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Studies on the ultrafiltration and electrodialysis of insulin solutions.

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Insulin (Lilly) was ultrafiltered through graded collodion membranes, and also subjected to electrophoresis and dialysis combined. No apparent purification was obtained by the first method, but by the second a definite fractionation into protein-like materials was accomplished. One of these fractions was very active, while the other showed very little activity. Each contained about 14.5% of nitrogen; and the active portion contained sulphur, while the inactive portion did not. The isoelectric point of the inactive material was pH 4.8 to 5.0, which is the same as that reported for the original material; while that of the active portion is apparently 5.0 to 5.2.

At pH of 3.6 all of the active material deposits on the negative membrane during the passage of the electric current, while the inactive material remains in solution. If the current is stopped there is immediate resolution, showing that the deposit is not an isoelectric precipitation. Adjustment of a solution of this material to a pH of 5.0 causes a precipitation. The ash from this material constituted about 2% of the total dry weight, and consisted only of silica. Repeated treatments did not remove this ash. The physiological activity of the active fraction is about 100% greater than that of the original insulin from which it was prepared, per unit of dry material.

Treatment of the active sediment with 25% sulphuric acid to which an equal volume of alcohol has been added causes a complete solution. On long standing of this solution, at room temperature, small needle crystals deposit. These crystals continue to form until all of the solid material originally dissolved appears in micro-crystalline form.

SUMMARY

The procedure described gives a method for further concentrating the active principle of insulin without the use of added

reagents, precipitants, adsorbants or whatnot. It accomplishes a separation of insulin, as prepared commercially, into active and inactive protein-like substances the isoelectric points of which are so close together that fractional precipitation as ordinarily practised is almost impossible.

Crystals, the chemical and physiological properties of which are being investigated, are formed from the active sediment, either by cision or by compound formation.

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The Ramon flocculation test in relation to the antigenic value of diphtheria toxoid (anatoxin).

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In a series of publications, Ramon[1] and Glenny, Hopkins and Pope[2] have claimed that the antigenic value of diphtheria toxoid (anatoxin) can be determined by the flocculation test described by Ramon. Since anatoxin, as defined by Ramon, is completely atoxic, the L+ and Lo cannot be used for determining the neutralizing and antigenic values of such a preparation. There are three other tests, however, that can be utilized:

1. The flocculation reaction of Ramon;
2. The combining value for antitoxin, as shown by the neutralization test;
3. The immunizing value in guinea pigs.

The flocculation reaction of Ramon, as will be shown below, is not a true index of the antigenic value of toxoid (anatoxin). Baecher, Kraus and Lowenstein[3] have also come to the same conclusion as a result of their work with guinea pigs.

In a recent communication Nelis[4] states that while anatoxin

[1] Ramon, G., *Ann. de l' Inst. Pasteur*, 1925, xxxix, 1-21.

[2] Glenny, A. T., Hopkins, B. E., and Pope, C. G., *J. Path. and Bact.*, 1924, xxvii, 261.

[3] Baecher, S., Kraus, R., and Lowenstein, E., *Zeitschr f. Immunitats f.*, 1925, xlii, 350.

[4] Nelis, P., *C. R. de la Soc. de Biol.*, 1925, xcii, 1112.