scribed by Du Bridge and Brown.[3] The circuit includes a Leeds & Northrop type K potentiometer and type R galvanometer; 1 mv. gives a deflection of about 4 mm. on the scale at one metre. No erratic behavior has been encountered on the part of these electrodes; there may be slight hysteresis after changing from one buffer solution to another of widely different pH but the effect wears off within a few minutes after careful washing. With all ordinarily well buffered solutions, including blood samples from spiders, we regularly obtain steady readings within ± 0.2 mv. or better. The value of the constant RT/nF became practically normal after the first few weeks of use.

Although the initial outlay may be greater than for many designs hitherto published, the assembly here described is so convenient that it seems probable that it might find a use in other laboratories. An assistant can be trained to make routine measurements without difficulty. The amplifier is simple to operate provided proper shielding requirements are observed, in particular the grid lead should be thoroughly shielded and insulated. As pointed out by Hill[4] the high input resistance of the Pliotron eliminates the need for making fragile glass membranes of very low resistance.

The electrodes described above were made by the Macalister Bicknell Company of Cambridge and New Haven. The amplifier was built by Dr. A. Petrunkevitch of this department, who has designed a very convenient non-capacity switch (unpublished). We are indebted to Mr. D. G. Brubaker and the Sloane Physics Laboratory of Yale University for measuring the constants of the Pliotron used and also for assistance in determining the resistance of the electrodes.

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Preparation and Properties of a Specific Polysaccharide from a Strain of *Vibrio Cholerae*.*

D. L. SHRIVASTAVA AND S. C. SEAL. (Introduced by R. W. Linton.)

From the Cholera Inquiry, Indian Research Fund Association, All-India Institute of Hygiene and Public Health, Calcutta.

After a number of trials the method described below gave satisfactory results in the preparation of a specific polysaccharide of the

[3] Du Bridge, L. A., and Brown, H., *Rev. Sci. Instr.*, 1933, **4**, 532.

[4] Hill, S. E., *Science*, 1931, **73**, 529.

* This work was done under the auspices of the Indian Research Fund Association and is published by the permission of the Secretary.

strain known as Inaba variant, which belongs to Group VI in the chemical classification of the vibrios (Linton, Shrivastava and Mitra[1]). The method is based on that of Heidelberger, Kendall and Scherp.[2]

To the 72-hour growth of Inaba variant in 21 liters of 1% peptone water (pH 8.0), phenol was added to make a final concentration of 0.5% and the mixture allowed to stand for an hour. The bacterial growth was then removed in a Sharples supercentrifuge. The peptone water was neutralized with acetic acid and concentrated *in vacuo* to 2 liters. To the concentrate 60 gm. of sodium acetate and 12 cc. of glacial acetic acid were added and the mixture precipitated with 2 volumes of absolute alcohol, or if necessary for complete precipitation, 3 volumes. Larger amounts of alcohol were found to bring down peptone, inorganic salts, etc., in quantities which greatly interfered with the subsequent purification. The precipitate was taken up in 150 cc. of water, 3 or 4 cc. of acetic acid and 10 cc. of saturated sodium acetate solution, and centrifuged; if the material thrown down gave only a faintly Molisch positive reaction it was discarded. If it gave a strong reaction, it was again dissolved in water, treated as above, and the 2 centrifuged solutions were mixed.

The solution was precipitated with a minimum amount of alcohol, one-half volume usually being found sufficient. This precipitate was taken off and further addition of alcohol to the supernatant usually yielded only a very small precipitate which could be neglected. The above process was repeated and the final precipitate was taken up in a minimum amount of saturated sodium acetate (20 cc.) and about 10 cc. of acetic acid, centrifuged, and the supernatant precipitated with chilled acetic acid. Usually 15 to 20 volumes were required to give a flocculent precipitate. After centrifuging, the precipitate was taken up in water and centrifuged, and the biuret-positive insoluble matter was discarded. The solution was tested for biuret, Molisch reaction, and serological activity with homologous antiserum. If it was still biuret-positive it was shaken with chloroform to remove the last trace of protein. For this purpose, the solution was made up to 200 cc. with water, 20 cc. of chloroform were added, together with 4 cc. of amyl alcohol, 5 g. of sodium acetate and 4 cc. of acetic acid. It was then shaken mechanically for about half an hour and centrifuged. The topmost layer contained the polysaccharide in solution and was decanted carefully and shaken again with chloroform and amyl alcohol until the

[1] Linton, R. W., Shrivastava, D. L., and Mitra, B. N., *Indian J. Med. Res.*, 1935, **22**, 633.

[2] Heidelberger, M., Kendall, F., and Scherp, H. W., *J. Exp. Med.*, 1936, **64**, 559.

amount of the denatured protein layer became extremely small. After the final centrifuging the top layer was filtered and the filtrate precipitated with 1 to 2 volumes of alcohol.

The precipitate was taken up in about 20 cc. of saturated sodium acetate, and an appreciable amount taken for the biuret test. To the acetate solution 3 cc. of acetic acid were added and the whole was made up to 100 cc. with water and precipitated again with one volume of absolute alcohol. At this stage the solution of the precipitate was biuret-negative, and 3 or 4 alcohol precipitations in the presence of sodium acetate and acetic acid at approximately pH 4.0 were carried out, centrifuging the water solution each time to free it from any extraneous matter. The final precipitate was taken up in water, and after centrifuging, redistilled alcohol was added until opalescence or a slight precipitate appeared. The minimum amount of sodium acetate necessary for a flocculum to appear was then added. This precipitate was washed twice with redistilled alcohol, followed by acetone and quickly filtered on hardened filter paper and dried *in vacuo*. Yield: 0.297 gm.

