LXIX. FRUCTOSE METABOLISM IN THE INTACT ANIMAL.

BY JAMES NORMAN DAVIDSON, WILLIAM OGILVIE KERMACK,
DORIS MIDDLETON MOWAT AND CORBET PAGE STEWART.¹

*From the Medical Chemistry Department, University of Edinburgh, and
The Royal College of Physicians Laboratory, Edinburgh.*

(Received January 31st, 1936.)

THE work described in this paper was undertaken to obtain fresh evidence on the metabolism of fructose in the intact rabbit. Many of the earlier workers, handicapped by the lack of a reliable method for estimating fructose in blood, estimated only the total sugar present or confined their attention to the amount of glycogen deposited in the liver and muscles after administration of fructose. With the development of new and reliable methods for the estimation of fructose in comparatively small quantities of blood it has become possible to attack the problem from the point of view of the blood chemistry, and this method has been employed in obtaining the results which follow.

MacLean and Smedley [1912] showed that the hearts of the dog and rabbit were unable to utilise fructose and this result was confirmed by Steinberg [1927] and by Stewart and Gaddie [1934] for frogs' hearts, while Ashford [1933] obtained similar negative results using brain tissue. Dickens and Greville [1932] found that some embryonic tissues were able to convert only glucose into lactic acid, whilst others were able to convert fructose as well. The intact animal is capable of utilising fructose but there are clearly two possibilities (1) that fructose must first be converted into glucose or into glycogen in the liver, and (2) that some of the tissues are able to utilise it directly.

Cori and Cori [1928] have concluded that fructose is utilised directly by the tissues without previous conversion into glucose, whilst Mann and Bollmann [1930], working with hepatectomised animals, support the view that fructose is first converted into glucose. Mann [1925, 1, 2] showed that glucose but not fructose could restore a moribund hepatectomised dog and further evidence of the importance of the liver in the disposal of fructose has been provided by Isaac [1920], Stroebe [1932] and Kimball [1932], who showed that fructose causes prolonged hyperglycaemia in cases of hepatic disease but not in normal animals, while Bodansky [1923] and Kimball [1932] have demonstrated the importance of fructose tolerance tests in indicating liver damage.

EXPERIMENTAL.

Estimation of fructose.

The estimation of fructose in presence of glucose in small amounts of blood involves certain difficulties. In the early experiments in this series fructose was estimated by a method depending on the reduction of phosphomolybdotungstic

¹ In receipt of a part time grant from the Medical Research Council.

acid. This method had certain advantages over the colorimetric method of Van Creveld [1927] and Radt [1928] but it suffered from the disadvantage that the reaction was incomplete and that any attempt to force it to completion caused interference by glucose. This method however, when the conditions were carefully controlled, gave satisfactory results which were found to be comparable with those obtained by Van Creveld's method.

Later a micro-scale modification of Patterson's [1935] adaptation of Radt's method was evolved which was more convenient and gave results similar to those obtained by the previous method. The results which follow were obtained in most cases by employing both methods.

1. *Phosphomolybdotungstic acid method.* To 2.0 ml. of Folin-Wu blood filtrate 2.0 ml. of the phosphomolybdotungstic acid reagent (35 g. molybdic acid and 5 g. sodium tungstate were dissolved in 200 ml. of 10 % NaOH and the mixture boiled vigorously, cooled, diluted to 350 ml., added to 125 ml. 85 % H_3PO_4 and diluted to 500 ml.) were added and the mixture heated on the boiling water-bath for 30 min. Simultaneously 0.5 ml. of a 0.05 % solution of fructose was similarly treated. After rapid cooling the solutions were titrated with $N/200$ KMnO_4 until the blue colour just disappeared. The amount of fructose present was calculated using standard fructose as a basis. A blank value obtained from fasting blood was subtracted from each result (the assumption being made that normal blood contains no fructose) since the colour so obtained was always greater than that given by the reagent alone.

2. *Diphenylamine method.* 0.2 ml. blood was diluted with water to 0.6 ml. and 0.2 ml. each of 10 % ZnSO_4 and 0.5 N NaOH added. After heating to 80° for 10 min. the mixture was filtered. 1.0 ml. of the filtrate was acidified with 2 drops of 0.1 % acetic acid and was evaporated to 0.4 ml. 0.4 ml. 6 N HCl and 1 drop 20 % alcoholic diphenylamine were added and the tube heated on the boiling water-bath for 15 min. After cooling for 2 min. 1.0 ml. butyl alcohol and 0.2 g. solid $(\text{NH}_4)_2\text{SO}_4$ were added. The tube was stoppered and briskly shaken. The upper alcoholic layer was pipetted off for comparison in the micro-colorimeter. The standard was prepared from suitable dilutions, in fasting blood filtrate, of a stock standard fructose solution, treated in exactly the same way as the blood filtrates.

