XXIX. THE EFFECT OF FRUCTOSE ON THE GLUCOSE TOLERANCE CURVE

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FRUCTOSE, when injected intravenously into fasting rabbits, was found by Davidson *et al.* [1936] to decrease the concentration of the glucose of the blood and to depress the glucose tolerance curve of rabbits. They interpreted both types of result as indicating that fructose stimulated the secretion of insulin by the pancreas, and that it was this excess insulin, so produced, which was responsible for the lowered blood glucose following the injection of fructose. Although this interpretation is in line with the conventional view that the glucose tolerance curve is determined primarily by the response of the pancreas to the ingested glucose (by the amount of extra insulin secreted), no facts were adduced in its support. It seemed to us very doubtful that fructose should possess this property of being able to promote increased secretion of insulin by the pancreas. Certainly the pancreas does not utilize fructose in perfusion experiments [Steinberg, 1927]. It is appreciated that it does not necessarily follow from this fact that pancreatic cells or more particularly the islet cells would not be stimulated.

To elucidate this action of fructose the obvious test object is the depancreatized animal in which the amount of available insulin can be rigidly controlled. We therefore planned to find out if fructose similarly affected the blood glucose first of the normal and then of the depancreatized dog. While we were unable to obtain any depression of the blood glucose of the fasted dog by an intravenous injection of fructose, we did obtain by this means a marked depression of the glucose tolerance curve. We could not detect any effect of fructose on the blood glucose of the depancreatized animal, in the complete absence of insulin. These experiments will be reported in greater detail later. It was then decided to repeat these experiments on the depancreatized dog, when the animal was receiving a steady supply of insulin during the period of the test. It seemed to us that the newly discovered protamine zinc insulin would serve this purpose conveniently. It was found that the subcutaneous injection of a fairly large dose of protamine zinc insulin into the fed depancreatized animal, in the late afternoon, resulted in a marked depression of the blood sugar by the following morning, and the concentration of sugar remained fixed at this low value (within a few mg. per 100 ml.) for at least a further 8 hr. Further, it was found that the feeding of a given amount of glucose during this latter period gave a glucose tolerance curve which could be reproduced with somewhat surprising exactness on subsequent experimental days, provided that the conditions were kept constant. With such conditions established it was an easy matter to determine if an intravenous injection of fructose effected any alteration in this glucose tolerance curve.

EXPERIMENTAL

Methods. Glucose was estimated in blood by the method of Shaffer & Somogyi [1933], but for convenience we used the combined tungstic acid reagent for protein precipitation, as described by Van Slyke & Hawkins [1928].

Occasionally true blood glucose values were obtained by fermentation tests. When necessary, galactose was estimated by the same method, after removal of glucose with yeast and making the required deduction for non-sugar reducing substances. Fructose was estimated by the titrimetric method of Campbell & Hanna [1926].

Some weeks were allowed to elapse after pancreatectomy¹ before the animals were used for these experiments. They were kept in separate metabolic cages, and fed regularly (twice daily) on a standard diet, adequate to maintain weight and including vitamin preparations. Each animal received a daily morning injection of protamine zinc insulin, in amount sufficient to allow a daily excretion of 20–60 g. glucose in the urine. On the day before the tolerance test the animals were fed as usual, but the morning dose of insulin was reduced to 6 units, while in the late afternoon an amount of insulin was given which would ensure a low concentration of blood glucose during the whole of the following working day. Too large a dose of insulin resulted in convulsions, while with too small an injection the blood sugar began spontaneously to increase before the end of the experiment. The same dosage and general procedure were employed while obtaining comparison curves.

The glucose tolerance curves of all animals, normal and depancreatized, were obtained after feeding by stomach tube 2.5 g. glucose per kg. body weight, in 25% solution. Graphs given below illustrate representative results. The blood glucose values given in these graphs include non-sugar reducing substances to the extent of approximately 15–20 mg. per 100 ml. of blood.

Fig. 1. The effect of fructose on the blood glucose concentration of the fasting normal dog and of the fasting depancreatized dog.

