What appears to have been the third type of specific carbohydrate was reported by Jermoljewa and Bujanowskaja[4] as having been isolated from an old Russian strain of *Vibrio cholerae.* The phenylosazone of this substance melted at 204° and the substance itself gave a specific rotation of +64.0 and was tentatively identified as glucose by these authors.

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## Proteins and Carbohydrates of the Cholera and Cholera-Like Vibrios.*

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The results of the van Slyke analyses of the proteins of the cholera and cholera-like vibrios, which we have published elsewhere,[1] may be briefly summarized. No differences in nitrogen distribution could be found between proteins of the agglutinating vibrios and of the non-agglutinating or water vibrios. In comparison with similar analyses of other bacteria, which have been reviewed by Hirsch,[2] the vibrios form a well-defined group with a relatively high content of the simpler amino acids (average, 55.7%) and a lower content of the basic amino-acids (average, 24.4%) than has previously been found in the bacteria. The figure for amide nitrogen, which averaged 6.8%, is about half that reported for other microorganisms. Taken altogether, the van Slyke analyses indicated that the vibrios had, relative to other bacteria, a comparatively simple structure, and as already stated the nitrogen distribution appeared identical in all of them from whatever source. An elementary analysis of the vibrio proteins was also made and in no case could any differences in these constituents be detected in the group.[3]

We have also studied the vibrio proteins by the method of "racemization" in dilute alkali, which was developed by Woodman[4] and

[4] Jermoljewa, Z. W., and Bujanowskaja, I. S., *Z. Immunitätsf.*, 1930, **68**, 346.

* This work was done with the support and under the auspices of the Indian Research Fund Association.

[1] Linton, Richard W., Mitra, B. N., and Shrivastava, D. L., *Indian J. Med. Res.*, 1934, **21**, 635.

[2] Hirsch, J., *Z. Hyg. and Infektionskr.*, 1931, **112**, 660.

[3] Linton, Richard W., Shrivastava, B. L., and Mitra, B. N., *Indian J. Med. Res.*, 1934, October, in press.

[4] Woodman, H. E., *Biochem. J.*, 1921, **15**, 187.

based on the earlier work of Dakin.[5] Woodman's method consists of following the optical activity of the protein in dilute alkali and plotting the degree of specific rotation against time, and was applied by him to the study of the globulins and albumins of cow sera and colostrum. Since it appeared from our previous work that the vibrios were closely related in their protein constituents, it seemed that this method might be applied to a possible differentiation of them. A typical example of the method and of the results obtained may be briefly outlined. Approximately 40 gm. of dried vibrio growth were collected, and the globulin separated out by repeated precipitations with ammonium sulphate. In no case was more than a trace of albumin present in any of the vibrios studied, and in most of them this constituent appeared to be absent. The globulin was dialyzed against running water followed by distilled water, until sulphates could no longer be detected, the pseudo- and euglobulin fractions separated, washed, dried and weighed.

The 2 globulins were then taken up in N/2 NaOH, to make a 1% solution, and polarimetric readings taken at intervals, using a 1 dm. tube and sodium light. No differences were found in the readings of the pseudo- and euglobulin solutions in any given vibrio. During the period of the readings the solutions were kept at 37°. The results obtained from the pseudoglobulins of a cholera vibrio (2027) and a water vibrio (W3075) in N/2 alkali are given in Table I.

TABLE I.
Specific Rotations of the Pseudoglobulins of Cholera Vibrio 2027 and Water Vibrio W3075 in N/2 NaOH.

| Hours | Cholera Vibrio 2027 Specific Rotation | Water Vibrio W3075 Specific Rotation |
|---|---|---|
| 1 | —76° | —71° |
| 5 | —65° | —60° |
| 24 | —44° | —39° |
| 48 | —36° | —31° |
| 96 | —27° | —22° |
| 120 | —25° | —20° |
| 145 | —23° | —18° |
| 196 | —20° | —15° |
| 217 | —19° | —14° |
| 264 | —19° | —14° |

After the period of about 200 hours the readings became constant and showed no further changes up to 350 hours, beyond which the experiment was not carried.