All the rabbits used were tested to ensure that they gave normal sugar curves when glucose alone was administered and each animal fasted for 24 hours before an experiment.

Total reducing sugar in the blood was estimated by the method of Hagedorn and Jensen. Preliminary experiments showed that in this method fructose had the same reducing power as glucose. Glucose was estimated by difference.

Blood samples were collected by pricking an ear vein and allowing the blood to drip into a watch glass containing oxalate. The specimens were collected as quickly as possible and samples were immediately pipetted into the precipitating reagents. In the injection experiments the sugar solution was warmed to 37° and injected into the marginal ear vein opposite to that from which blood samples were taken. In the feeding experiments the sugar was at first fed by stomach tube but later it was found to be less disturbing to the animal to feed the sugar as a syrupy paste between two pieces of cabbage leaf.

The curves shown below are specimens which approximate closely to the average of the series and are not necessarily the "best" of the series.

RESULTS AND DISCUSSION.

1. *Fructose injection.*

Injected fructose was found to disappear from the blood stream at about the same rate as glucose (Fig. 1).

Fig. 1. 0.25 g. fructose injected at A.

When fructose alone was injected there was invariably found to be a slight fall (about 10 mg./100 ml.) in the blood glucose. The tables given by Corley [1929] show a similar slight fall in some cases but this is in contradiction to the findings of several other authors, notably Van Creveld and Ladenius [1928] and Harding *et al.* [1933] who found that the rise in total reducing sugar after fructose ingestion could not wholly be accounted for by the fructose present in the blood.

It is possible, and in view of other evidence distinctly probable, that fructose causes the secretion of insulin by the pancreas, and that this insulin is responsible for the fall in glucose (see section 3).

2. *Fructose ingestion.*

The effect of ingestion of 1.25 g. fructose was irregular. In some cases there was no rise in the total reducing sugar of the blood whilst in others there was a delayed rise. In a few cases a curve somewhat similar to that obtained by feeding glucose but with a much smaller rise in total sugar was obtained. This is in agreement with the work of Kimball [1932], Harding *et al.* [1933] and Corley [1929]. Such a rise in fructose was accompanied, as before, by a slight fall in glucose.

3. *Ingestion of glucose followed by injection of fructose.*

The hypothesis that fructose stimulates the secretion of insulin was tested by feeding glucose and injecting fructose 15 min. later while the glucose curve was still rising. The injection of fructose was found to convert the rise in glucose into an immediate fall and the glucose curve returned to the fasting level much more quickly than when glucose was ingested alone (Fig. 2).

Fig. 2. 1.25 g. glucose fed at A. 0.25 g. fructose injected at B.

The fructose disappeared at the same rate as when it alone was injected. Obviously the injection of fructose has caused an immediate acceleration of the disappearance of glucose from the blood and it is most probable that it has done so by stimulating the pancreas to produce insulin.

4. *Ingestion of glucose followed by injection of insulin.*

The glucose curve obtained in the previous experiment could be closely reproduced by feeding glucose and injecting insulin (intravenously) instead of fructose

(Fig. 3). The rise in the glucose curve was converted into an immediate fall and it appears exceedingly probable that the fall in the glucose curve in the previous experiment was due to insulin produced under the influence of fructose.

Fig. 3. 1.25 g. glucose fed at A. 0.8 unit insulin injected at B.

5. *Injection of fructose followed by injection of glucose.*

As a variation to previous experiments glucose was injected 4 min. after fructose had been injected. The extra glucose disappeared more rapidly from the blood and the glucose curve returned to the fasting level more rapidly than when glucose alone was injected (Fig. 4). The fructose curve was identical with those previously obtained. We can explain these results by assuming that the injection of fructose stimulated the secretion of a certain amount of insulin which acted on the glucose subsequently injected, causing it to disappear more rapidly than it would otherwise have done. The insulin however had no effect on the rate of removal of fructose from the blood.

This explanation is supported by the fact that the glucose curve shown in Fig. 4 was closely reproduced when insulin was injected along with glucose.

6. *Ingestion of glucose followed by injection of glucose.*

When glucose was injected after glucose had been fed the rise in the total reducing sugar was greater than when fructose was injected after the ingestion of glucose. The extra glucose disappeared rapidly from the blood stream (more rapidly than when glucose was injected or ingested alone) but the fall was less rapid and the return to the fasting level more delayed than when fructose injection followed the feeding of glucose (Fig. 5).

