CCLXXVII. CUTANEOUS BLOOD-SUGAR CURVES AFTER THE ADMINISTRATION OF FRUCTOSE, MANNOSE AND XYLOSE.

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THE oral administration of galactose to man results not only in the finding of galactose in the blood-stream but of an increased concentration of glucose (estimated as fermentable sugar) [Harding and Van Nostrand, 1929-30; Roe and Schwartzmann, 1932; Harding and Grant, 1932-33]. The increases in blood-glucose are variable in amount but can usually be found in arterial blood, if a sufficient number of samples be taken. In the fasting rabbit and the rat neither Corley [1927] nor Cori and Cori [1928] had found such increases, though Blanco [1928] reported them in the well-fed rabbit. The conditions and methods of these last three observations make them non-comparable with the findings on man. A previous meal of carbohydrate or fat, however, appears to cause no difference in the human results.

The oral administration of fructose in man is also presumably accompanied by increases in blood-glucose. Isaac [1920] first emphasised this possibility, pointing out that the increases in total blood-sugar during fructose tolerance tests by no means represent the amount of circulating ketose. Direct determination of blood-fructose by modifications of the Selivanoff reaction [Folin and Berglund, 1922] or by the Van Creveld reaction [Kronenberger and Radt, 1927; Radt, 1928] show that the amount of circulating fructose, after fructose ingestion, in normal man is very small, and that such amounts do not account for the observed increases in total blood-sugar. In the rabbit Corley [1929] observed the same discrepancy.

Are the increases in blood-glucose following the administration of galactose and fructose to man evidence of a general stimulation or irritation of a glucose-producing mechanism, due to the presence in the blood-stream and tissues of a large amount of a foreign sugar? Or do they represent the conversion of the entering sugar into glucose, either directly or through some intermediate? Harding and Grant [1932-33] concluded that blood-sugar studies after galactose administration gave evidence too limited and uncertain in character. To some extent the former view may be true, for Fishberg [1930] found the intravenous injection of galactose or xylose into the rabbit to produce a sharp increase in blood-fermentable sugar, though Corley's results with *l*-arabinose and *d*-xylose are less clear [1926; 1928]. Orally administered, neither pentose produces an increase in blood-glucose in the rabbit [Corley, 1926; 1928].

As both the amount of foreign sugar entering the blood-stream and its nature are likely to influence the result we have examined the blood-sugars in man after

the oral administration of varying doses of fructose, mannose and xylose. The use of *Proteus vulgaris* [Harding and Nicholson, 1933] forms an exact method for the separation of glucose from fructose or mannose. Glucose and xylose can be separated by the same method or by the action of baker's yeast. The methods allow of the use of a small amount of cutaneous blood and thus of the collection of a reasonable number of blood specimens. Whilst fructose tolerance tests in man have been frequently reported there are no records of the use of mannose and no blood-sugar observations after the oral administration of xylose to man. We have compared the results with those observed after 40 g. and 80 g. galactose by Harding and Grant [1932-33].

ANALYTICAL METHODS.

Separation of glucose from fructose or mannose in cutaneous blood.

0.5 cc. finger blood is laked in 8.5 cc. water in a centrifuge-tube, 0.5 cc. 10 % sodium tungstate and 0.5 cc. 0.66 *N* H₂SO₄ are successively added from accurate micro-burettes, the mixture well stirred, allowed to stand 10 mins. and centrifuged. The centrifugate is decanted and by means of a knife blade sufficient solid Na₂HPO₄ is added to adjust the *p*_H to 6.7-7.2. 3 cc. centrifugate are added to 0.25 g. wet-weight *Proteus vulgaris* [Harding and Nicholson, 1933] contained in a flat, high-efficiency tube belonging to the Lündgren high angle centrifuge and incubated with agitation for 30 mins. at 38°. The bacillus is separated in the centrifuge and the supernatant fluid decanted. The residual reducing substances are determined in 1 cc. of this fluid by mixing with 1 cc. of the sensitive copper reagent [Harding and Downs, 1933], heating for 10 mins. in a rapidly boiling water-bath, adding 1 cc. 1 % KI solution and 1 cc. *N* H₂SO₄, allowing to stand with occasional agitation for 3 mins. and titrating the excess iodine with 0.0025 *N* thiosulphate. At the same time, the total sugar is determined in 1 cc. of the original Folin-Wu centrifugate. The difference represents glucose. The value for the residual reducing substances in the fasting specimen of blood is subtracted from the values obtained after fructose or mannose administration. This gives the fructose or mannose value in cc. 0.0025 *N* thio-sulphate.

