Octopine picrate melted at 224° and a mixed melting point was 219° (all uncorr.). The new picrate when analyzed by the Jorpes modification of the Sakaguchi method gave 97% of the expected color when octopine was used as a standard. The color given by the new picrate with the Sakaguchi reagents was identical with the purple color given by octopine. Picric acid analysis yielded the following results: Calculated for $C_9H_{18}N_4O_4.C_6H_3N_3O_7$ 48.20%, found 48.31%. The compound showed no free amino nitrogen with the Van Slyke method. The specific rotation, after removal of the picric acid, was +10° while the specific rotation of natural octopine is +20°. The free compound was crystallized from water by adding alcohol. The synthetic material melted at 257-60°; octopine melted at 266-68° and a mixture melted at 264-67° (uncorrected. All melted with gas evolution). The pH of dilute water solutions of both the synthetic and natural compounds was about 6.4. These results suggest that we have prepared partially inactive octopine. We plan to treat d-arginine with optically active bromopropionic acid.

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Quantitative Assay of Insulin Effect.

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In the post-absorptive state the constancy of the blood sugar level is an expression of a well-maintained balance between glycogenolysis and the withdrawal of sugar from the blood stream. The fall of the blood sugar level subsequent to an injection of insulin is regarded as the result of an inhibition of the glycogenolytic process, the withdrawal of blood sugar going on unaltered.[1, 2, 3] The degree to which the blood sugar is lowered is not in direct proportion to the insulin dosage.[4] One may increase the insulin dosage considerably in the lower and higher ranges with little or no increase in the degree of blood sugar depression. The effect of the larger dose expresses

[1] Issekutz, B. von, *Biochem. Z.*, 1927, **147**, 264; **148**, 283.

[2] Sahyum, N., and Luck, J. M., *J. Biol. Chem.*, 1929, **85**, 1.

[3] Cori, G. T., Cori, C. F., and Buchwald, K. W., *Am. J. Physiol.*, 1930, **93**, 273.

[4] Scott, E. L., and Dotti, Louis B., *Arch. Int. Med.*, 1932, **50**, 511.

itself in a maintenance of the maximum depression for a longer time period and in a slowing of the rate of return to the initial level. It follows that the effect of injected insulin lasts as long as the blood sugar is below the post-absorptive level. The quantitative determination of insulin action must, therefore, involve the measurement of both the intensity of blood sugar depression and its duration.

The times chosen by MacLeod and his coworkers[5] for determining blood sugar as a measure of insulin effect are suitable on the basis of the above postulate when relatively large doses are employed (0.5 units per kilo or over). Himsworth[6] has sought to measure the sensitivity of man to insulin by measuring the area enclosed by the depression curve below the resting level for the first 15 minutes. Scott and Dotti (loc. cit.) measured insulin response in terms of blood sugar depression at what they regard as optimum interval: 30 minutes after administration. They found that the proportionality between the blood sugar change and insulin dosage over a range of dosages from 1/16 to ½ unit per kilo followed a logarithmic rather than a direct relationship. Neither of the 2 latter insulin blood sugar relationships is an adequate measure of insulin action in the sense that it is an inhibitor of the glycogenolytic process in the liver. Scott and Dotti's method applicable in the study of large groups does not serve well for small numbers.

Insulin action in its entirety seems best expressed by the area confined by the blood sugar curve and a line parallel to the abscissa which intersects the curve at the initial post-absorptive level. No direct proportionality exists between insulin dosage and such areas even under strictly uniform experimental conditions in the same animal on a uniform diet. For example, in 4 dogs given 0.3 and 0.15 units per kilo at a 2-day interval the average ratio of the areas was 1.57, the individual variations being considerable. In 12 normal dogs given 0.5 and 0.25 units per kilo the areas of response at a similar interval showed a ratio of 3.73, the individual variations being less than in the previous group. It was found consistently that in the lower dosage ranges doubling the dosage causes considerably less increase in insulin action than in the higher dosage ranges. The inference is made that in the former a relatively larger quantity of insulin is required for the initiation of the insulin effect than is left to carry it on. Variable factors in the glycogenolytic system preclude proportionality in the complete insulin effect. The effect of 2 insulin samples or of 2 different dosages of the same

[5] MacLeod, J. J. R., *Carbohydrate Metabolism and Insulin*, 1936, Longmans, Green and Company, Ltd., London.

[6] Himsworth, H. P., *Clin. Science*, 1935, **2**, 67.

insulin preparation is considered identical when they produce blood sugar curves of similar form and magnitude. Under this condition all the variables, known and unknown, are so integrated as to represent the equivalent of a single factor, the insulin dosage.

Such a method of insulin assay has been roughly applied by us to measure the change in insulin response in dogs at 4 weeks and at 12 months after hypophysectomy. Under uniform conditions it was found that intravenous insulin 0.25 units per kilo produced blood sugar curves similar to those obtained by the use of 0.5 units per kilo before operation (Fig. 1). Twelve months after operation these animals were approximately 4 times as sensitive as when normal.

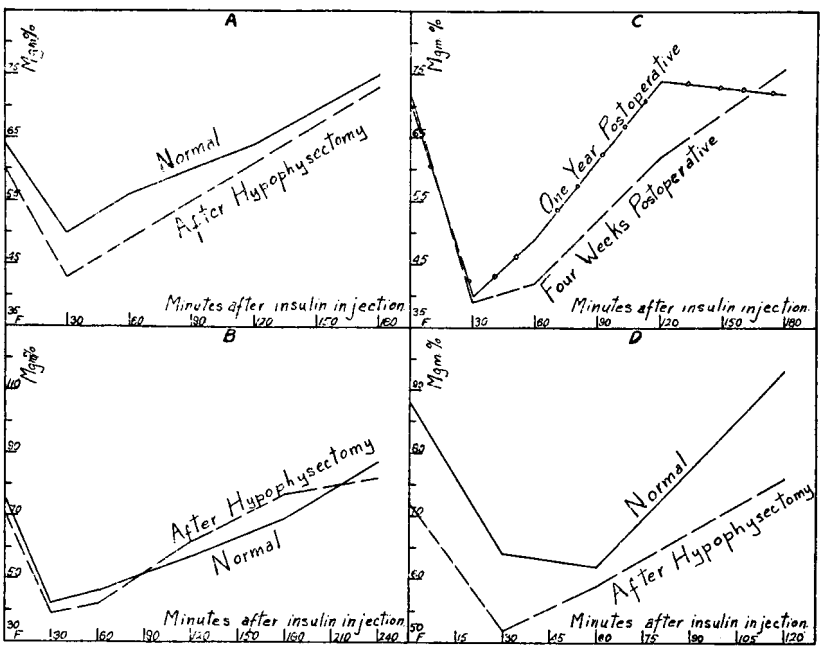

FIG. 1.

A. Blood sugar curves illustrating insulin effect in a dog before and 4 weeks after hypophysectomy. Preoperative dosage was 0.5, postoperative 0.25 units per kilo. The curves are approximately comparable.—Normal curve unbroken curve, after hypophysectomy broken.

B. Similar curves on a second dog 4 weeks after hypophysectomy. Insulin dosages as in A.

C. Blood sugar curves from same animal as for B. Dosages were 0.25 units 4 weeks after operation and 0.125 units per kilo one year after hypophysectomy.

D. Blood sugar curves on same animal as for C preoperatively and one year after operation. Preoperative dosage 0.25 units, postoperative 0.06 units per kilo. Note that C and D both indicate a postoperative insulin effect approximately four times that found for this dog preoperatively.