Fig. 4. 0.25 g. fructose injected at *A*. 0.25 g. glucose injected at *B*.

Fig. 5. 1.25 g. glucose fed at *A*. 0.25 g. glucose injected at *B*.

A possible explanation of the higher rise in the total reducing sugar obtained in this experiment as compared with the rise obtained when fructose injection followed glucose ingestion is that fructose is more effective than glucose in stimulating the pancreas to secrete insulin. In the absence of further evidence however it is impossible to arrive at any definite conclusion regarding the relative stimulating powers of the two sugars, since the one sugar stimulates insulin production but does not utilise the insulin so produced while the other both produces and utilises insulin.

7. *Injection of fructose and insulin.*

The intravenous injection of insulin (in doses up to 0.6 unit) simultaneously with fructose was found to produce no effect on the rate of disappearance of fructose from the blood (Fig. 6). This is in agreement with the results of Corley

Fig. 6. 0.25 g. fructose and 0.3 unit insulin injected at A.

[1929] who found that fructose could protect rabbits against insulin although the latter had no effect on the rate of removal of the fructose from the blood. Wierzechowski [1926] also states that insulin has no effect on the rate of removal of fructose.

Cori and Cori [1927, 1, 2; 1928] found that insulin affected the disposal of fructose and that surplus led to an increased oxidation although it did not influence the tolerance for intravenously injected fructose.

All these results are consistent with the hypothesis that the first stage in fructose metabolism is the conversion of fructose into glucose (or some similar substance) and that the rate of this conversion is independent of insulin, whilst the second stage is the disposal of the glucose (or similar substance) and is controlled by insulin.

The question of the metabolism of fructose is of course by no means settled by these experiments. They do indicate very strongly however that in all probability fructose is converted into glucose or some similar substance (*e.g.* hexosephosphoric acid) independently of insulin. It is likely that this conversion takes place in the liver in view of the known importance of the liver in fructose metabolism. The results also suggest that fructose stimulates the secretion of insulin although the insulin so produced can be required only in the second stage of fructose metabolism. The results appear to us to be of interest in connection with the known tolerance of diabetics for fructose since, if the pancreas is still capable of producing insulin at all, administration of fructose will (*a*) provide a sugar which is converted into glucose at a rate small enough for the deficient supply of insulin to deal with and (*b*) stimulate the flagging production of insulin.

SUMMARY.

1. Two methods for the estimation of fructose in small amounts of blood are described.

2. These methods have been used to investigate the fate of ingested and injected fructose in rabbits.

3. The results obtained suggest that fructose stimulates the pancreas to secrete insulin.

4. Insulin however has been found to have no effect on the rate of removal of fructose from the blood and it is therefore suggested that the metabolism of fructose takes place in two stages, (*a*) the conversion, independently of insulin, of fructose into an unknown substance which is most probably glucose, (*b*) the disposal of this substance under the influence of insulin.

The expenses of this work were defrayed by a grant from the Moray Fund of the University of Edinburgh to one of us (C. P. S.).

REFERENCES.

- Ashford (1933). *Biochem. J.* **27**, 903.
Bodansky (1923). *J. Biol. Chem.* **56**, 387.
Cori and Cori (1927, 1). *J. Biol. Chem.* **72**, 597.
—— — (1927, 2). *J. Biol. Chem.* **73**, 555.
—— — (1928). *J. Biol. Chem.* **76**, 755.
Corley (1929). *J. Biol. Chem.* **81**, 81.
Dickens and Greville (1932). *Biochem. J.* **26**, 1251.
Harding, Nicholson and Armstrong (1933). *Biochem. J.* **27**, 2035.
Isaac (1920). *Med. Klin.* **16**, 1207.
Kimball (1932). *Guy's Hospital Rep.* **82**, 157.

- MacLean and Smedley (1912). *J. Physiol.* **45**, 462.
Mann (1925, 1). *J. Amer. Med. Assoc.* **85**, 1472.
—— (1925, 2). *Amer. J. Physiol.* **74**, 238.
—— and Bollmann (1930). *Amer. J. Physiol.* **93**, 671.
Patterson (1935). *Biochem. J.* **29**, 1398.
Radt (1928). *Biochem. Z.* **198**, 195.
Steinberg (1927). *Pflüger's Arch.* **217**, 686.
Stewart and Gaddie (1934). *J. Physiol.* **80**, 457.
Stroebe (1932). *Z. klin. Med.* **119**, 564.
Van Creveld (1927). *Klin. Woch.* **6**, 697.
—— and Ladenius (1928). *Z. klin. Med.* **107**, 328.
Wierzuchowski (1926). *J. Biol. Chem.* **68**, 631.