The method follows that used by Harding and Grant [1932-33] for the fractionation of glucose, galactose and residual reducing substances. It was shown that the residual reducing substances after removal of glucose and galactose in a Folin-Wu blood-filtrate remained very constant during galactose tolerance tests. The constancy of this non-sugar fraction has been assumed in the present work. As control, we have occasionally determined the total glucose-fructose fraction by using *Monilia krusei* to remove both sugars. The total as determined in this manner agrees satisfactorily with that obtained by the method just detailed. Added fructose is satisfactorily determined. It is absolutely essential to control the *p*_H of the Folin-Wu filtrate by the addition of Na₂HPO₄. The *p*_H of a Folin-Wu filtrate prepared with the usual larger quantities of venous blood is very constant and about 6.8. In these filtrates we never experienced any difficulty in separating glucose from fructose or mannose. The *p*_H of corresponding centrifugates from the small amounts of capillary blood is quite variable, sometimes being less than 6.0. The *Proteus* does not completely remove glucose under these conditions.

Separation of glucose and xylose in cutaneous blood.

The procedure detailed above can be used if desired, but owing to the difficulties connected with the use and lack of keeping qualities of *Proteus*, it is more convenient to use 0.1 g. wet-weight washed baker's yeast. This removes glucose quantitatively from the xylose.

The determination can also be made by allowing the yeast action on whole blood to precede the precipitation of proteins by the Herbert and Bourne technique. In one centrifuge-tube is placed 0.25 g. washed baker's yeast. To this and to a second centrifuge-tube are added 2.5 cc. of a solution containing 3 % sodium sulphate and 0.6 % sodium tungstate. 0.3 cc. of blood is placed in each tube and the contents well mixed. The yeast-containing tube is now incubated for 8 mins. at 38° and then to each is added 0.2 cc. 0.5 *N* H₂SO₄. The contents of both tubes are very thoroughly stirred, centrifuged and decanted, and the reducing value is determined on 1 cc. samples from each tube. The value of the yeast-containing tube represents xylose when a small correction (about 3 mg./100 cc.) is applied for non-sugar reducing material. The difference between the values of the two tubes is glucose.

Examination of urine for fructose or mannose.

The urine is diluted to an appropriate degree, usually 5 or 10 times, if negative to Benedict's reagent. The diluted urine is treated with H₂SO₄ and Lloyd's reagent [Harding and Selby, 1931]. The *p_H* of the filtrate is corrected to 7.0 by the addition of solid Na₃PO₄. 15 cc. of the above neutral filtrate are poured onto 0.75 g. wet-weight washed *P. vulgaris* and incubated for 30 mins. at 38° with stirring, 0.3 g. NaH₂PO₄ is added and the mixture centrifuged in the high angle centrifuge. 0.6 g. light calcined MgO is added to the centrifugate which is then shaken for 15 mins. The mixture is filtered and the filtrate adjusted to *p_H* 7.0 by a few drops of 1 : 2 H₂SO₄ from a very thin glass rod. The reducing power is determined on 1 cc. (as in the previous blood-filtrates) and represents the fructose or mannose *plus* the usual non-fermentable "sugar". 3 cc. of the filtrate from the above MgO treatment are poured onto 0.5 g. wet-weight *M. krusei* [Harding and Nicholson, 1933] in a centrifuge-tube, incubated for 30 mins. at 38° with stirring, centrifuged and the reducing power determined on 1 cc. of the centrifugate. The difference between this and the previous determination represents fructose or mannose. If only small amounts of known fructose or mannose are present baker's yeast can be substituted for *M. krusei*.

Determination of xylose in urine.