△—△ Glucose	} Fructose (0.5 g. per kg. body weight) injected into normal dog.
△---△ Fructose	
○—○ Glucose	} Fructose (0.67 g. per kg. body weight) injected into fasting depancreatized dog (receiving insulin) with high blood sugar.
○---○ Fructose	
●—● Glucose	} Fructose (0.5 g. per kg. body weight) injected into fasting depancreatized dog (receiving insulin) with low blood sugar.
●---● Fructose	
- (Arrow indicates time of sugar injections.)

Fig. 1 illustrates the effect of fructose on the blood glucose of the normal fasted dog, on the depancreatized dog (receiving protamine insulin), when the blood glucose concentration is low and also when the concentration of blood glucose is comparatively high. It will be observed that fructose has no depressant effect upon the blood glucose of the normal fasted dog or of the depancreatized dog when the blood glucose concentration is low; when in the depancreatized dog receiving insulin, however, the concentration of blood glucose is relatively

¹ We are indebted to Dr C. H. Best, who performed these operations for us.

high, intravenous injection of fructose is followed by a decrease in the glucose concentration. Only occasionally were we able to obtain these high blood glucose values. More sugar was given with the afternoon meal of the day preceding the experiment and the animal was sometimes given less insulin in the morning of that day. But the exact conditions required seemed difficult to reproduce, and when high blood sugar values were obtained they sometimes varied spontaneously, and in such instances it was doubtful or impossible to evaluate the effect of the fructose injection.

Fig. 2 illustrates the marked effect of fructose, given intravenously, on the glucose tolerance curve of the normal dog.

Fig. 2. The effect of fructose on the glucose tolerance curve of the normal dog.

- Glucose tolerance curve (control).
- Glucose tolerance curve after intravenous injection of fructose (0.5 g. per kg. body weight) at time marked with arrow.
- Blood fructose values.

(Arrow indicates time of sugar injection.)

Fig. 3. The effect of fructose on the glucose tolerance curve of the depancreatized dog.

Dog M. Weight 16.3 kg. Insulin: daily 18 units, day before experiment 6 units a.m., 28 units p.m.

- Glucose tolerance curve (control).
- △—△ Glucose tolerance curve after injecting galactose (0.67 g. per kg. body weight).
- Glucose tolerance curve after injecting fructose (0.67 g. per kg. body weight).
- Blood fructose values after injecting fructose.

(Arrow indicates time of sugar injections.)

Fig. 3 shows the results obtained on a depancreatized animal. In these experiments it was our practice to alternate control glucose tolerance curves with tests on the influence of fructose on these curves. We found little variation in our control glucose tolerance curves, but for clearness only one such curve is

reproduced in the figures. Fructose exercises a marked depressant action on the glucose tolerance curve. To be assured that this effect was not a non-specific one we injected at other times an equal quantity of galactose. The glucose tolerance curve remains uninfluenced by the galactose.

Similar findings are illustrated in Fig. 4, as well as another point of interest, namely, that a small dose of fructose has a smaller effect than a large dose.

Fig. 4. The depression of the glucose tolerance curve is proportional to the amount of fructose injected.

Dog F. Weight 13.5 kg. Insulin: daily 6 units, day before experiment 6 units a.m., 18 units p.m.

- Glucose tolerance curve (control).
- △—△ Glucose tolerance curve after injection of galactose (0.33 g. per kg. body weight).
- Glucose tolerance curve after injection of fructose (0.33 g. per kg. body weight).
- Glucose tolerance curve after injection of fructose (0.1 g. per kg. body weight).
- Blood fructose after larger injection.
- Blood fructose after smaller injection.

(Arrow indicates time of sugar injections.)

A number of other substances have been tested, including pyruvic and laevulic acids, injected in the form of their Na salts. These substances resemble fructose in that they possess a ketone group and, further, they are possible intermediary products of fructose oxidation. Neither substance has any detectable influence on the glucose tolerance curve of the depancreatized dogs, when given in amounts of 0.5 g. per kg. body weight.

DISCUSSION

The lowered glucose tolerance curve which is obtained in the depancreatized dog receiving insulin, when fructose is injected shortly after feeding the glucose, is not due to an increase in available insulin. For there is no reason to suppose that the fructose leads to more rapid absorption of the protamine insulin from the site of injection. Nor in the normal animal, therefore, is it necessary to assume that fructose increases the secretion of insulin by the pancreas. In order that fructose shall lower the glucose tolerance curve, insulin must be present in the tissues of the animal, but no increased secretion of this hormone appears to take place.

