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Ultrafiltration in the Concentration and Purification of Pneumococcus Specific Polysaccharides.

RACHEL BROWN.

From the Division of Laboratories and Research, New York State Department of Health, Albany, N. Y.

In continuance of the chemical and immunological studies of the pneumococcus,[1,2] methods of ultrafiltration previously described for use in the purification and concentration of other products[3,4] have proved distinctly advantageous in the preparation of the type-specific carbohydrate from broth cultures. Simultaneous concentration and purification are accomplished and the injurious effects of heat and chemicals are avoided. In one instance a 16-hour culture of pneumococcus type VII grown in an infusion-free peptone broth was run through a Sharples supercentrifuge and the effluent ultrafiltered and washed on a 4% nitrocellulose (Parlodion) membrane at room temperature. The residue, which contained all of the specific polysaccharide, represented a 100-fold concentration of the original material; at the same time 98.26% of the nitrogen had been removed. Similar results in the concentration of the specific polysaccharide and the elimination of inert material have been obtained by the passage of from 10 to 12 liters of clear broth culture through a single nitrocellulose-coated alumina thimble (127 x 45 mm.).

When larger amounts are to be handled, an initial concentration with ammonium sulfate can conveniently be used, followed by ultrafiltration for purification. If the broth-culture effluent from the Sharples supercentrifuge is treated with the optimum amount of ammonium sulfate (dependent upon the type of pneumococcus), the major portion of the specific polysaccharide is found in the scum which forms. This scum, when dissolved in water to give approximately a 50-fold concentration of the original, can then be purified by ultrafiltration.

Several alcoholic precipitations of the ultrafiltered residues give, in excellent yield, a product comparable in purity to those obtained by other methods and of high serologic activity.

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These methods have been found applicable to the preparation of the specific polysaccharides of pneumococcus types I, II, III, V, VII and VIII.

8812 P

Fixation of Ascorbic Acid by Tissues.

H. C. HOU.

From the Division of Physiological Sciences, Henry Lester Institute of Medical Research, Shanghai.

During the last few years considerable data have been accumulated to show that scorbutic animals suffer a marked depletion of the ascorbic acid content of the tissues particularly in organs such as the adrenal, pituitary, intestine, and liver. This depletion is readily made up when vitamin C rich foods are fed to the animals. The importance of the presence of ascorbic acid has been subsequently linked up with its function as an oxygen carrier, particularly through the studies of Harrison[1] and Euler and Klussmann.[2] They showed that tissue slices from scorbutic animals have a low oxygen uptake which is restored by the addition of ascorbic acid. Söderström and Törnblom[3] further show that in scorbutic animals the oxygen consumption is diminished.

The ability of tissues to fix ascorbic acid was suggested by the report of Harris, Ray and Ward.[4]. They believed from their study of the urinary output of vitamin C that the amount excreted might serve as a gauge of the nutritional state of the individual. This suggests that the tissues must be saturated with ascorbic acid before it will overflow through the kidney. This conception is supported by further observations of Harris and Ray,[5] Johnson and Zilva,[6] Ippen,[7] Euler and Malmberg,[8] Hou[9] and others on the urinary

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