THE SEROLOGICAL GROUPING OF ROUGH VIBRIOS

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From the investigations of past workers, from those recently made with modern refinements by Gardner and Venkatraman (1935) and from my own studies, it is clear that in their serological diversity the vibrios rival the group of coliform bacilli.

In the differentiation of the "cholera-like" vibrios the heat-stable antigens have been found to supply the readiest if not the only basis of study (Abdoosh, 1932; White, 1934*b*; Gardner and Venkatraman, 1935).

Analysis on such lines of strains isolated in India, the Far East and El Tor, from human subjects, in cholera and in health, and from water, reveals two frequently occurring serological groups, each including three or more subtypes. To one of these groups we may still, pending convincing differentiation of pathogenic and harmless races, apply the term *Vibrio cholerae* (O Group I of Gardner and Venkatraman); for the other, represented in my collection by some fifteen strains from Nanking and a few from India and derived from cholera stools and water sources, I adopt on Dr Y. N. Yang's suggestion the title *V. nanking* (O Group II of Gardner and Venkatraman). Gardner and Venkatraman recognise four seemingly rarer O groups (III–VI)—some of which I am able to augment—and present a list of eight strain specific races—for a few of which I have been able to discover equivalents in my collection, making at the same time some additions to the list of as yet "individual" strains.

In the hope of discovering some broader basis of classifying these vibrios, of finding a means of assembling in groups the seemingly isolated strains, perhaps of demarcating the pathogenic and non-pathogenic forms, I have undertaken, in complement to Dr Gardner's investigation of S vibrios, to examine the relationships of R variants of various groups and types.

The isolation of R forms

The first problem of this research was to collect material for study. R colonies of vibrios are seldom to be found—as R colonies of colon-dysentery-*Salmonella* bacilli may be found—by simple inspection of platings of normal laboratory cultures. Some strains, on the other hand, are wholly rough, and smooth races of these are not available.

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Where active A type choleraphage is to hand suitable R forms may usually be obtained by its aid, but the A type choleraphages which I have used have proved variously limited in the range of their action on cholera strains and are, of course, valueless in dealing with other vibrios.

Recourse was therefore made to the method of exposing young cultures of vibrios to the homologous activated S antiserum (White, see Yang and White, 1934). The mixtures have contained 1 : 15 fresh guinea-pig serum and specific antiserum, varied, according to its O agglutinating titre, between 1 : 35 and 1 : 350. With antisera with O titres about 1 : 1000 concentrations such as 1 : 35 and 1 : 70 have given best results; with stronger sera (titre 1 : 2000–5000) concentrations between 1 : 70 and 1 : 150.

Granted good smooth specific sera of adequate titre, not overcharged with preservative, the organisms which survived a single exposure to the bactericidal mixtures have yielded, over a certain serum range, a considerable proportion, often 80–99 per cent., of R colonies on plating. Some few of these have shown a tendency to revert in subculture but the majority have proved stable. Where too much specific serum has been employed, the surviving vibrios have been for the most part smooth serologically, though culturally abnormal: this zonation was possibly in part due to traces of R antibody and to the 0·3 per cent. of phenol in the undiluted sera. Weak antisera with O titres below 1 : 500 gave consistently poor results.

It has been clearly established that bacteriophage is not in any way involved in this effect; it is due to specific serum and complement in suitable proportions and is almost certainly a simple selection of pre-existing R elements in the culture by destruction of the diluting mass of S organisms. In one case in which the bactericidal method failed, rough variants were obtained by prolonged culture in 1/10 specific antiserum.

By treatment of R cultures with R antiserum and complement ρ races (White, 1934 *a*) of several vibrios were obtained. They showed greatly reduced agglutinability with R serum.

The strains for which R variants were secured are listed below:

Typical V. cholerae

R variants by specific bactericidal method:

Type Inaba: strain Inaba*.

Type Hikojima: strain Hikojima.

Type Ogawa: strains Ogawa, Kasauli 92/1*, Shillong 610 and Shillong 1077*.

Unclassified: strains Kasauli 78/2, 1485, 1486.

R variants by bacteriophage action:

Strains Ogawa, Shillong 610, 1077, Kasauli 78/2.

R variants from totally R cultures:

Strains "Wada" and Kasauli 1410.

* An asterisk indicates that ρ races were also isolated.

