LXXV. THE INFLUENCE OF HEXOSEDIPHOSPHORIC ACID AND HEXOSEMONOPHOSPHORIC ACID UPON INSULIN HYPOGLYCAEMIA.

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DURING the last twelve years a considerable amount of evidence has accumulated which points to a carbohydrate-phosphorus complex as being an essential intermediate in the normal carbohydrate metabolism. The work of Embden and his co-workers has made it appear probable that a hexosediphosphoric acid, similar to that isolated by Harden and Young [1906] from the products of yeast fermentation, is formed as an intermediate stage in the conversion of glycogen or carbohydrate to lactic acid in the muscle.

A reduction in the concentration of the inorganic phosphate in the blood following the administration of insulin has been observed by many workers, while the exact relationship between the concentrations of the inorganic phosphate and sugar in the blood has been investigated by Eadie, Macleod and Noble [1925] and by Chaikoff, Macleod, Markowitz and Simpson [1925], who found that the inorganic phosphate in the blood showed an almost parallel decrease with the blood-sugar. If, as the evidence suggests, a carbohydrate-phosphorus compound is formed as an intermediate stage in carbohydrate metabolism, the administration of this compound might result in the complete or partial amelioration of hypoglycaemic symptoms. We have therefore tried the effect of subcutaneous injections, under such conditions, of two naturally occurring phosphoric esters of this type, hexosediphosphoric ester, and hexosemonophosphoric ester.

Robison [1922] isolated from the products of yeast fermentations a hexosemonophosphoric acid which he believed was probably a mixture of glucose- and fructose-monophosphoric acids. This conclusion has recently been supported by Meyerhof [1926], who, by determining the reducing power of the substance by Willstätter and Schudel's [1918] hypoidite method, was able to distinguish between the aldose and ketose types of acid, since this method only estimates the reducing power of the aldose type. Meyerhof concluded

that Robison's acid was a mixture of about 60 % glucosemonophosphoric acid and 40 % of fructosemonophosphoric acid. Application of this method of analysis to hexosediphosphoric acid supports the conclusions of Young [1909] and Neuberg and Kretschmer [1911] that it consists almost entirely of fructose-diphosphoric acid.

Preparation of the acids.

The hexosemonophosphoric acid used was prepared from fructose by fermentation with a preparation of dried yeast. The general conditions of separation were similar to those used by Robison in his original investigation. The final barium salt, after drying over phosphorus pentoxide at 80° and 20 mm., possessed the following constants:

$$[\alpha]_{\text{H}_g \text{ green}}^{18^\circ} + 16.7^\circ. \quad \text{Phosphorus } 7.45 \text{ \%}.$$

The barium salt was converted into sodium salt by shaking with the exact amount of a solution of sodium sulphate. The barium sulphate formed was removed by the centrifuge and the clear solution of sodium hexosemonophosphate was concentrated by evaporation in a vacuum desiccator.

The hexosediphosphoric acid was also prepared from fructose by fermentation with dried yeast. The final product was converted into sodium salt as described above for the monophosphate. The solution of the sodium salt was free from inorganic phosphate.

Injection experiments.

The ability of the diphosphate to relieve the symptoms of insulin hypoglycaemia was first tested on rabbits, with entirely negative results. Rabbits in insulin convulsions injected with the diphosphate showed no greater recovery than the untreated animals. Certainly there was no such dramatic recovery as is observed after the injection of a corresponding amount (1 g.) of glucose.

On account of the large quantity of material required to obtain a convincing result when using rabbits, it was decided to employ mice for further tests, since they exhibit unmistakable recovery after an injection of 100 mg. of glucose.

The mice, after receiving 0.025 unit of insulin subcutaneously, were kept in special boxes heated to 37°, since under these conditions the insulin intoxication follows a well-defined course with fatal termination, unless relieved by an injection of glucose or its equivalent. In about half an hour the mice began to show typical symptoms of insulin hypoglycaemia, namely, a condition of collapse, with legs sprawling and tails erect, or actual convulsions. Five of the affected mice received a subcutaneous injection of 0.1 g. glucose; four, an injection of an equivalent amount of hexosediphosphate; five, an injection of an equivalent amount of hexosemonophosphate; and six, the same volume of saline (0.4 cc.).

All the mice which received glucose showed definite recovery in one to two minutes and survived until the end of the experiment. Although in some cases the animals were not normal until 10 or 15 minutes later, they regained the use of their legs almost immediately.

On the other hand, those which received saline showed no amelioration of symptoms. Some of them seemed to remain alive longer than would be expected in the absence of any injection, but all were dead within $\frac{1}{2}$ to 1 hour after the injection of saline.

The effect of both hexosediphosphate and hexosemonophosphate was in all respects similar to that of saline. One of the mice, which was the last to be injected with the diphosphate, was killed in a collapsed condition at the end of the experiment. All the others were already dead.

These experiments, therefore, afford no evidence that these two compounds can replace glucose in the relief of insulin hypoglycaemia, or that they are readily convertible into that substance in the animal body.

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