It is clear from the data given in Table I that the "racemization" of the 2 proteins begins, proceeds and ends differently, and it is

[5] Dakin, H. D., *J. Biol. Chem.*, 1912, **13**, 357.

probable that this difference may depend upon the differing structure of the 2, since the rate at which the change in rotation occurs will be an expression of the rate of change within the protein molecule and will vary with the structure of the molecule (Woodman,[4] Jordan-Lloyd[6]). In both cases the data yield a perfectly smooth curve. As we have already found that the nitrogen distribution is the same in both these proteins it would follow from these results that the difference observed might be due to the different manner in which the amino-acids were grouped together within the respective molecules of the two proteins.[7]

Altogether 20 vibrios have been studied by the "racemization" method, and we have found that these 2 curves are the only ones obtained. It should be emphasized that in the case of either kind of curve the agreement between similar proteins is extremely close, and the readings do not vary more than a degree and are usually

TABLE II.
Origin, Agglutinability, Protein, and Specific Carbohydrate Content of a Series of Cholera and Cholera-like Vibrios.

| Number | Origin | Agglutinability | Protein No. | Carbohydrate No.* |
|---|---|---|---|---|
| 1617 | Cholera | Agglutinable | I | I |
| 1676 | " | " | I | I |
| 79A | " | " | I | I |
| Rangoon Smooth | " | " | I | I |
| Rangoon Rough (1) | From Rangoon Smooth | Non-agglutinable | I | I |
| Rangoon Rough (2) | From Rangoon Rough (1) | " | II | III |
| 2027 | Cholera | Agglutinable | I | II |
| 505 | " | " | I | II |
| E | " | " | I | II |
| W880 | Water | Non-agglutinable | II | II |
| W3075 | " | " | II | II |
| 79B | Cholera | Irregular | II | I |
| El Tor | Human; non-cholera | Agglutinable | II | I |
| " " I† | " " | " | II | I |
| " " II | " " | " | II | I |
| " " III | " " | " | II | I |
| Basrah I | Cholera | Irregular | II | I |
| " II | " | " | II | III |
| " III | " | " | II | I & III |
| " IV | " | " | II | II & III |

*Carbohydrate No. I is the galactose-containing specific substance; No. II is the arabinose-containing; and No. III the glucose-containing. The details are given in the preceding paper.

†El Tor strains I, II, and III represent respectively strains Nos. 3657, 3658, and 3659 of the National Type Collection, London.

[6] Jordan-Lloyd, D., Chemistry of the Proteins, Churchill, London, 1926.

[7] Linton, Richard W., Mitra, B. N., and Shrivastava, D. L., *Indian J. Med. Res.*, 1934, **21**, 749.

identical. We have designated the protein giving the first type of data above (2027) as Protein I and the second protein (W3075) as Protein II. In Table II are given the results of the racemization method in relation to origin, agglutinability and type of specific carbohydrate in the series of vibrios studied.

In the preceding paper we have given an account of the Rangoon strains and their specific carbohydrates, and it is only necessary here to point out that the dissociant Rangoon Rough (2) belongs to the second protein group in contrast to its parent strains Rangoon Smooth and Rangoon Rough (1), and this finding taken in conjunction with the different types of specific carbohydrate present in the two is to be correlated with the complete non-identity of the 2 strains in cross-absorption tests. Rangoon Rough (2) had its origin from a colony picked from a plate streaked with Rangoon Rough (1). Whether it arose as a mutation or whether it was simply by the chance of streaking that a colony of this distinctive "medusa-head" type was isolated is of course impossible to determine. Rangoon Rough (2) is extremely slow in its growth, and would be easily overgrown and hidden under ordinary conditions when mixed with rapidly growing strains like Rangoon Smooth and Rangoon Rough (1). On the other hand, during the 12 months in which these 2 strains have been under continual observation in this laboratory, a colony like that of Rangoon Rough (2) has been observed only on the one occasion, a fact which might be considered to favor the mutation hypothesis.

Whichever idea of the origin may be the correct one, it is clear that from a smooth, agglutinating cholera vibrio there has been derived a rough non-agglutinating vibrio differing from the parent in having the protein constituent found in the water vibrios and an entirely distinct type of specific carbohydrate substance. At the same time the derived form is wholly different serologically from its parent form.

