many instances, there was obvious enhancement of the local response.

The striking thing about the growth of *H. pertussis* culture number 778 in liquid veal brain broth media was the formation of a thick tenacious substance. The resemblance between this material and the thick stringy sputum coughed up by children after an attack of whooping cough was obvious.

Culture number 778 was also planted on many types of solid media, 1½% agar with broth, Pelouze's gonococcus and Bordet-Gengou media. On the broth agar and Pelouze's media, the growth was rapid and in about 5 to 6 days the surface began to appear dully glazed. After this time, the growth stuck tenaciously to the medium so that separation was not possible with the ordinary platinum loop. In marked contrast, however, there was never any stickiness to the growth that appeared on the surface of the Bordet-Gengou media.

Growths of culture number 778 on Bordet-Gengou, as well as on veal brain broth media, were blown, dropped and syringed into the nasal passages of 5 *Macacus rhesus* monkeys weighing on an average of 6 pounds. In no instance was there a response that suggested whooping cough, either clinically or hematologically. This may be explained by the fact that culture number 778 was old and probably had lost its pathogenicity.

7146 C

Further Studies on the Specific Carbohydrates of Vibrio Cholerae and Related Organisms.*

RICHARD W. LINTON AND D. L. SHRAVASTAVA.

From the All-India Institute of Hygiene and Public Health, Calcutta.

In a previous note[1] on the specific carbohydrates of cholera and cholera-like vibrios some preliminary results were indicated. Since then our work has been considerably broadened, and we are able to extend our conclusions.

The method of extracting the carbohydrates used at first con-

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

sisted essentially of coagulating the vibrios by heat in acid solution and then separating the specific substances from the supernatant fluid by alcoholic precipitation. The vibrios were grown on agar, and further study has convinced us that vibrios so grown carry agar along with them in amounts sufficient to lead to considerable confusion, and whose removal is a matter of some difficulty. Organisms of the 2 vibrio types, whose carbohydrates contain galactose and arabinose respectively, were then grown for 5 days in 30 liters of 1% peptone water at pH 8.0, and the 2 characteristic sugars were obtained from this growth. In this method, however, the polysaccharide yield is too small to be of practical use.

We have therefore adopted a method used by Furth and Landsteiner[2] in which the organisms themselves, and not the fluids in which they have been growing, are used as the source of the polysaccharides. The organisms are washed repeatedly in distilled water, and centrifuged, until the supernatant fluid after hydrolysis no longer reduces Benedict's solution. The washed vibrios are dissolved in alkali, precipitated in alkaline solution with 1.5 volumes of alcohol, the precipitate dissolved in water and protein removed by the addition of acid. The polysaccharide in solution is then finally precipitated by alcohol in alkaline solution, washed in alcohol, and dried. The method has the advantages of being more rapid and less expensive than the first one, and of giving a good yield of the polysaccharide.

Types of Vibrios. By this method we have now prepared and analyzed the specific carbohydrates of 38 cholera and cholera-like vibrios. These have fallen into 3 groups as follows: 26 vibrios from clinical cholera with galactose-containing polysaccharide. Agglutinable. 8 vibrios from clinical cholera with arabinose-containing polysaccharide. Agglutinable. 4 vibrios from water with arabinose-containing polysaccharide. Non-agglutinable.

All the vibrios in the first group are agglutinable, and 15 of them have been used in the manufacture of cholera vaccines. It is interesting to note that the one "El Tor" vibrio investigated fell into this group. All the arabinose-containing organisms from clinical cholera in the second group are also agglutinable. Four of them have been used in vaccines. The water vibrios are non-agglutinable and are arabinose-containing as in Group 2.

The first and third groups of vibrios were described previously. We must now recognize an intermediate group of vibrios, having the same carbohydrate as the water vibrios and occurring in cholera,

[2] Furth, J., and Landsteiner, K., *J. Exp. Med.*, 1928, **47**, 171.

and at the same time being "typical" enough in the characteristics usually considered to be placed among the carefully chosen vibrios used in vaccines. The interrelationships between the 3 groups are not yet clear. It appears, however, that the agglutination reaction among the vibrios does not depend upon the presence of arabinose-containing carbohydrate.

