To elucidate this question the isolation of these substances was undertaken from twelve-hour cultures of Gram-positive organisms, by removing the protein from the carbohydrate solution with the chloroform method.[1] The Type I polysaccharide thus isolated produced active immunity in mice and is chemically different from those hitherto described.

The present report is an extension of the above study. To prevent enzymic autolysis strictly Gram-positive, young organisms of Type I were used. They were grown in beef broth containing catalase to remove the hydrogen peroxide produced as a metabolic product. A polysaccharide was obtained which is identical with the one previously isolated.[1] After repeated precipitation from methyl and ethyl alcohol and finally from 60 per cent. ethyl alcohol, the analysis of the polysaccharide gave 6.35 per cent. nitrogen; 2.78 per cent. phosphorus; and 23.0 per cent. reducing sugar.

Serologically, this polysaccharide in dilution of one to one million gives precipitates with monovalent horse sera of Types I, II and III. Type I serum, absorbed with purified species-specific carbohydrate "C" prepared from young Gram-positive rough pneumococci, still reacted to titer with the conjugated polysaccharide derived from Type I, but not at all with a conjugated polysaccharide derived from Type II. Similarly, a Type II serum, absorbed with "C," reacted to titer with Type II but not at all with Type I conjugated polysaccharide. This type I carbohydrate removed antibody against "C" from Type II and Type III sera, and both type and species-specific antibodies from Type I serum.

The presence of phosphorus might be attributed either to inorganic phosphorus, phosphatides or the species-specific "C" carbohydrate as a mixture or in chemical combination with the type-specific polysaccharide.

To remove any phosphatide that might be present in the polysaccharide in one form or another, the following treatments were used:

(a) The dry carbohydrate was refluxed for one hour successively with each of the following solvents: pure acetone, one to one ethyl alcohol and ethyl ether solution, benzene, followed by ethyl ether and alcohol solution, and finally with low-boiling petroleum ether. The carbohydrate was then precipitated from its water solution with two parts of 95 per cent. alcohol.

(b) The carbohydrate was refluxed on a boiling water bath for four hours in N/5 acetic acid, cooled and extracted with ethyl and petroleum ether neutralized and extracted again with the same reagents. The carbohydrate was then precipitated with two parts of 95 per cent. alcohol.

[1] M. G. Sevag, *Bioch. Zt.*, 273: 419, 1934.

The treatments (a) and (b) produced chemically and serologically no change, thus eliminating the possibility of the presence of phosphatide in this polysaccharide. Furthermore, pure "C" in a mixture with the present carbohydrate is readily removed by treatment with two parts of alcohol; therefore the present substance must contain "C" in combination with the type specific carbohydrate.

(c) To remove any inorganic phosphorus which might be present as impurity, the solution of carbohydrate was brought to pH 2.5 with glacial acetic acid and precipitated with two parts of 95 per cent. alcohol. This treatment was repeated five times. Chemically and serologically no change was produced.

(d) The aqueous solution of the polysaccharide prepared as in (C) was treated with five volumes of glacial acetic acid; no precipitate was produced. Upon subsequent treatment with 1.5 volumes of 95 per cent. alcohol and two grams of sodium acetate per 100 cc solution, it flocculated immediately. The aqueous solution of the alcohol and ether-washed precipitate containing 2.0 grams of sodium acetate per 100 cc solution was further fractionated twice with two parts of 95 per cent. alcohol.

(e) 20 cc of the acidified carbohydrate solution was treated with 2 gms of sodium acetate. It was then chilled and treated with 7 cc of chilled 95 per cent. alcohol and centrifuged. The supernatant fluid was further treated with 7 cc of chilled 95 per cent. alcohol. These treatments were repeated several times on the sediments, and the carbohydrates thus obtained were similar to the one obtained above.

These treatments resulted in no change chemically or serologically. These findings would seem to indicate that in actively growing Gram-positive pneumococcus the type- and species-specific carbohydrates exist in a combined complex form. This complex alone or in mixture with swine serum did not produce immunity in rabbits. It is therefore reasonable to assume that this new complex carbohydrate may be in an antigenically active union with a protein component in the organism as the complete antigen. Studies are in progress in this laboratory to isolate this protein-carbohydrate complex in native form.

