firmed by the fact that mice lived in an atmosphere of 21% oxygen and 79% helium without apparent injury for periods as long as 2½ months.

Sayers and Yant[2] exposed animals to 10 atmospheres of helium-oxygen mixtures and then decompressed them in ⅓ of the time necessary for animals exposed to similar nitrogen-oxygen mixtures. They suggested the use of helium and oxygen for caisson workers and divers exposed to excessively high barometric pressures because helium has a lower solubility and a greater diffusivity than nitrogen. The physical basis for the use of helium in clinical disease is its decreased specific gravity in comparison to nitrogen. According to the formula F = Ma, in which F is force, M mass and A acceleration, the movement of air requires a force three times that of a mixture of 20% oxygen and 80% helium. The velocity of gas mixtures diffusing through small orifices is proportional to the square root of the molecular weights, approximately twice as fast in the helium-oxygen mixtures than air. In conditions in which there is resistance to the movement of an adequate volume of air to and from the lungs in any part of the respiratory system from the pharynx to the alveoli, helium-oxygen mixtures may be transported with less effort. When large pulmonary ventilations are maintained over long periods of time, the saving of pulmonary effort is relatively great. Clinical studies in asthma, pneumonia and cardiac failure are in progress.

7725

Types of Specific Carbohydrates in the Cholera and Cholera-Like Vibrios.*

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We reported the finding of 2 types of specific carbohydrate in the vibrios.[1] Both types contained an aldobionic acid from which on prolonged hydrolysis galactose and glucuronic acid could be

[2] Sayers, R. R., and Yant, W. P., *Anesthesia and Analgesia*, 1926, **5**, 127.

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

[1] Linton, Richard W., and Shrivastava, D. L., PROC. SOC. EXP. BIOL. AND MED., 1933, **30**, 600; 1933, **31**, 406.

isolated. The first type, which has been found only in vibrios isolated from cholera, also contained a second sugar, which proved to be galactose. The second type of specific carbohydrate, which occurred in a few of the vibrios from cholera and in all of the non-agglutinating water vibrios, had arabinose in place of the galactose of the first type. Both galactose and arabinose were found to be readily separable from the specific substance by mild hydrolysis. The structure and constituents of these carbohydrates have been more extensively considered in other publications.[2]

As an example of the second type of carbohydrate and of the results of its analysis we may outline the study of vibrio W880, which was isolated from water in an endemic cholera area (Calcutta), and is a smooth, non-agglutinating organism. Three and one-half grams of the purified specific polysaccharide was added to 5.0 cc. of 50% H_2SO_4 and the mixture placed at 37° for 20 hours. Reducing substances, 18%, calculated as glucose. The sulphuric acid was removed from the hydrolysate by $CaCO_3$, and the calcium salt of the aldobionic acid prepared and precipitated out by the addition of alcohol. The supernatant fluid was concentrated and a phenylosazone prepared from it which had a melting point of 158° to 160°. On the basis of our previous experience with the vibrio carbohydrates, we were able to take this result as an indication of the presence of arabinose.[2]

The decomposed calcium salt was found to have a reducing power equivalent to 80 mg. of glucose. On hydrolysis on the steam bath it showed a gradual increase in reducing power up to 21 hours, when 252 mg. (219% increase) were present. At 23 hours a slight decline had occurred, and the heating was stopped. From the hydrolyzed material mucic acid (m.p. 222°) and potassium acid saccharate were prepared, indicating the presence of galactose, and under these conditions, of glucuronic acid in the complex.[2]

We have now found in certain vibrios a third type of specific carbohydrate. In studying a smooth, agglutinating vibrio from a case of cholera in Rangoon (designated Rangoon Smooth) we found that its specific carbohydrate was of the first kind described above, *i. e.,* it contained galactose, and an aldobionic acid of the usual type. From Rangoon Smooth a rough colony (Rangoon Rough (1) was isolated, which contained non-agglutinating organisms only. The carbohydrate of this strain proved to have the same structure as that of the smooth parent, but was present in only

[2] Linton, Richard W., and Shrivastava, D. L., *Indian J. Med. Res.,* 1933, **21,** 91, 379; January, 1935, in press.

