

Tabulation of the relative protective action of these drugs against ordinarily lethal doses of *Streptococcus hemolyticus* shows the following (Table I).

The Prontylin and para-tolyl-sulfonamide were selected because of the former's (NH_2) and the latter's (CH_3) groups. The results in these experiments show that in these drugs the substitution of the (CH_3) for (NH_2) in sulfanilamide reduced, to a definite extent, the protective action of this drug. Phenyl-alanine-sulfonamide was found to protect rabbits from ordinarily lethal inoculations of *Streptococcus hemolyticus* even more completely than sulfanilamide; this substance did not show any toxicity in the doses given while sulfanilamide definitely showed some toxicity. The acid amide group ($\text{CO} \cdot \text{NH}_2$) has no protective action compared with the glutamine structures.

Conclusions. 1. The protective action induced in rabbits against the lethal effects of *Streptococcus hemolyticus* infections depends chiefly on the presence of the SO_2NH_2 group, not so much on the free NH_2 group. 2. Phenyl-alanine-sulfonamide was found to exert a more complete protective action against ordinarily lethal inoculation in rabbits of *Streptococcus hemolyticus* suspensions than the better known sulfanilamide.

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Absorption of Insulin by Yeasts.

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The absorption experiments were performed in the following manner: a cake of fresh yeast (Fleischmann) was suspended in 40 cc. of physiological salt solution, vigorously shaken for a few minutes, and centrifuged. The supernatant fluid was discarded. This procedure was repeated in order to remove extraneous matter present in the cake. The washed yeast cells were tested on a slide for viability by mixing a drop of the yeast suspension with a drop of Loeffler's methylene-blue solution and examining microscopically. Viable cells remained unstained, while dead cells readily took up the dye. Usually, only about 5-10% of the cells were stained. If the percentage of dead cells was higher, the cake was not used.

The washed yeast cells were suspended in a mixture of 20 cc. of

insulin U-40 of pH 3.5 and 60 cc. of saline. The suspension containing 800 units of insulin, or 10 units per cc., was allowed to stand for 2 hours at room temperature, occasionally stirred, and then centrifuged for 5 minutes at 1200 r.p.m. The supernatant fluid was decanted from the sediment and the latter washed once with 80 cc. saline in order to remove traces of the supernatant fluid, and then suspended in sufficient saline to make 80 cc. The insulin content of both the supernatant fluid and yeast cells suspension was determined in the usual way by injection into a series of fasting rabbits. The testing was performed several times with freshly prepared mixtures of insulin and yeasts. One cc. of the sediment suspension induced hypoglycemic shock in 12 of a series of 14 rabbits in from 2 hr. 10 min. to 4 hr. 50 min. The drop in blood sugar varied from 58 to 114 mg. %, with an average fasting glycemia of 98 mg. %, and an average glycemia during the shock of 24 mg. %. The minimal dose of yeast cell suspension which caused shock in 9 out of 12 fasting rabbits was 0.4 cc. to 0.6 cc. Inasmuch as that amount, according to the existing standard, corresponded to 3 units we may assume that 1 cc. of the suspension contained approximately 5-7.5 units of insulin. Control injections, with non-treated yeasts, failed to produce any change in the blood sugar.

The supernatant fluid was likewise bio-assayed for insulin content. 1.5 cc. injected into a series of 20 rabbits caused insulin shock in 15 animals in from 25 min. to 2 hr. 10 min. The drop in blood sugar varied from 64 to 88 mg. %, with an average fasting glycemia during shock of 32 mg. %. The minimal doses of supernatant fluid required to induce insulin shock in 12 out of 20 fasting rabbits was 0.6 cc. to 1.5 cc., thus showing that the supernatant fluid contained from 2 to 5 units per cc. instead of the original 10 units.

By comparing the insulin content of the yeast cells with that of the supernatant fluid we may assume that the yeast cells absorb from 50% to 75% of the insulin of a solution assaying 10 units per cc.

Substantially the same results were obtained when the absorption experiments were performed at 20° and 37° or by overnight incubation in the refrigerator. At 45° no absorption took place. Yeasts killed by heating at 100° or by chloroform did not show any evidence of insulin absorption.

Thus it follows that only viable yeast cells are able to absorb insulin. We are at present investigating the effect of peroral administration of "insulinated" yeast.