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Further observations on the chemical and physical properties of insulin.

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Probably all active workers with insulin have observed considerable variation in the potency of the final product by whatever method obtained. Experience in this laboratory had led us to reinvestigate especially the influence of the exact H ion concentration upon potency at the several stages of the aqueous method.[1]

It has been found that after thorough extraction in N/5 HCl neutralizing with N/1 NaOH to P_H of 5.0 to 5.7 gives the largest yield of potency in the first filtrate.

The reaction is controlled both titrometrically to phenolphthalein and electrometrically (error $\pm 0.2 P_H$). Stopping at a P_H of 4.0 to 4.4 gives more rapid filtration but not so much available potency at once. However if the reaction after filtration is at once readjusted to 5.3 or thereabouts the potency is rendered available or, if it be allowed to stand for 24 hours at P_H 4.0 to 4.4 the potency increases. In fact the filtrate may be kept at room temperature until there is an abundant growth of bacteria and yeasts without destroying the potency.

In the final concentration it has been found that potency is best preserved in a fairly acid medium .06 to .08 N HCl; though a reaction as high as P_H 5.7 preserves for several weeks if an antiseptic like tricresol is added at once.

Pasteurization temperature maintained for half an hour does not destroy the potency. In fact the concentrated extract purified of proteins can be heated to 80° for half an hour, the exact effect depending upon the reaction. At a P_H of 6.2 to 7.0 or higher heating to this point seems regularly to increase the potency on rabbits. Heating at a P_H of 4.4 to 5.7 the potency is usually diminished or carried down with the coagulum if proteins are

[1] Murlin, Clough, Gibbs and Stokes: *Jour. Biol. Chem.*, 1923.

present. Heating the first filtrate (containing much protein) at P_H of 4.0 does not always produce a coagulum and if not, may increase the potency.

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Precipitation reactions of insulin.

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We here report the results obtained by the addition of various reagents to the aqueous solution of insulin as it is prepared for injection in human cases. Potency tests were made on normal rabbits, the dose given being equivalent to 20 gms. of pancreas except in a few of the earlier experiments where the equivalent of 40 gms. was taken. Only those instances where some positive test was obtained with the same material treated at the same time are reported.

Up to date 29 reagents embracing a wide range in chemical nature, have been tried of which two (formaldehyde and ethyl acetate) gave no precipitate of any kind. Next comes a list of 6 which gave a definitely negative result, *i. e.*, contemporary experiments with the same material gave some positive tests with other reagents. It should here be explained that on the addition of the reagents the precipitate is thrown down by centrifuge taken up in sterile water and injected, the supernatant liquid is dried down, with or without dialysis, depending on the nature of the precipitant, and similarly dissolved and injected. The following 6 gave definite negatives: ether, petroleum ether, toluene, xylene, chloroform and cadmium chloride. Five more are probably destructive to the principle, phosphotungstic, phosphomolybdic and tannic acids, $MgSO_4$ and $NaSO_4$ although the evidence is not yet conclusive. Mechanical difficulties were encountered with phenyl hydrazine, pyrogallic and picric acids, which have eliminated them from our list. Too little has been done with UO_2Ac_2, Zn SO_4 and NaCl to justify any inferences.

Turning now to the positive results we find that absolute ethyl alcohol is unique in being the only reagent encountered so far