LXXXII. THE METABOLISM OF CARBOHYDRATES. PART II.

ON THE POSSIBLE OCCURRENCE OF STEREOCHEMICAL CHANGES IN EQUILIBRATED SOLUTIONS OF REDUCING SUGARS INTRODUCED INTO THE CIRCULATION.

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IN view of the existence of stereoisomerides of glucose [Fischer 1914, Irvine, Fyffe and Hogg 1915], fructose [Irvine and Robertson 1916] and galactose [Hudson 1915] much more reactive than the crystalline α - or β -modifications, and the recognition of the formation of certain of these substances under the influence of the living intact intestinal wall either prior to or during absorption [Hewitt and Pryde 1920], it became a matter of interest to determine whether any similar isomeric change occurred in these hexoses when introduced into the circulation by routes other than the alimentary canal¹. It was thought that by investigating this point information might be obtained on such questions as the condition of glucose in the blood and on the function of γ -sugars in carbohydrate metabolism. It may be presumed on *a priori* grounds that a structural change of the nature referred to has some fundamental reason and the relation of glucose to glycogen has therefore also to be considered.

The scheme of research formulated included:—(1) Observations on the stereochemical condition of certain hexoses as soon as possible after removal from the circulating blood of anaesthetised, but otherwise normal animals; (2) similar observations on the condition of glucose formed *in vivo* by hydrolysis of glycogen. It is the first part of the work which is now communicated.

In view of the rapidity of the change from the γ - form to the equilibrium mixture of α - and β -glucoses, and further owing to the necessity for avoiding as far as possible chemical manipulations, the only method suitable for the purpose seemed to be the removal of the sugar from the blood by the animal's own kidneys and its collection in the urine secreted. By simple filtration, material could be obtained sufficiently clear for the determination of optical activity in a 2 dm. tube if amount permitted, or otherwise in a 1 dm. tube. The general technique consisted in intravenous injection into rabbits or dogs

¹ It may be recalled that the last authors could find no evidence for assuming such a change to take place in glucose introduced into the peritoneal cavity.

of glucose, fructose or galactose, collection of the urine secreted in the following five minutes and examination of its optical behaviour. In each case the constant optical value was subsequently determined after addition of a trace of caustic alkali. As further controls the reducing powers of the same samples were obtained by Bertrand's method and from the same material also the phenylosazones were isolated, their crystalline appearances examined and, when any doubt existed, the melting point recorded, alone and when mixed with an osazone prepared from pure specimens of the sugar in question. Since anaesthesia is commonly considered to cause glucosuria, as a preliminary to most experiments observations were made on the amount of reducing substances in the urine prior to the injection of diuretics. It may be said that without induced diuresis the amount of sugar or other reducing substance found in the urine as the result of ether anaesthesia in the rabbits used was so small as to be considered negligible. Urea, sodium chloride and sodium sulphate have been employed as diuretics. Of the three the last has with rabbits given by far the best results. The diuresis is rapid and sufficiently prolonged to enable an adequate quantity of urine to be collected in the arbitrary time chosen, five minutes. The urine secreted under the action of these diuretics invariably contained reducing substances, usually in small amount, and these were submitted to the same examination as the urine after injection of sugar, that is optical activity, reducing power and the isolation of the osazone. This investigation is the second part of the research and will be communicated subsequently. We do not propose to discuss this point, but we may note that the observations made show, with some certainty, that reducing substances other than glucose are excreted under the action of diuretics. Some of these substances have a lower *initial* specific rotation than that accepted in the literature for glucose, and the quantitative estimations by polarimetry and by reduction methods frequently yield conflicting results. A certain amount of evidence is in our possession to enable the conclusion to be drawn tentatively that in the rapid excretion of glucose, formed presumably by hydrolysis of glycogen, part, if not all, of the glucose excreted has a much lower optical value than either of the butylene oxide modifications. The evidence does not permit us to say that the ethylene oxide modification is formed, though the optical behaviour is significant. The definite establishment of this fact would lead to important conclusions as to the constitution of glycogen. The work is being continued.

Under the influence of concentrated sugar solutions intravenously injected temporary diuresis is produced and the administered sugar is excreted rapidly and in great part into the urine. According to Croftan [1909], but denied by Grube [1909], part is excreted into the intestine and subsequently reabsorbed into the circulation. In view of the previous work quoted above, this point may be of considerable importance. We became aware of these communications, however, after the completion of the experiments recorded below, but it is thought that, under the conditions of the present research, even if the

results of Croftan are accepted, only an inappreciable amount would have traversed this route in the time between the injection and the excretion of the sugar. Any sugar which took this route would presumably undergo stereochemical changes. We think that a complication from this possibility is remote. We assume therefore that the reducing sugar of the urines examined is composed of (a) hexose injected, (b) sugar formed from glycogen under the influence of the diuretic.