Carbohydrates in "Rice-Water" Stools. Between one and 2 liters of the typical "rice-water" stool was collected from each of a series of 11 cholera patients. This material was concentrated to 300 or 400 cc. in acid solution on the water bath, the heavy flocculum removed in the centrifuge, and the concentrate treated as in the method of Furth and Landsteiner. The flocculum which appeared after the final alcoholic precipitation in alkaline solution was biuret-negative, and until hydrolyzed did not reduce Benedict's solution. The quantities obtained, while small, enabled us to identify the sugars they contained as the 2 characteristic ones of the cholera vibrios. Seven of the stools yielded galactose-containing and 3 arabinose-containing polysaccharide. In the eleventh stool, taken from a patient who recovered, no definite conclusion as to the nature of the sugar could be drawn.

Antibactericidal Effect of Specific Polysaccharides. The identification of the specific polysaccharides of *Vibrio cholera* in the stools of cholera patients gains in interest from several experiments which have been made to demonstrate an antibactericidal effect of the carbohydrate, since bacteriolysis is probably a means of defense of the body against vibrios. Such an experiment is as follows:

TABLE I.
Antibactericidal Effect of Specific Carbohydrate of Cholera Vibrio No. 79.

Dilution of 24 Hour Culture.	Serum Dilutions: 1:25	1:250	1:2500	1:25	1:250	1:2500	Control
10^5	+	+	+	0	0	0	+
10^6	+	+	+	0	0	0	+
	Carbohydrate added			No carbohydrate added			

+ = Growth; 0 = No Growth.

Each tube contained 0.5 cc. of fresh guinea pig complement diluted 1:10, 1.0 cc. of bacteriolytic antiserum, and 0.5 cc. of the dilution of organisms. One-half cc. of a 0.1% solution of the specific carbohydrate was added to the first series of tubes, and an equal amount of normal saline to the second. The tubes were incubated for 24 hours, the entire contents then placed in broth and the readings made after a further 24 hours' incubation. The car-

bohydrate solution was derived from the organism used, and contained arabinose. Similar antibacteriolytic power was shown in an experiment in which galactose-containing organisms and carbohydrate were used.

Conclusions. The vibrios which have been studied in this endemic area for cholera (Bengal) appear to fall into 3 groups on the basis of their carbohydrate content and agglutinability. In the majority of vibrios derived from cholera, galactose is the characteristic sugar. A second smaller group of vibrios, also from cholera, contain arabinose. These do not appear to differ from the first in agglutinability or in virulence, to judge from the type of case in which they have been found. The third group consists of non-agglutinable vibrios derived from water, some of which differ from *Vibrio cholerae* only in their failure to react with cholera agglutinating serum. All of these are arabinose-containing vibrios.

Polysaccharides containing both arabinose and galactose have been isolated from "rice-water" stools of cholera cases.

Both types of carbohydrate have an antibacteriolytic effect.

7147 P

Experimental Poliomyelitis. Active Immunization with Neutralized Mixtures of Virus and Serum.*

S. D. KRAMER AND M. SCHAEFFER. (Introduced by A. J. Goldforb.)

From the Laboratory of the Infantile Paralysis Commission, Long Island College of Medicine, Brooklyn, and the Bureau of Laboratories, Department of Health, City of New York.

Work on the nature of virus-antiserum combinations has indicated that although the virus in such mixtures is completely inactivated, the effect on the virus is not a destructive one, but more in the nature of a "neutralization" phenomenon. It is possible to recover active virus from neutral virus-serum unions by such means as dilution, and cataphoresis.[1-5] It is therefore not surprising that

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1 Andrews, C. H., *J. Path. and Bact.*, 1928, **31**, 671.

2 Douglas, S. R., and Smith, W., *Brit. J. Exp. Path.*, 1928, **9**, 213.

3 Long, Perrin H., and Olitsky, Peter K., *J. Exp. Med.*, 1930, **51**, 209.

4 Olitsky, Peter K., and Long, Perrin H., *J. Exp. Med.*, 1929, **50**, 263.

5 Olitsky, Peter K., Rhoads, C. P., and Long, Perrin H., *J. Exp. Med.*, 1929 **50**, 273.