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THE BITTERLING OVIPOSITOR REACTION TO CORTICOSTERONE

IN 1932 we reported that female bitterlings (*Rhodeus amarus*) show an enlargement of the ovipositor following injection of an estrogenic preparation, while physiological solution of sodium chloride and an anterior pituitary extract yielded no reaction.[1] This

[1] W. Fleischmann and S. Kann, *Pflügers Arch. f. d. ges. Physiol.*, 230: 662, 1932.

effect could also be produced by adding estrogenic preparations to the water of the aquarium inhabited by the fish.[2] This observation was confirmed by the work of several authors. (Ehrhardt und Kühn,[3] Wunder,[4] Matousek,[5] Macht and Bryan.[6]) Kanter, Bauer and Klawans[7] showed that by using standardized bitterlings the ovipositor lengthening reaction could be used for detecting excesses of estrogenic hormones in the urine of pregnant women. These findings are confirmed by recent papers (Stiasny,[8] Marcus[9]). Whilst urines of women who were neither pregnant nor suffered from any endocrine disturbance gave negative results, urine from adult males gave a positive reaction. It was suggested by Kleiner, Weisman and Mishkind,[10] that it was the male sex hormone, which caused the reaction. Barnes, Kanter and Klawans[11] tried to locate the source of the material in the urine, which caused the reaction, by means of tissue extracts. The only extracts giving a positive response came from the adrenals. Kleiner, Weisman and Mishkind[12] found that purified active extracts of adrenal cortex gave a positive reaction. They suggested that the active principle, giving the bitterling reaction, might be identical with the corticosterone isolated from the adrenal cortex by Reichstein.[13]

We are indebted to Professor Reichstein, of Zurich, for giving us 20 mg of pure corticosterone for our experiments. We found that the ovipositor reaction following the injection of pure corticosterone was very great. We dissolved 3 mg of corticosterone in 0,3 cc of warm ethyl alcohol and added 2,7 cc of physiological solution of sodium chloride. Injection of 0,1 cc of this solution into two female bitterlings gave lengthening of the ovipositors from 4 and 3 mm to 16 and 15 mm in 48 hours. 2 cc of the solution added to one liter of water caused the ovipositors of three bitterlings placed in the water to grow from 3,4 and 4 mm to 12,12 and 15 mm in 48 hours. These experiments were repeated with the same results. An active extract of the adrenal cortex (Cortin from the firm Organen), of which 2 cc were added to one liter of water, caused the ovipositors of bitterlings, placed in the water to react in the same way. By using the colchicine technique (Dustin,[14] Allen, Smith and Gardner[15]) we found that the application or corticosterone was followed by high mitogenetic activity in the ovipositor.

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SCIENTIFIC APPARATUS AND LABORATORY METHODS

MICRO-SCISSORS

ANY one who has made dissections of minute objects under the dissecting binocular microscope has felt the need of scissors more delicate and better adapted for such work than even the smallest scissors now available. The writer ordinarily grinds his dissecting needles so as to form knives with straight cutting edges. Some time ago it was realized that two such needle-knives mounted so as to oppose each other would serve as scissors (Fig. 1), and would, moreover, avoid certain disadvantages common to scissors of the conventional type, in which blades rotate on a pin thrust through the blades themselves. The mechanical difficulties inherent in a minute pin would be obviated and there would be the minimum area of overlapping of the blades in which bits of tissue could be lost. The chief problem was to mount the needles so that they could be readily and securely adjusted and be capable of easily controlled movement upon one another.

In early attempts to make such micro-scissors, the needles were mounted by a variety of methods so that, in effect, they replaced the tips of ordinary fine forceps. In one case the points of forceps were cut off and replaced by shafts of pure silver tipped with small chucks for holding the needles. The silver could be bent easily and would remain in adjustment reasonably well. Although somewhat awkward, such instruments made possible dissections such as slitting the body-wall of mosquito and sandfly larvae without much disturbance of the internal organs.

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