PHYSIOLOGIC RESPONSE TO D-MANNOHEPTULOSE:
FRUCTOSE TOLERANCE CURVES

By

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ABSTRACT

Male rats were given fructose tolerance tests in the presence and absence of d-mannoheptulose. In the presence of mannoheptulose, fructose uptake was normal, but glucose uptake was inhibited. Insulin was able to reduce the hyperglycaemia. These findings support the hypothesis that the inhibition of glucose utilization produced by mannoheptulose is due to insulin lack.

The aim of this study was to explore further the mechanism of the action of mannoheptulose on the carbohydrate metabolism of the rat, by determining the effect of the presence of mannoheptulose on the uptake of fructose. All existing evidence is consistent with the hypothesis that administration of mannoheptulose causes a decrease in available insulin (Coore et al. 1963; Simon et al. 1961). Concomitant with the apparent decrease in insulin, there is increased gluconeogenesis (Simon et al. 1962). As a result of the simultaneous overproduction and underutilization of glucose, the rat undergoes a short hyperglycaemia. In order to verify that, following administration of mannoheptulose, the utilization of glucose is inhibited in a specific way, rats were given a fructose tolerance test.

The uptake and utilization of fructose from the blood, in contrast to that of glucose, seems to be unaffected by insulin (Price et al. 1945; Baker et al.

This investigation was supported in part by the Public Health Service Research Grant No. AM-05071-01 MET from the National Institute of Arthritis and Metabolic Diseases, Bethesda, Maryland.
1952; Miller et al. 1952). Therefore, the fructose tolerance test should be of use in distinguishing decreased glucose utilization due to insulin lack from that due to blockage of utilization of carbohydrate in general (Chernick et al. 1951). If mannoheptulose had blocked glucose utilization by some other means, for example, inhibition of glycolysis, fructose uptake should be inhibited by mannoheptulose just as is glucose uptake. These experiments were performed to test the hypothesis that insulin lack is sufficient to account for the decreased glucose tolerance produced by mannoheptulose.

**MATERIALS AND METHODS**

**Rats**

Thirty-three young male rats of the Weizmann Institute colony were used. They were selected on the basis of normal growth curves and were used when they weighed 200 g. After a preliminary overnight fast, they were divided into four groups: Group I, 7 rats; II, 7 rats; III, 9 rats; and IV, 10 rats. Injections were given between 8:30 and 9:30 a.m.

Mannoheptulose was prepared in our laboratory. It was injected sc, as 2 ml of 0.95 M aqueous solutions, to groups II, III and IV.

Fructose was reagent grade, and was used as purchased. It was injected sc, as 2 ml of 0.95 M aqueous solution, to all 4 groups. In groups II, III and IV, the fructose was injected 30 minutes after the mannoheptulose.

**Insulin**

Regular beef insulin solution (40 IU per ml) was diluted with insulin diluting medium immediately before use so that the dose was contained in 0.25 ml of solution. Injections were subcutaneous, simultaneous with the mannoheptulose. Group III was given 0.2 IU and group IV, 0.5 IU.

**Blood analysis**

Blood was taken from the cut tip of the tail at the time of the mannoheptulose injection, if given, the fructose injection, and 0.5, 1, 2, 3, 4 and 6 h thereafter. The methods of glucose and mannoheptulose estimation have been described in earlier publications (Simon & Kraicer 1957). Fructose was determined by the method of Kulka (1956). In this procedure, glucose did not interfere measurably when present in low concentrations. At high concentrations the fructose values were corrected for the small amount of colour due to glucose. The fructose concentration was calculated by subtraction of the contribution of mannoheptulose to the colour produced with the Kulka reagent. The glucose concentration was calculated as total reducing sugar minus mannoheptulose and fructose.

**Quantitation of Hyperglycaemia**

The changes in blood glucose during the period of observation after injection of mannoheptulose or fructose or both was determined by measuring the areas under the glucose response curves. It was sufficiently accurate to calculate the area of the irregular polygon whose successive altitudes are the concentrations of glucose measured at the various times. The results are expressed as $\frac{\mu\text{mole}}{\text{ml}} \cdot \text{h}$ or more compactly $\mu\text{mole} \cdot \text{h/ml}$. 

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Fructose tolerance curves. The fructose concentration in the blood of rats injected with 350 mg of fructose sc. The control group (Group I), solid circles, ●, was injected with only fructose; the other group (II), open circles, ○, was pretreated with 400 mg of mannoheptulose 30 min before fructose injection.

The glucose concentration in the blood of fasted rats after mannoheptulose (lower curve from Simon & Kraicer 1957) or mannoheptulose + fructose (upper curve). The points are means; ranges around the means are ± one SE.

RESULTS

Fructose Tolerance

The presence of mannoheptulose had little effect on the fructose tolerance curves. In Fig. 1 the fructose tolerance curves of groups I and II are shown. The only difference in the concentrations occurs 2 h after injection. In the mannoheptulose-treated group, the concentration has dropped to 1.6 μmole
The glucose concentration in the blood of fasted rats after fructose administration. The difference between the curves of 2 a is shown (upper curve) with the response to fructose alone (lower curve). The points are means; ranges around the means are ± one SE.

per ml as compared to 1.0 in the control group. Later values, i. e. at 3 and 4 h after injection, are equal. In groups III and IV, which received insulin in addition to the fructose and mannoheptulose, fructose tolerance curves were identical to that of group I.

