TABLE II.

No.*	pH of liquor	pH after addition	$\frac{-\Delta B}{-\Delta pH} \times 10^{-3}$
4	6.95	5.64	7.6
8	7.15	4.85	4.3
9	6.97	4.55	4.1
10	6.92	5.30	6.2
11	6.88	4.70	4.6
12	6.92	3.50	2.9
13	6.95	3.15	2.6
14	7.06	4.30	3.6
15	6.87	4.05	3.5
16	6.94	4.85	4.8
17	6.98	4.10	3.5
18	6.92	2.75	2.4
19	6.98	3.50	2.9
			Mean 4.1 $\times 10^{-3}$

* Numbers are taken from Table I.

Bay shows little variation from the established norm of pH 6.95. The hydrogen-ion concentration of the liquor was not influenced by the hydrogen-ion concentration of the environmental sea water in the number of samples studied. The buffer capacities of the oyster liquors showed a wide variation.

The author wishes to acknowledge his indebtedness to Mr. S. J. Caskey for the collection of the oysters, and to Mr. J. F. Muller for the preparation of the graph, each from the Bureau of Sanitary Engineering of the State of Maryland Department of Health.

6589

Carbohydrate Fractions from Vibrio Cholerae, and Related Organisms.*

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We have shown[1] that the carbohydrate substances extracted from *Vibrio cholerae*, and related organisms, are so closely allied that cross-reactions will occur between them and immune sera at high dilutions. This cross-relationship was independent of the agglutination reaction, to differentiate the presumably pathogenic from

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[1] Linton, R. W., *Ind. J. Med. Res.*, 1932, **20**, 347.

non-pathogenic vibrios, since carbohydrate from a water vibrio, for example, would precipitate with serum against a pathogenic form, or against a rough non-agglutinable form. The vibrios included pathogenic smooth agglutinating forms, rough forms, some of which agglutinated and some of which did not, from human sources and from water, and non-agglutinating smooth water vibrios. These vibrios were characterized by the possession of a carbohydrate which was very similar if not identical in all.

A closer study has now been made of the carbohydrates of these organisms. The method of carbohydrate extraction will be given in detail subsequently.[2] The 48-hour growth of 300 to 500 Roux flasks is washed off in normal saline, of which 10 cc. or less is used for each bottle. Glacial acetic acid is added to the washings to make a N/20 solution, and the organisms are then boiled under reflux until coagulation. Upon cooling, clumps fall to the bottom and leave a clear yellow supernatant fluid. Coagulation time varies with the type of organism. With rough organisms coagulation and clumping may take place between 10 minutes and one hour; smooth organisms, from 3 to 6 hours. The organisms are removed from the extract in a Sharples supercentrifuge, and the extract filtered to a water clearness in Seitz filters. After concentration to approximately 500 cc., the extract is precipitated with 3 volumes of absolute alcohol. The alcohol precipitation is repeated at least 6 times and the usual protein reactions become negative after the second or third precipitations. The water solution, each time after the first, is concentrated to 250 cc. The final precipitate is dried *in vacuo,* and yields in some cases a brittle brownish gum, translucent in thin layers, and in other cases a white friable powder. The former is found in extracts from the non-pathogenic vibrios, and the latter appears in extracts of the pathogenic forms. The yield is between 10 and 15 gm.

After hydrolysis with normal sulphuric acid for 2 or 3 hours on sandbath, the yield of reducing substances is usually between 30% and 40%, calculated as glucose. By fractional precipitation with absolute ethyl alcohol, the hydrolysate may be separated into 3 portions. Fraction I, which separates from the hydrolysate upon the addition of 3 volumes of alcohol, is a dark reddish gum, containing no reducing substances even after prolonged boiling with acid. Fraction II, which separates upon the addition of 6 volumes of alcohol, and Fraction III, which remains behind in the hydrolysate,

[2] Linton, R. W., and Shrivastava, D. L., *Ind. J. Med. Res.*, in press.

have been qualitatively determined, and the results are given in Table I, for 4 different vibrios. Fraction II is the same in all the organisms, and appears to consist of glycuronic acid and galactose. It appears to be an aldobionic acid of the type found widely among bacterial carbohydrates and plant gums. The reducing power of this fraction is increased by 25% to 30% by prolonged boiling or autoclaving in acid solution, indicating that the compound is undergoing further hydrolysis. It has also been separated from a mixture of Fractions II and III as the barium salt.

