibrios, used a 48 hr. test as routine. The majority of the haemolytic strains in my series attained their maximum haemolysin production at 24–48 hr. and maintained it at 72 hr., and none which was haemolytic at 24 hr. had lost activity at 72 hr. On the other hand, one strain (30a) became haemolytic only at 48 hr., and two strains (9 and 10), though quite strongly haemolytic at 4 hr., were not haemolytic at 24 hr. Two of the strains, 3b and 6a, were found to be non-haemolytic when first isolated, but when retested several months later, haemolysed goat cells. This phenomenon has been observed by other authors.

The haemolysins of two of the strains, selected as being typical members of the series and strongly haemolytic, were further investigated and found to fulfil the criteria required of a true toxin in that they were heat labile, being destroyed by heating at 56° for 10 min., filterable, and antigenic.

Filterability of the haemolysin. Greig (1914), in his original experiments, filtered haemolytic vibrio cultures through Pasteur-Chamberland candles and, testing the filtrates, found that a considerable diminution had occurred in the process, or in a few cases complete loss of haemolytic activity. He concluded that 'the haemolysin producing substance... is to a considerable extent non-filterable'. Goyle (1939) used a Pasteur-Chamberland candle, a Seitz E.K. disk and a Berkfeld N filter, and collected the filtrates in successive 10 ml. fractions. He found that the haemolysin was definitely filterable, but tended to be adsorbed on the filter disks or candles. This adsorption was most marked with the Seitz disks and least with the Berkfeld N filter, though even when the latter was used the final filtrate fractions did not attain the haemolytic titres of the unfiltered cultures. In the present investigation supernatant fluid from a centrifuged haemolytic peptone water culture was passed through an Elford gradacol filter membrane of 0.75 μ pore diameter. The filtrate was collected in successive 10 ml. fractions and tested for haemolysin in parallel with the unfiltered culture. The first fraction was found to be only weakly haemolytic, with a titre of only 1/1 as against 1/512 of the unfiltered culture, the second produced haemolysis at a dilution of 1/32, and the third was as powerfully haemolytic as the parent culture. The superiority of this type of filter for the purpose was thus demonstrated and Goyle's claim of the filterability of the haemolysin was confirmed.

Antigenicity of the haemolysin. Using Goyle's method, an anti-haemolytic serum was prepared against vibrio 6b and tested against the haemolysins of other vibrios in the series. The anti-haemolytic activity of the serum was not as marked as in Goyle's sera, and a serum concentration of 1/10 was required for complete inhibition of the haemolysin of the vibrio 6b. Control series were therefore run with all tests, using 1/10 normal serum from the same rabbit. It was found that the serum inhibited the haemolysins of all the British water vibrios and also that of the only El Tor vibrio tested (strain Doorenbos 34D 13).

Serological characters of the strains isolated

Antisera were prepared against 23 of the British strains (19 against the O antigens and 11 against the whole organisms), against the typical cholera vibrios 'Inaba' and Ogawa 1077 (O and OH antisera) and against the O antigens of nine strains representing as many of Taylor & Ahuja's (1937) Indian vibrio types from water, from carriers, and patients with cholera.

The agglutination technique was similar to that described by Taylor *et al.* (1937). Suspensions of agar-grown vibrios in 0.25 % formol saline were made of all the British strains, of the true cholera vibrios and of 22 'inagglutinable' Indian strains from various sources.

After preliminary selection by slide agglutination tests, tube agglutination

tests were made with all positive reactors, using serum dilutions between 1/25 and 1/25,000. The tubes were incubated in a water-bath at 52° for 4 hr. and readings taken at this term and again after the tubes had stood on the bench overnight.

The results of these agglutination tests are summarized in Table 2. For simplicity cross-reactions amounting to more than 15 % but less than 25 % of the homologous titres of the sera concerned are recorded by the sign < 25 %, while those falling below 15 % are entered as negative. It is thought that cross-reactions of this order are not sufficiently significant as indices of relationship to warrant their more detailed statement.

As indicated in the table, the British water vibrios proved to be serologically heterogeneous. Of the 50 strains only five (1, 2, 7, 9 and 10) were closely related in both their O and H antigens, forming a compact group. Two others (14*a* and 25*a*) showed a measure of similarity limited to the O antigens; another three or four (4*a*, 6*a*, 15*a* and 15*b*) showed relationship restricted to the H antigens, and the same was true of the strains 5*a* and 5*b* in which the H antigen appeared to be identical with, or to resemble closely, that of *V. cholerae*. The remaining races were apparently strain specific.

None of the strains reacted appreciably with cholera O agglutinins and only two to as much as 25 % of the homologous titre with any of the O antisera prepared against Indian and Far Eastern vibrio types—vibrio 23 with the O antiserum of vibrio 630/3T (a Calcutta 'case' strain, 'strain 9' of Taylor *et al.*), and vibrio 25*a* with that of vibrio Nanking 32/124 (also a 'case' strain, Group II, Gardner & Venkatraman (1935), and 'strain 6' of Taylor *et al.*).

Phage sensitivity of the strains

All the strains were tested, as nascent agar-plate cultures, with droplets of concentrated preparations of the Indian cholera phage types A-L and three were found sensitive to certain of these.

Strain 5 <i>a</i> reacted with phage type B to give 'cloudy lysis'				
Strain 6 <i>a</i>	„	„	B	„ total clearing
	„	„	F	„ partial clearing
	„	„	J	„ partial clearing
Strain 22	„	„	B	„ partial clearing
	„	„	J	„ partial clearing

It is interesting to note that these three strains, 5*a*, 6*a* and 22, were the only members of the series to give a positive cholera-red reaction.

I wish to thank Major-General Sir John Taylor and Mr P. Bruce White for their invaluable encouragement and advice, and Miss Irene Gammon, Miss Edna Schultz and Miss Marie French for their technical assistance.

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(Received 15 January 1948)