These experiments have been conducted on four depancreatized dogs, receiving protamine zinc insulin. The glucose tolerance curves of three of these

animals were depressed by fructose, whereas in the fourth animal no change could be detected even when fructose equivalent to 1 g. per kg. body weight was injected. The animals which responded showed differences in sensitivity in their response to fructose. Thus one animal showed a significant response (in a lowered glucose tolerance curve) with only 0.1 g. per kg. body weight of fructose, while in another animal which responded to a larger amount of fructose this small amount had no apparent effect. Increasing amounts of fructose gave greater depressions below control tolerance curves. In this sense there is a quantitative relationship between the amount of fructose injected and the corresponding effect on the glucose tolerance curve.

Fructose caused no decrease in the blood glucose of the fasting normal dog. Neither was any such effect obtained with fasting depancreatized dogs, nor when these animals were receiving protamine insulin and the blood glucose value was equal to or less than that of a normal fasting animal. But when the blood glucose concentration of the fasting depancreatized dog receiving protamine insulin was relatively high, then a fall to a lower value was obtained following fructose administration.

Evidently fructose administration, under certain conditions, leads to a more rapid utilization of glucose. This may be due to increased oxidation of glucose or increased glycogen formation from glucose or to both processes. We are of the opinion that the chief effect is on the glycogenesis mechanism, and that the tissue mainly involved is the liver, but the possible participation of other tissues should not be ignored. This view is consistent with the facts enumerated above: that fructose exercises its effect in the presence of insulin, but not in its absence, and that it does so when the blood glucose concentration is high, but not when low. That is, when conditions are favourable to glycogen synthesis the action is positive, when unfavourable it is negative. Further, the different degrees of sensitivity of different animals to fructose might be regarded as an indication of the hepatic efficiencies of these animals, with respect to glycogen formation.

We would suggest that fructose exercises a stimulating or catalytic action on the glycogen synthetic mechanism of the liver cells. The influence of fructose on the blood glucose of the hepatectomized animal is of interest. Griffiths & Waters [1936] injected fructose (0.5 g. per kg. body weight) into hepatectomized dogs and found no change in the concentration of blood glucose. But in these experiments the blood glucose concentration was that of fasting normal animals, and, as pointed out above, at such levels fructose has no effect on the normal animal. The negative evidence provided by these experiments indicating the liver as the site of the catalytic influence of fructose, must therefore be accepted with caution.

A number of investigators have confirmed the finding of Lusk [1892] that fructose is a better hepatic glycogen precursor than glucose. In an endeavour to account for this fact Fletcher & Waters [1937] attempted, unsuccessfully, to adduce evidence of a preferential absorption of fructose over glucose by the hepatic cell. As indicated at the time, we believe that the experimental results described in the present paper throw some light on this problem. We would adhere to the view that the absorbed fructose is rapidly converted into glucose in the liver cells, but, further, we believe that the fructose exercises a stimulating effect on the glycogenesis mechanism of the cells. Certainly such a view would provide an explanation for the experimental fact that fructose is a better hepatic glycogen precursor than glucose, which up to the present has been inexplicable.

It is possible that this influence of fructose on blood glucose concentration is not due directly to fructose *per se*, but to some produce of fructose metabolism.

Also it is possible that this property is shared by a number of substances. Other ketonic substances so far tested, including pyruvic and laevulic acids, have been found to have no effect.

SUMMARY

1. Fructose, when injected intravenously, does not decrease the blood glucose concentration of the fasting dog.
2. Fructose, when injected intravenously, lowers the glucose tolerance curve of normal dogs, and also the glucose tolerance curve of depancreatized dogs receiving a steady supply of insulin.
3. Therefore it is concluded that this effect in normal animals is not to be interpreted as due to a stimulation of the pancreas to give increased secretion of insulin.
4. The quantitative aspect of this influence of fructose is shown.
5. A hypothesis is advanced that fructose exercises a catalytic influence on the utilization of glucose, the site of this influence, probably, being in the glycogenesis mechanism of the liver.

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