A further point of interest in the Table lies in the position occupied by the El Tor strains. As is well known, these strains have always been anomalous among the vibrios because they are agglutinating organisms isolated from human non-cholera cases under conditions of pilgrimage, where cholera outbreaks were to be expected, but have not in fact occurred. On the basis of their chemical structure the 4 El Tor strains in our series occupy an intermediate position between the cholera and the water vibrios; that is, their protein belongs to the same type as that of the water vibrios while their specific substance (Type I) is identical with that of the major-

ity of vibrios found in clinical cholera. Two other strains in the Table, both of them also variants from the type of vibrio generally found in cholera, show the same structure: 79B, which on isolation from a case of cholera in Calcutta was non-agglutinating, and which has since shown considerable irregularities in this reaction, and Basrah I which is also an irregularly agglutinating strain from cholera. It may be that the basis for the peculiarities of this group of 6 vibrios lies in the hybrid relationships of the protein and carbohydrate types of which they are composed.

The Basrah strains all possess the same kind of protein (Type II). The structure of Basrah I and II has been discussed above and in the preceding paper. Basrah III and Basrah IV are of especial interest because they appear to be mixed strains in the sense that 2 types of specific carbohydrate can be isolated from them. In the case of Basrah III the galactose- and glucose-containing specific substances are present, and in Basrah IV the arabinose- and glucose-containing types. These are the first mixed strains which we have found in an analysis of the carbohydrates of about 40 vibrios. It is probable that we are dealing here with 2 groups of organisms in the same culture, some of which have one and some the other of the 2 carbohydrates. From the repeated analyses of these vibrios which we have carried out it appears that the proportions of the organisms containing the 2 types of carbohydrate may vary from time to time.

As a result of the work on the proteins and carbohydrates of the vibrios it has become possible as we have shown to distinguish 2 types of protein and 3 types of specific carbohydrate in the group. On the basis of the various combinations which we have found of these constituents the vibrios in our series may be divided into several groups as follows:

Group I (Protein I and Specific Carbohydrate I) contains most of the vibrios isolated from clinical cholera.

Group II (Protein I and Specific Carbohydrate II) contains some of the vibrios found in clinical cholera. These are, however, somewhat less frequent than those of Group I. In its composition Group II is intermediate between the cholera vibrios of Group I and the non-agglutinating vibrios of Group III.

Group III (Protein II and Specific Carbohydrate II) contains the non-agglutinating water vibrios.

Group IV (Protein II and Specific Carbohydrate I) contains the El Tor strains and at least 2 other aberrant vibrios.

Group V (Protein II and Specific Carbohydrate III) contains at

present only 2 vibrios, the dissociant Rangoon Rough (2) and Basrah II.

Group VI (Protein I and Specific Carbohydrate III). Of this combination no examples have as yet been found, but their existence among dissociants of the other groups is not improbable.

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## Pantothenic Acid Content of Animal Tissues.

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Evidence has been presented[1] for the existence in all types of living tissue of a substance which has been named "pantothenic acid". A quantitative biological test based upon yeast growth which is specific for this substance has been developed.

While marked progress has been made in this laboratory in concentrating and purifying the acid it is unlikely that any chemical method for its determination can be devised for some time to come. In order to learn something of its functions, however, it seemed desirable to obtain approximate information as to the content of various animal tissues.

Each of the tissues indicated below was thoroughly ground and extracted with a large volume of hot water, usually 100 times its weight. The pantothenic acid which is not "bound" in the tissues is thus extracted, and that which in some cases, at least, is "bound" is not determined. The numerical values are based upon the pantothenic acid extracted from a unit weight of moist tissue, in comparison with that in a unit weight of an arbitrary standard preparation. This "standard" was prepared by extracting rice bran with 60% methanol and evaporating to dryness. Our most potent concentrate is approximately 8,000 times as effective on a weight basis as this standard.

Duplicate or triplicate determinations were made in every case. These usually agreed within about 10%. In order to save space, only averages are given: Skeletal muscle dog No. 1, 0.032, dog No. 2, 0.037, rat, fresh, 0.034, rat autolyzed at 37°, 0.147; smooth

[1] Williams, R. J., Lyman, C. M., Goodyear, G. H., Truesdail, J. H., and Holaday, D., *J. Am. Chem. Soc.*, 1933, **55**, 2912.