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V. nanking

R variants by specific bactericidal method:
Strains Nanking 1932–77*, 121, 124, 126.

"Atypical" vibrios from cholera or cholera-like cases*

R variants by specific bactericidal method:
Strains Manila Ha 10 and Bulacan, Nanking 1932–82 and 123, Kasauli 11*, 73, 77.
R variant from totally R culture:
Strain Manila Ha 11.

Water vibrios

R variants by specific bactericidal method:
Strains Nanking 1932–101, 102, 106, 110.
R variant by prolonged culture in specific antiserum:
Strain Kohat.

NOTES ON THE SMOOTH PARENT CULTURES

The smooth strains of *V. cholerae* all showed normal agglutinability with anti-cholera sera with those variations in titre which are associated with their absorption type. No appreciable O cross-agglutination occurred with the serum of any other organism considered.

The four strains of *V. nanking* (O group II, Gardner and Venkatraman), deriving from the Nanking epidemics of 1932, form a similar group of vigorously cross-agglutinating absorption types, in which strain 1932–126 stands midway between strains 1932–77 and 121 on the one hand and strain 1932–124 on the other. These strains are sharply distinct in O agglutination properties from all others in the list. Five of the seven smooth "atypical" vibrios employed are strain specific, while two (Bulacan and Nanking 1932–123) represent O group III of Gardner and Venkatraman. Kasauli 77 (and its R variant) has a tendency to autolyse during agglutination tests and gives poor somatic clumping: it is probably a bacteriophage-resistant variant and has not been much studied in the present series of tests.

The water vibrios Nanking 1932–102, 106, 110 form an agglutinative group (IV of Gardner and Venkatraman) and from absorption tests appear identical. Nanking 1932–101 and Kohat are distinct from these, from one another, and from all other strains tested. The Kohat vibrio, kindly sent by Colonel J. Taylor, I.M.S., is of interest in that it was suspected of having yielded, while under laboratory study in India, a variant readily agglutinable by anticholera serum.

* The term "atypical vibrios" is here used for vibrios isolated from cases of cholera-like disease, which though culturally similar to *V. cholerae*, differ from that organism serologically.

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Serological methods

The antisera employed were prepared against vaccines heated at 100° C. for 60 min. Agglutination tests were made with living agar-grown vibrios suspended in 0·42 (or 0·3) per cent. salt solution, in which medium spontaneous clumping of R forms seldom occurs.

The tubes were incubated in the water bath at 45–50° C. for at least 2 hours: final readings were taken after the racks had stood in the cold overnight.

Identity of R variants within the S agglutination groups

Examination was first made of various R races derived from the same strain or type or from different types belonging to a single S agglutination group, in order to determine whether the R variant was a constant or variable product of the S form and whether type differences survived roughening or disappeared. In the case of *V. cholerae*, twenty-seven R races, occurring naturally, isolated after action of bacteriophage in the laboratory or after exposure to specific bactericidal mixtures and derived from eleven strains representative of the three known types, were examined and found to be serologically identical.

In the same way several R *V. nanking* races, obtained from four strains belonging to three absorption types, showed uniform serological characters; so too the R forms of the water vibrios Nanking 1932–102, 106 and 110 of Gardner and Venkatraman's Group IV.

It seems that the R form of a S type is fixed in serological properties and that types belonging to the same S agglutination group have, to speak serologically, a common R variant.

ρ races from three different strains of *V. cholerae* were likewise found to be serologically identical.

Cross-agglutination tests with R and ρ antisera

None of the R forms utilised in these studies were agglutinated by the homologous smooth O sera (titres 1 : 1000–1 : 5000) at greater dilutions than 1 : 100—and at that dilution but partially and slowly. The parent S strains showed scarcely greater reaction with the O antisera of their R derivatives.

It does not appear necessary to support these statements with protocols: examples of cross-tests between R and S vibrios and sera are given by Yang and White (1934) and White (1934 *b*).

A selection of the results of agglutination tests with the series of R vibrios and some of the corresponding antisera are given in Table I: other tests either duplicated these observations or failed to show groupings of any interest.