No specific method is available for xylose determination. Corley [1926] has used extra non-fermentable sugar after xylose administration as an index of excretion. McCance and Madders [1930] have estimated xylose in urine as extra pentose, or furfuraldehyde-yielding material. After xylose administration to man, the amount appearing in the urine is usually sufficient to give a positive Benedict's test. The urine thus requires a high dilution, and it is sufficiently accurate to term xylose any non-fermentable "sugar" left after treatment with yeast.

The factors requisite for the conversion of cc. 0.005 *N* thiosulphate to fructose, mannose or xylose can be found in Table I of Harding and Downs [1933].

EXPERIMENTAL.

Normal male subjects fasted from 6.0 p.m. to the next morning. The bladder was emptied at 9.0 a.m. The desired amount of sugar ~~was dissolved in~~ 200 ml

400 cc. water was taken. Finger blood-samples were drawn every 15 mins. during the first hour, and at 30 min. intervals during the second hour. Urine was collected at 11.0 a.m. The sugars were bought as pure, but the xylose was found to contain a small amount of reducing substance removable by yeast.

RESULTS AND DISCUSSION.

Fructose. The amounts of fructose appearing in the arterial blood after ingestion of 50 g. are very small. The small amounts, determined by the use of micro-organisms as a method of analysis, agree in general with those obtained by colorimetric chemical methods (Fig. 1). The main increases which occur in

Fig. 1. Showing glucose and fructose in cutaneous blood after oral administration of 50 g. fructose.

the total blood-sugar are due to glucose. Similar, though perhaps not so large, increases are found after the ingestion of 25 g. fructose (not shown). Small amounts of fructose were also found in the urine, varying from 2 to 54 mg. in the two-hour period. These results support the supposition of Harding and Selby [1931] that part of the fermentable sugar of a daily urine can be fructose, arising from its ingestion in the form of invert sugar in fruits, honey, etc.

Mannose. The blood analyses after ingestion of either 25 or 50 g. mannose are devoid of positive findings (Fig. 2). Neither the glucose nor the mannose fraction showed variations outside the biological or experimental variation. Of the so-called fermentable sugars, mannose shows the lowest rate of absorption. Using Cori's [1925] figures on the rat as a general guide, mannose is absorbed from the intestine at slightly less than one-half the rate of fructose, yet this cannot be advanced as the entire reason for our failure to demonstrate its presence in the blood-stream. Some absorption unquestionably took place, for its presence in small amounts was readily shown in the urine. Moreover, the presence of xylose, which in the same series possesses only a very slightly inferior absorption rate, can readily be shown in arterial blood after its oral ingestion. The rate of utilisation of mannose must equal or exceed its rate of absorption. The few mg. which escape into the urine provide evidence for the

practical non-existence of a renal threshold for mannose. From our results on fructose the renal threshold for this sugar must also be non-existent or extremely low [Folin and Berglund, 1922]. The renal threshold for galactose appears to be equally low [Folin and Berglund, 1922; Harding and Van Nostrand, 1929-30; Harding and Grant, 1932-33], and glucose thus seems to be the only utilisable sugar which shows the interesting phenomenon of a high renal threshold.

Xylose. This sugar is not utilised by the mammalian organism. True, coefficients of utilisation have been calculated from time to time on this and other pentoses, but the use of the term utilisation is based upon a lack of complete recovery of an administered amount, rather than on definite proof that the sugar

Fig. 2. Showing the lack of rise of blood-glucose after oral administration of mannose. Mannose was not detected in the blood. Subjects Ag and Gt received 25 g. mannose; subjects Ad and Nn 50 g.

takes an active part in daily metabolism. In recent studies Miller and Lewis [1932] have been unable to demonstrate satisfactory glycogen formation in the rat. Very active tissue, such as tumour tissue [Russell, 1923] or embryonic kidney tissue [Watchorn and Holmes, 1931], causes the disappearance of xylose from a nutrient solution, coincidentally with an increase in area of the growth, but the mechanism of the utilisation of xylose evidently differs from that accompanying the similar disappearance of glucose, fructose and galactose. The loss of xylose from the nutrient solution is not accompanied by any diminution of production of urea and ammonia as is the case invariably with glucose or fructose, and irregularly with galactose [Watchorn and Holmes, 1931].