about one-fifth the amount, *i. e.,* 1% as against 5% in the smooth form. That is, this rough strain had shown the usual phenomenon of roughening being accompanied by loss of specific carbohydrate which we have noted in the vibrios,[3] and which appears widespread among bacteria. From Rangoon Rough (1) a further organism was then isolated (designated as Rangoon Rough (2)) whose colonies were of the extremely rough "medusa-head" type, with a dry corrugated surface, extremely tenacious growth on agar, scanty growth in broth, slow fermentation of glucose alone out of the several sugars tried, loss of indol formation; and the strain was in every way a complete dissociant from the original Rangoon Smooth. Serologically also it appeared to have nothing in common with the parent strain. Although the mass growth on agar was extremely slow and poor, the percentage of specific carbohydrate which was present was the same as that found in the parent smooth strain, *i. e.,* 5%. The method for separating the polysaccharides from the vibrios has been reported previously.[2]

4.222 gm. of the gum from Rangoon Rough (2) were taken up in 10 cc. of 50% H_2SO_4 and kept at 37° for 18 hours. Reducing power, 10.9% (460 mg.) calculated as glucose. After dilution to bring the acid concentration to 3% the mixture was further hydrolyzed on the water bath for 2 hours; reducing power, 12.2% (514 mg.); a further heating for 2 hours gave 9.4% reducing power (415 mg.) and hydrolysis was stopped.

The hydrolysate was treated exactly as in the case of W880 carbohydrate. No precipitate of calcium aldobionate could be obtained. The solution presumably containing the aldobionic acid had a reducing value equivalent to 7.6 mg.; on hydrolysis this increased to 9.0 mg. after 2 hours and decreased to 6.0 mg. at 4 hours, when the experiment was terminated. In a second experiment, in which 4.0 gm. of the carbohydrate were used, the initial reducing power of the presumed aldobionic acid solution was *nil,* increased to 8.0 mg. after 2 hours heating and was again negative after 5 hours heating. It is probable that these small amounts represent reducing substance carried over into the presumed aldobionic acid solution from the hydrolysate. It was evident throughout these experiments that we were not dealing with a carbohydrate of the type we had previously found among the vibrios.

The portion of the hydrolysate remaining after the attempted separation of the calcium aldobionate was then studied. It yielded

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

a phenylosazone melting sharply at 210°. It was concentrated *in vacuo* until it had a syrupy consistency, seeded with a few crystals of glucose, a little absolute alcohol added and the mixture placed in the refrigerator under vacuum. After 4 days a solid mass of white crystals had appeared. They were washed with a little ethyl alcohol, followed by methyl alcohol and finally with ethyl alcohol and dried *in vacuo*. Yield: 0.3350 gm.

0.15 gm. made up in distilled water to form a 1% solution, which was perfectly clear, gave a specific rotation of +54.0°.

The crystals did not have a definite melting point, but showed irregular changes between 110° and 125°. The phenylosazone melted sharply at 214°, and the melting point was unchanged when it was mixed with known glucose-phenylosazone. The characteristic crystals of potassium acid saccharate were also readily obtained.

From this evidence it appears possible to identify the substance as glucose, and to conclude that it is the sole constituent of the specific carbohydrate. The finding of a dissociant with a polysaccharide completely different from that of its parent strain is of some interest, especially in view of the fact that we have found by cross-absorption tests that the 2 vibrios are unrelated serologically, and as the succeeding paper will show are also different in their protein constituents.

The same type of glucose-containing polysaccharide has been found in another vibrio strain, Basrah II, a rough strain which was isolated along with 3 other strains from cholera cases in Basrah in 1931. It is a strain of considerable variability in its serological and biochemical reactions. Working with 4.2 gm. of specific polysaccharide of this organism by the methods outlined above, what appeared to be unequivocal evidence for the presence of glucose as the sole constituent of the carbohydrate was obtained, and as in Rangoon Rough (2) no aldobionic acid could be isolated.

It thus appears that at least 3 types of specific polysaccharide are present in the vibrios:

	Constituents	Source
Type I	Galactose + an aldobionic acid consisting of galactose and glucuronic acid.	In most vibrios from cholera cases.
Type II	Arabinose + an aldobionic acid consisting of galactose and glucuronic acid.	In a few vibrios from cholera cases, and in all the non-agglutinating water vibrios studied.
Type III	Glucose only; no aldobionic acid.	In a dissociant (Rangoon Rough (2), and in an aberrant strain (Basrah II).

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.

7726

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.