It is seen that the tests with R sera indicate certain groupings quite inevident in the case of the S reagents. The R race of strain specific Kasauli II behaves like an R variant of *V. cholerae*, the R forms of the isolated strains

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Table I

Living vibrio tested for agglutination	Greatest active dilutions of: *V. cholerae* Shillong 1077 R-O serum	*V. cholerae* Shillong 1077 ρ-O serum	Atypical vibrio Kasauli 11 R-O serum	Atypical vibrio Manila Ha 10 R-O serum	Atypical vibrio Manila Ha 11 R-O serum	*V. nanking* 1932–121 R-O serum	*V. nanking* 1932–124 R-O serum	Water vibrio Nanking 1932–101 R-O serum	Water vibrio Nanking 1932–102 R-O serum	Water vibrio Nanking 1932–110 R-O serum	Atypical vibrio Manila Bulacan R-O serum	Control in 0·42 % NaCl solution
V. cholerae												
Inaba type, Inaba R	**>2000**	**1000**	**>2000**	100 (t.)	200 (#)	<200	400 (+)	<200	<100	100 (##)	200 (+)	0
Ogawa type, Shillong 1077 R	**>2000**	**1000**	**>2000**	100 (+)	200 (#)	<200	400 (#)	<200	<100	100 (+)	200 (+)	0
Ogawa type, Shillong 1077 ρ	400 (#)	**1000**	200 (##)	100 (+)	200 (#)	<200	400 (+)	<200	<100	100 (+)	200 (+)	0
V. nanking												
1932– 77 R	200 (##)	**1000**	200 (#)	100 (+)	200 (#)	**2000** (+)	**2000** (+)	<200	<100	100 (##)	200 (+)	0
1932–121 R	400 (+)	**500**	200 (#)	<100	200 (#)	**1000** (##)	**1000** (##)	<200	<100	100 (##)	200 (+)	0
1932–124 R	400 (+)	**1000**	200 (##)	100 (t.)	200 (#)	**2000** (+)	**2000** (+)	<200	<100	100 (#)	200 (+)	0
1932–126 R	200 (#)	**1000**	200 (##)	100 (+)	200 (##)	**2000** (+)	**2000** (+)	<200	<100	100 (#)	200 (#)	0
Atypical vibrio												
Manila Ha 10 R	400 (+)	**1000**	200 (#)	**>2000**	400 (+)	200 (+)	400 (+)	**2000** (+)	<100	100 (+)	200 (+)	0
Manila Ha 11 R	400 (#)	**1000**	200 (+)	100 (##)	**>2000**	<200	400 (#)	<200	<100	100 (#)	200 (+)	0
Manila Bulacan R	200 (#)	**1000**	200 (#)	100 (##)	200 (#)	<200	400 (+)	<200	**1000** (+)	**2000** (+)	**2000** (+)	0
Kasauli 11 R	**>2000**	**1000**	**>2000**	100 (##)	400 (#)	<200	400 (+)	<200	<100	100 (#)	200 (#)	0
Kasauli 73 R	200 (#)	**1000**	200 (#)	100 (+)	<200	<200	400 (+)	<200	<100	100 (+)	100 (#)	0
Nanking 1932– 82 R	400 (+)	**1000**	200 (+)	100 (#)	200 (#)	<200	400 (+)	<200	<100	100 (+)	200 (+)	0
Nanking 1932–123 R	200 (#)	**500**	200 (+)	100 (+)	200 (#)	<200	200 (+)	<200	**500** (#)	**1000** (+)	**1000** (t.)	0
Water vibrio												
Nanking 1932–101 R	200 (#)	**1000**	200 (#)	**>2000**	200 (+)	<200	400 (+)	**2000**	<100	100 (#)	200 (+)	0
Nanking 1932–102 R	200 (#)	**500**	200 (+)	100 (#)	200 (#)	<200	400 (+)	<200	**1000** (+)	**1000** (+)	**2000** (+)	0
Nanking 1932–106 R	400 (+)	**1000**	200 (+)	100 (+)	200 (#)	<200	400 (+)	<200	**1000** (#)	**2000** (+)	**2000** (#)	0
Nanking 1932–110 R	400 (+)	**1000**	200 (+)	100 (+)	200 (+)	<200	400 (+)	<200	**1000** (+)	**1000** (##)	**2000** (#)	0
Kohat R	<100	100 (t.)	<100	<100	<200	<200	<200	<200	<100	<100	100 (t.)	0

##, #, + and t. indicate degrees of agglutination, ranging from "almost complete" to "traces", observed at the accepted titre limits.
0 indicates no agglutination.
Black type is used to emphasise the most important items in the table.