TABLE I.

Analysis of Fractions II and III of *Vibrio cholerae*, and Related Organisms.

	Fraction II	Fraction III
1. A smooth agglutinating vibrio, recently derived from a case of cholera.	glycuronic acid + galactose	galactose
2. A rough, non-agglutinating strain derived from the above by the action of bacteriophage.	,, ,, ,, ,,	,,
3. A rough, weakly agglutinating strain.	,, ,, ,, ,,	arabinose
4. A water vibrio, smooth and non-agglutinating.	,, ,, ,, ,,	,,

The third fraction of the hydrolysate consists of a single sugar, which differs according to the type of vibrio from which the carbohydrate has been derived. In a smooth, agglutinating vibrio recently derived from a case of cholera, this fraction consisted of galactose. The same sugar is found in a rough non-agglutinating vibrio which is a secondary growth obtained from the first after the action of bacteriophage. The third organism in the table is a rough, weakly agglutinating strain, whose complete history cannot be traced, but which is supposed to be non-pathogenic. In it Fraction III consisted of arabinose. The last organism is a smooth, non-agglutinating water vibrio, in which Fraction III also contained arabinose.

Complete details as to the identification of these substances will be given later.[2] The following compounds have been prepared, and their melting points determined. For galactose, phenylosazone, methylphenylhydrozone, *o*-tolylhydrozone, mucic acid, and the formation of crystalline galactose; for arabinose, the phenylosazone, the diphenylhydrozone, and the formation of the crystalline sugar itself; for glycuronic acid, potassium acid saccharate, the barium *p*-bromphenylhydrozone of glycuronic acid, and the phenylosazone,

which also gave the characteristic reaction with naphthoresorcin and benzol.[3]

While the vibrios contain carbohydrate factors so closely allied that they cross-react throughout the group at high dilution, yet the vibrios themselves are not identical, since their specific substances are different. So far as our present analysis goes, the chemical difference between the specific substances of the pathogenic vibrio and the water vibrio lies in the possession of galactose by the first and of arabinose by the second. It appears further that the smooth-rough transition, brought about by bacteriophage action, does not change the character of the carbohydrate, nor does the change from the agglutinable to the inagglutinable type have any effect upon the specific substance.

6590

Studies on Sensitization. I. Skin Sensitivity and Serum Precipitin Response Following Intracutaneous Injections.*

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This report is the first of a series undertaken with the aim of throwing light on the rôle of sensitization in infection and immunity. Our early studies deal with the tissue hypersensitiveness that results from repeated injections of protein substances in animals, a condition generally classed under the Arthus phenomenon. Albino rabbits served as the experimental animals and the sensitizing injections were made, in most instances, intracutaneously.[1] The white skin in these animals rendered the effects of the injections readily discernible. This fact, in turn, permitted quantitative studies of the skin (local) response and its ready correlation with the serum (general) response.

The present study utilizes the method for measuring skin sensitivity recently reported.[2] Briefly, into the hair-clipped skin of an

[3] Abderhalden, E., *Handbuch d. biol. Arbeitsmethoden*, Abt. I, Tl. 5, Kohlenhydrate, 99.

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[1] A review of the literature on immunization by cutaneous means is given by Tuft, L., *J. Immunol.*, 1931, **21**, 85.

[2] Kahn, R. L., *J. Bact.*, 1933, **25**, 81.