As subjects dogs and rabbits were employed, the latter in by far the larger number. The sugars investigated were *d*-glucose, *d*-fructose and *d*-galactose. The glucose and fructose were the same as used by Hewitt and Pryde in the research quoted and gave permanent optical values corresponding with those accepted as standard; the galactose was not so pure and gave in 5.6% solution $[\alpha]_D^{15} + 77^\circ$. The Bertrand solutions were standardised against pure glucose and gave results within the limits of experimental error. Copper values for fructose were taken from Bertrand's tables for invert sugar. The general technique has been indicated above. The sodium sulphate and sodium chloride were 20 % solutions in tap water and the quantity injected at a time usually was 5 cc. of the former or 10 cc. of the latter. The sugar solutions were in the cases of glucose and fructose 10 %, galactose 20 %, and were made up in tap water at least 24 hours before required to ensure that equilibrium was attained and a constant optical value reached. In rabbits 10 cc. were usually injected at once, occasionally 20 cc. Polarimetric observations were made immediately and, when a constant value was reached, the solution was treated with a trace of caustic alkali, filtered and again examined. Subsequently the phenylosazone was isolated. In view of the similarity of the results obtained with all three sugars it is proposed to give in detail only one typical experiment in which glucose was employed.

23. ix. 21. *Rabbit, male, weight 2 kilos, ether anaesthesia.*

Cannulae in right jugular vein and in bladder. Neck of bladder ligatured. Bladder washed out with warm 0.9 % NaCl.

11.25 a.m.	5 cc. 20 % Na_2SO_4 in tap water injected.
11.25–11.33 a.m.	Urine 5 cc. Labelled No. 1. Freely reducing.
11.33 a.m.	5 cc. 20 % Na_2SO_4 in tap water injected.
11.33–11.38 a.m.	Urine 11.5 cc. Polarimeter specimen <i>A</i> .
11.38–11.58 „	„ 14.0 cc.
12.3 p.m.	10 cc. 10 % glucose injected.
12.6 „	„ „ „
12.3–12.8 p.m.	Urine 8.3 cc. Polarimeter specimen <i>B</i> .
12.8–12.32 p.m.	„ 14.0 cc. Labelled No. 2.
12.45 p.m.	Animal died.

ANALYTICAL NOTES.

Polarimeter *A*. 1 dcm. tube, sodium light, examined at once, $\frac{1}{2}$ minute readings.

11.42 a.m.	0.08°	0.17°	0.17°	0.17°	0.20°	0.19°	0.20°
	0.19°	0.23°	0.23°	0.25°	0.30°	0.33°	0.27°
	0.33°	0.33°	0.30°	0.30°	0.32°	0.30°	0.32°.

Average final rotation 0.31°

Reducing material, estimated as glucose 0.59 %

After addition of a trace of alkali and filtration, mean of nine concordant readings:—

Permanent rotation 0.25°

Reducing material, estimated as glucose 0.47 %

Bertrand estimation gave concentration, estimated as glucose ... 0.36 %

Polarimeter *B*. 1 dcm. tube, sodium light, examined at once, $\frac{1}{2}$ minute readings. (Readings not so sharp as in *A* above.)

12.23 p.m.	1.32°	1.28°	1.20°	1.18°	1.18°	1.20°	1.23°
	1.28°	1.28°	1.27°	1.27°	1.30°	1.30°	1.28°
	1.27°	1.28°	1.27°	1.27°	1.32°		

Average rotation 1.26°

Concentration, estimated as glucose 2.40 %

After addition of a trace of alkali, filtering and making up to twice original volume, mean of nine concordant readings:—

Permanent rotation 0.58°

Concentration, estimated as glucose in undiluted urine 2.22 %

Bertrand estimation gave concentration for undiluted urine, estimated as glucose 2.18 %

Characteristic phenylosazones isolated from Nos. 1 and 2.

The one experiment given in detail above is typical of the results obtained generally; it shows a considerably better agreement between polarimetric and reduction estimations of "glucose" excreted under the influence of the diuretic than was sometimes found; with this proviso the figures are representative and the following can be noted:—

- In *A*. (1) The slight but marked mutarotation from a low to a permanent higher optical value.
 (2) A large change in observed rotation on addition of alkali.
 (3) The variation in amount of reducing substance (calculated as glucose only) determined by optical and reduction methods.

- In *B*. (4) The absence of any evidence of mutarotation.
 (5) The small change in observed rotation on addition of alkali.
 (6) The relatively good agreement between polarimetric and reduction methods of estimation.

It is probably legitimate to assume that the factors causing the variations noted in (2) are due to injection of diuretic and would thus continue to be operative in (5), and it is seen that the absolute percentage difference in (2) and (5) is, within experimental error, the same, viz. 0.12 % and 0.18 % (estimated as glucose). If this assumption is granted, it must be concluded that the glucose (or other reducing sugar) excreted in these experiments is an equilibrated mixture of the α - and β -isomerides and that no stereochemical changes of the nature found by Hewitt and Pryde have occurred. Although no explanation can as yet be given of the discrepancies in the estimations of reducing substances under the action of diuretics it can be stated that errors are not introduced by substances interfering with the accuracy of the estimations since glucose added to such urines can be recovered quantitatively. It may also be concluded that polarimetric estimation of glucose in urine, uncontrolled by other methods, may lead to fallacious results.

SUMMARY.

1. Equilibrated solutions of *d*-glucose, *d*-fructose and *d*-galactose on injection intravenously into rabbits or dogs undergo no stereochemical changes and the equilibrium is unaltered in the urine excreted.

2. Polarimetric estimation of reducing sugar in urine may give fallacious results unless controlled by other methods.

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