The curve of blood-xylose after the oral administration of 50 or 25 g. differs from that of the other sugars. Large amounts are present, and at the end of two hours there is only a slight diminution in its concentration in three of the four experiments. Even after ingestion of only 10 g. the curve of blood-xylose has not reached zero level in two hours (Fig. 3). The contrast between the blood-sugar curves of this sugar and galactose (where the zero level is reached in two hours after ingestion of 40 g.) emphasises their different metabolic behaviour. Galactose belongs to the class of utilisable sugars, a fact which is now being more widely recognised than formerly.

Despite the large amounts of xylose in the blood-stream there is no marked rise in blood-glucose. The amounts of non-glucose sugar are larger than after either galactose or fructose. Only after ingestion of 50 g. of xylose is there, in one subject, any increase in blood-glucose which might be legitimately reckoned as outside the normal fasting variation. The presence of a considerable quantity of foreign sugar in the blood-stream evidently need not produce a hyperglycaemia. These results on man agree with those of Corley on the rabbit. The amounts of xylose found in the urine are comparable with those noted by McCance and Madders [1930].

Fig. 3. Showing glucose and xylose in cutaneous blood after oral ingestion of xylose. Subjects Jn and Ds received 50 g. xylose; subjects Ag and Nn 25 g.; subjects Oc and Wd 10 g.

Galactose. Figs. 1 and 2 in the previous paper of Harding and Grant [1932-33] show the average curves of blood-galactose and glucose after the ingestion of 40 and 80 g. galactose by man.

What significance must be attached to the increase in blood-glucose occurring after the oral administration of galactose and fructose? Harding and Van Nostrand, and Roe and Schwartzmann, attributed it to direct conversion of galactose into glucose. Harding and Grant felt, however, that the increases were too variable and too independent of the amount of galactose to have their origin in a direct simple interconversion of the sugars. Yet, as we have just seen, the presence of similar amounts of foreign sugar in the shape of xylose will not produce a corresponding hyperglycaemia. Galactose and fructose are apparently a class apart, and produce a hyperglycaemia as a result of some specific action.

The most rational explanation to adopt at present seems to be to regard the hyperglycaemia as a result of the production of some precursor of glucose. Fructose has been known to produce lactic acid on ingestion in man [Campbell and Maltby, 1928] and galactose has recently been shown to give rise to lactic acid in dogs [Wierzuchowsky and Laniewski, 1931]. Whether the production of lactic acid from these two sugars reflects the primary path of metabolism or a secondary one is immaterial. Any large production would give rise to a temporary hyperglycaemia. Mannose is perhaps too slowly absorbed to give rise to a large amount of any glucose-former. Xylose, as a non-utilisable sugar, would possess no such action.

While such a view may harmonise the facts as known after the oral ingestion of non-glucose sugars, it would seem necessary to assume that extremely large amounts, such as are temporarily introduced into the blood-stream by intravenous injection, can give rise to hyperglycaemia, independently of metabolic change, as suggested by Fishberg. The one subject of our xylose series who showed a very slight hyperglycaemia after ingestion of 50 g. may have been at the commencement of such a change. The high dosage, however, produced a strong diarrhoea and precluded further experiments.

SUMMARY.

1. The oral administration of fructose to man produces an increase in blood-glucose. This is similar to the behaviour of galactose. Small concentrations of blood-fructose were also measured.

2. The micro-organism method of analysis shows small amounts of urinary fructose after fructose feeding.

3. It was not possible to demonstrate either the presence of mannose in the blood-stream or a hyperglycaemia after the oral administration of mannose. The slow rate of absorption of this sugar, coupled with a rapid utilisation, may account for these results.

4. Mannose was detected in the urine after ingestion of mannose.

5. The oral administration of xylose results in large amounts of circulating xylose in the blood. The rate of removal of xylose from the blood must be small, as easily measurable amounts are found at the end of two hours even after the ingestion of only 10 g.

6. Large amounts of xylose are found in the urine after ingestion of xylose.

7. It is suggested that the hyperglycaemias following the ingestion of fructose and galactose are the results of the production of a glucose-precursor. Any foreign sugar, however, if its blood concentration is sufficiently great, may produce a hyperglycaemia.

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