The dried substance was white in color and extremely fibrous, resembling fibers of dried filter paper pulp. It was soluble in water with difficulty, and gave a solution of high viscosity even at a concentration of less than 3 mg. per cc. It was free from glycogen and phosphorus.

The Molisch test was positive at a dilution of 1:6,000,000. The material had a nitrogen content of 2.62%, 7.8% of ash (as Na_2O), and a specific rotation of +58.0°. Hydrolyzed in the boiling water bath with 3% H_2SO_4 (by volume), it gave the following reductions:

Time Interval. Hr.	% of Reducing Substance as Glucose
1	22.2
2	33.0
3	36.6
4	57.1
5	62.2
6	57.9

Beginning at the 4-hour period, the hydrolysate was inactive with homologous antiserum. The sudden rise in reducing power between the 3d and 4th hours appeared to indicate the presence of a second substance in the solution whose rate of hydrolysis differed from that which hydrolyzed first; this observation recalled earlier work, in which certain preparations showed the presence of a difficultly hydrolyzable aldobionic acid (Linton and Shrivastava[3]). The phenyl-

[3] Linton, R. W., and Shrivastava, D. L., *Indian J. Med. Res.*, 1933, **21**, 91.

osazone obtained from the hydrolysate had a melting point of 206°; mixed with known glucosazone the melting point was unchanged. Attempts were made to isolate other reducing substances in the hydrolysate, but up to the present they have not been successful. Earlier work[1] on the glucose-containing type of polysaccharide was also unable to bring to light any other constituent than glucose, in contrast to the arabinose- and galactose-containing types of vibrio polysaccharide in which an aldobionic acid is present.

With homologous antiserum, the polysaccharide reacted strongly. A zone phenomenon was present between dilutions of 1:4,800 and 1:150,000, but beginning at the latter dilution a gradually increasing floccular reaction was evident up to 1:12,000,000, after which it disappeared. The maximum reaction lay at about 1:2,500,000. Readings were taken after the tubes had been for 2 hours at 52°C. and finally after having been kept overnight in the ice-chamber.

The specificity of the polysaccharide was also tested by the use of antisera representative of each of the 6 groups into which the vibrios as a whole are divisible on the basis of their chemical constitution (Linton[4]; Linton and Mitra[5]; Linton, Shrivastava and Mitra[1]).

TABLE I.

Precipitin reactions of Inaba Variant (Group VI) Polysaccharide and Inaba (Group I) Polysaccharide with antisera derived from members of the six vibrio groups.

Group	Antisera (1:10)	Inaba Variant Polysaccharide	Inaba Polysaccharide
I	Inaba	0	1:400,000
	Kasauli 11	0	1:400,000
	10404	0	1:25,000
	Rangoon Smooth	0	1:100,000
II	653 Original	0	0
	984 Habigunj	0	0
	3065 Habigunj	0	0
III	W880	0	0
	653 (11-D)	0	0
IV	El Tor	0	0
	676	0	0
	Nanking 32/109	0	0
V	El Tor 34/D/19	0	0
	Doorenbos 34/11 (El Tor)	0	0
	6380 of 14/1/35	0	0
VI	Ogawa	1:2,500,000	0
	Inaba Variant	1:12,000,000	0

All controls were negative.

[4] Linton, R. W., *Bull. Off. Internat. d'Hyg. Publique*, 1935, **27**, No. 6.

[5] Linton, R. W., and Mitra, B. N., PROC. SOC. EXP. BIOL. AND MED., 1934, **32**, 468.

The results are given in Table I, which also includes a similar test with the polysaccharide of a Group I strain, Inaba. This polysaccharide had been prepared in one of the earlier attempts to perfect the method, and hence does not react highly with its homologous antiserum. It is, however, specific for the Group I strains.

Although the number of strains studied is few as yet, it is evident that the polysaccharides give group reactions so far as the data go. It is further interesting to note that the 3 El Tor antisera are not reactive with the Inaba polysaccharide. El Tor strains cannot be differentiated from cholera strains by "O" agglutination (Gardner and Venkatraman[6]); or by the vibrio polysaccharides prepared by Bruce White,[7] but they appear to be differentiated serologically by the polysaccharide used in the present experiments.

A further example may be given to show the relationship which is being demonstrated between serological reactivity and chemical structure in the vibrios. In April, 1935, chemical analysis of strain 1200 which had been isolated from a carrier in the previous December, showed that the organism belonged to Group VI, *i. e.*, its polysaccharide was of the glucose-containing type, similar to that of Inaba variant. A second analysis of the polysaccharide fraction carried out in May, 1936, indicated the presence in the hydrolysate of a large amount of galactose, accompanied by some glucose. When the antiserum against this strain was tested with Inaba variant polysaccharide in January, 1937, no reaction was obtained, and accordingly a third analysis of the polysaccharide was done. This revealed the presence of galactose in pure form, and a subsequent test of the antiserum with Inaba polysaccharide (which is of the galactose-containing type) showed a strong reaction. The shift in chemical structure was thus indicated by the precipitin reaction of the polysaccharide. It would appear from this experience that serological methods may enable us to follow the changes in chemical structure which occur in the vibrio group, and which have previously been described at length.[1, 4]

Polysaccharides from strains representative of all the chemical groups are now in course of preparation.

[6] Gardner, A. D., and Venkatraman, K., *J. Hyg.*, 1935, **35**, 262.

[7] Bruce White, P., *Brit. J. Exp. Path.*, 1936, **17**, 229.