**Blood Glucose Response**

The administration of fructose to fasted rats causes only a slight increase in blood glucose, which may be considered to be the effect of a small »re-feeding« (Fig. 2 b). Compared with this small rise, the effect of the addition of mannoheptulose is very large. The hyperglycaemic response to mannoheptulose is 33.0 μmole · h/ml (Kraicer et al. 1962). The hyperglycaemia attributable to mannoheptulose plus fructose is 47.3 μmole · h/ml during the 6.5 hours fol-

**Table 1.**

The reduction by insulin of the hyperglycaemia caused by mannoheptulose + fructose. Total hyperglycaemia was measured as the area under the glucose response curve during the 6.5 hours following the administration of mannoheptulose and insulin, and is presented as mean ± SE. All values are significantly different from one another (t-test).

<table>
<thead>
<tr>
<th>Dose of Insulin, IU</th>
<th>0</th>
<th>0.2</th>
<th>0.5</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total hyperglycaemia μmole h/ml</td>
<td>46.5 ± 2.2</td>
<td>25.7 ± 5.5</td>
<td>10.2 ± 4.9</td>
</tr>
<tr>
<td>Number of rats</td>
<td>7</td>
<td>9</td>
<td>10</td>
</tr>
</tbody>
</table>
lowing the mannoheptulose injection. When fructose is given alone, the rise in blood glucose during the parallel period is only 4.8 μmole · h/ml.

The exacerbated hyperglycaemia is sensitive to the action of exogenous insulin (Table 1). When 0.2 or 0.5 IU of beef insulin is administered together with the mannoheptulose, the maximum blood sugar response is delayed to 3 and 4 hours after heptulose injection, respectively (Groups III and IV).

**DISCUSSION**

The mannoheptulose-treated rat is unable to remove glucose from its blood, and becomes hyperglycaemic. While this metabolic block is present, the same rat experiences no such difficulty in absorbing fructose from the blood. This demonstrates that the defect in carbohydrate metabolism, which is induced by mannoheptulose, is directed specifically against glucose. This supports the hypothesis that mannoheptulose blocks secretion of insulin.

The mannoheptulose-treated rats has little or no impairment in its fructose tolerance. However, after absorption from the blood, the bulk of the fructose is converted to glucose. If this glucose enters the blood, it is »trapped« there and exacerbates the hyperglycaemia. In an earlier study it was shown that the shape of the graph of glucose response to mannoheptulose varied with the dose of mannoheptulose administered. For a dose level of 400 mg (1.9 μmol), the curve showed a typical maximum glucose concentration of 8.5 μmol/ml approximately 2 hours after injection of mannoheptulose, with a hyperglycaemic response of 33 μmol · h/ml. In this study, we have shown that the addition of glucose from a source other than gluconeogenesis causes a further rise in blood glucose; maximum hyperglycaemia, 2 hours after mannoheptulose administration, was 14 μmol/ml and the hyperglycaemic response was increased to 47.3 μmol · h/ml.

The results of this and other studies are in accord with the hypothesis that mannoheptulose blocks the secretion of insulin. However, since the normal stimulus for insulin secretion is the blood glucose itself, there are two possible modes of inhibition which would explain the action of mannoheptulose: a total block of the stimulatory action of glucose concentrations on the beta-islet cells; or a rise in the threshold concentration of glucose which gives the minimum effective stimulus. If mannoheptulose caused a rise in threshold, the new threshold would determine the intensity of the hyperglycaemia, or at least the maximum concentration of blood glucose after any dose of mannoheptulose. The blood glucose response production results in increased hyperglycaemia. Therefore, the secretion of insulin in response to hyperglycaemia is apparently totally blocked. The blockade of endogenous insulin explains the efficacy of exogenous insulin, which reduces the hyperglycaemia in relatively small doses.
This result was expected, since it has been previously shown that insulin can reduce or prevent the hyperglycaemic response to mannoheptulose, and is consistent with the postulated block to insulin secretion by mannoheptulose.

Many studies of carbohydrate metabolism in the diabetic rat have depended on treatment of the rats with sub-lethal doses of alloxan to produce the diabetes. The use of alloxan has many distinct disadvantages, the major one in the present context being its toxicity to the liver. With the discovery of the diabetogenic action of mannoheptulose, an experimental alternative to alloxan has become available. Unlike alloxan, it does not produce its physiological effect by irreversible damage. The diabetes produced by mannoheptulose in the intact, fasted rat is transient and leaves no known aftereffects. The main actions of mannoheptulose seem to be on the pancreas and on the liver. In the pancreas, it apparently prevents secretion of insulin in response of hyperglycaemia. In the liver, it accelerates conversion of protein to carbohydrate via an adrenal-cortex dependent pathway.

ACKNOWLEDGEMENTS

Our warmest thanks are due to Mr. A. Almoznino for his excellent technical assistance. This work was done with the guidance of Professor M. C. Shelesnyak to whom we express our gratitude.

REFERENCES


Received on April 22nd, 1964.