Ha 10 and Nanking 1932–101 are indistinguishable by agglutination; and, what is particularly interesting, the R forms of Gardner and Venkatraman's groups III and IV behave alike. The groupings indicated in this small series of strains are summarised in Table II. But there is no general merging: the R groups and certain strains remain distinct, save at the lowest serum dilutions shown, where generalised minor reactions, of somewhat variable proportion to the main titre, occur with most sera. It will be noted that in the case of cholera antiserum these minor reactions, which are much greater than those of the corresponding smooth strains, simulate those of the cholera ρ variant included.

Table II

Strains studied	S serology	R serology	R group
V. cholerae	O group I subtypes: Inaba, Hikojima, Ogawa	No subtypes	A
"Kasauli 11"	Strain specific	Similar to but not identical with R *V. cholerae*	A
V. nanking	O group II 3 or more subtypes	No subtypes	B
Manila Bulacan	O group III	R variants similar or identical	C
Nanking 1932–123	O group III	R variants similar or identical	C
Water vibrios, Nanking 1932–102, 106 and 110	O group IV	R variants similar or identical	C
Manila Ha 10	Strain specific	R variants identical	D
Water vibrio, Nanking 1932–101	Strain specific	R variants identical	D
Kasauli 73	Strain specific	Strain specific	
Nanking 1932–82	,,	,,	
Manila Ha 11	,,	,,	
Water vibrio, Kohat	,,	,,	

The serum of this and other ρ forms has a very different action from that of the R sera just considered. It agglutinates with little preference all the R and ρ vibrios which have been tested with the exception of Kohat R. Absorption tests have, however, shown that the similarity of the ρ receptors of the different groups of vibrios does not in all cases extend to identity.

Other tests have shown that most at least of the minor cross-reactions between the R groups of vibrios and R antisera are due to ρ agglutinins.

Discussion

The results of this exploratory examination of the R vibrio are of some interest. They show that the R form of a given type has fixed serological characters, that with roughening type differences within a S agglutination group disappear more or less completely, and that forms quite distinct in the S state are often similar or identical in R serology. The general display of affinities and differences is very similar to that which I have found to pertain among the R variants of the coliform-dysentery-*Salmonella* group. The homogeneity of R variants derived from each recognised S agglutination group

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gives some confidence in accepting as real relationships disclosed only in the R state. On such grounds there is reason to believe that Manila Ha 10 and the O group III of Gardner and Venkatraman, derived from cases of cholera-like symptoms, are very close congeners of water vibrios, believed harmless. There is, therefore, a *prima facie* case for their own non-pathogenicity. On the other hand Kasauli 11, alone among "atypical" vibrios studied, displays unequivocal affinities with true *V. cholerae* and so comes under suspicion of having been the cause of the condition in which it was discovered. This line of argument may prove invalid, more particularly since many strains simulating epidemic *V. cholerae* in serology are apparently non-infective; but, in the present state of uncertainty, it is worth consideration: if this method of study were prosecuted it is possible that the association of certain R-defined groups with disease might be more readily established than when dealing with sporadically occurring S types.

A second point of interest is the generalised character of the ρ form in which even the group specificity of roughness has largely disappeared.

CONCLUSIONS

1. Vibrios belonging to the same S agglutination group, even if of different absorption type, have the same serological R variant.

2. R variants of vibrios belonging to sharply distinct S types or groups may show such similarities as to allow their fusion in larger R agglutination groups.

3. The serology of the ρ vibrio variant is overwhelmingly generalised.

REFERENCES

ABDOOSH (1932). *Brit. J. Exper. Pathol.* **13**, 42.
GARDNER, A. D. and VENKATRAMAN, K. V. (1935). *J. Hygiene*, **35**, 262.
WHITE, P. BRUCE (1934 *a*). *J. Pathol. and Bacteriol.* **39**, 530.
—— (1934 *b*). *Bulletin de l'office international d'hygiène publique*, **26**, No. 7, Suppl., p. 73.
YANG, Y. N. and WHITE, P. BRUCE (1934). *J. Pathol. and Bacteriol.* **38**, 187.

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