CONCENTRATION OF INSULIN BY ADSORPTION ON BENZOIC ACID.

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(Received for publication, July 17, 1923.)

In the preparation of insulin on a large scale, a problem which is of considerable importance is the concentration of dilute aqueous solutions of the potent material. On account of the expense and loss of potency which result in boiling down such solutions, it seemed desirable to investigate the possibilities of adsorption. It was found that the potent material could be almost completely removed from aqueous solutions by certain materials, such as decolorizing carbons, but attempts to redissolve the insulin from the solid by heating, altering the acidity, using a different solvent, etc., were only partially successful.[1] It was decided to try some reagent which could be used in a finely divided form, and which could be subsequently dissolved. For this purpose certain organic acids, such for example as benzoic and salicylic, suggested themselves on account of their relative insolubility in water and the fineness of division in which they come down when a dissolved salt is treated with an acid.

If a dilute solution of sodium benzoate in an impure aqueous solution of insulin is acidified, finely divided benzoic acid is precipitated and carries down with it a large percentage of potent material. The following is a detailed procedure for concentrating insulin by this method. To 1 liter of a crude aqueous extract 50 cc. of a 25 per cent sodium benzoate solution are added and the solution is acidified with 12.5 cc. of concentrated hydrochloric acid. This is usually sufficient to saturate the solution with benzoic acid and hence to give a lasting precipitate; these quan-

[1] We have later worked out a successful method of removing insulin from carbon, the details of which will be reported shortly.

tities can be increased or diminished proportionately, depending on the amount necessary to give the first lasting precipitate. Then 40 cc. of 25 per cent sodium benzoate and 10 cc. of concentrated hydrochloric acid are added, and after allowing sufficient time for complete precipitation the solution is filtered. This precipitate usually contains about two-thirds of the potent material. The filtrate is again treated with 40 cc. of 25 per cent sodium benzoate and 10 cc. of concentrated hydrochloric acid for a second precipitation. This is filtered and the resulting filtrate reprecipitated if it still contains sufficient potent material. The benzoic precipitate containing the insulin may be washed on the filter paper with a saturated aqueous solution of benzoic acid. A small amount of potent material comes off in the wash water which may either be reprecipitated along with the mother liquor, or may be collected and reconcentrated separately.

If this method of concentration is carried out on a highly purified solution the amount adsorbed is not so large as in an impure solution. This is probably due to the relative size of the particles of benzoic acid; the benzoic acid from the impure solution giving more adsorbing surface than that from a more purified solution.

Further, it is to be noted that even in very acid solutions the potent material is carried down in part. For example, at pH 1 one precipitation of a solution containing 150 units of insulin carried down 40 units of potent material. This is well out of the isoelectric range for insulin.

The insulin may be recovered in aqueous solution from the benzoic precipitate by various methods. If the original solution is comparatively free from protein the benzoic precipitate may be treated directly with ether and water to form two layers in a separating funnel. The benzoic acid is largely in the ether layer, and the potent material in the water layer. This water layer is washed with ether to remove traces of benzoic acid and the resulting aqueous solution is then boiled under vacuum to remove dissolved ether. It was noted that traces of ether had a distinct opposing or retarding effect on the action of insulin *in vivo*. If the solution to be concentrated is grossly impure, then sufficient inert material will come down with benzoic acid to form a distinct jelly when the benzoic precipitate is ethered directly.

One procedure in this case is to make up the moist precipitate to 80 per cent with ethyl alcohol, which dissolves the insulin and the benzoic acid; certain materials are allowed to settle out in the ice chest and the solution is filtered. The filtrate is boiled down under vacuum and ethered out as before. Another method of dealing with a precipitate which cannot be ethered directly is to dissolve the precipitate in glacial acetic acid. This solution is filtered after standing in the ice chest and either the potent material is precipitated in solid form by adding sufficient ether, or sufficient ether and water are added so that two layers form; the water layer containing the insulin. Traces of acetic and benzoic acids are removed from the aqueous solution by washing with ether.

This method of precipitating insulin with benzoic acid is successfully used in solutions containing 5 units in each 30 cc., the resulting solutions readily contain 5 units per cc. in a highly purified form. This method has been used on a large scale on the concentrate from the original alcoholic extract of the gland.

CONCLUSIONS.

A new type of adsorbing reagent has been successfully used. Insulin is by this means readily concentrated and greatly purified.

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J. Biol. Chem. 1923, 57:359-361.