XXXIII. THE METABOLISM OF CARBOHYDRATES. PART I.

STEREOCHEMICAL CHANGES UNDERGONE BY EQUILIBRATED SOLUTIONS OF REDUCING SUGARS IN THE ALIMENTARY CANAL AND IN THE PERITONEAL CAVITY.

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THE following communication describes a number of abnormal and unexpected results encountered in an experimental study of the rates of absorption of simple carbohydrates by living tissues. As originally formulated, the scheme of research included the determination of the relative rates of absorption of simple reducing sugars such as glucose, and also of closely related derivatives such as the synthetic alkyl glucosides. The opportunity was open to use in these experiments a number of carbohydrates of exceptional purity, and it was also hoped that the influence of configuration on the rate of absorption would be determined by the employment of definite α - and β -derivatives of glucose and by the substitution of galactose and mannose for glucose in such derivatives.

At the same time it was recognised that all problems connected with the reactions of sugars are much affected by the recent recognition of the specially reactive type of which the so-called " γ -glucose" is a representative, and as the study of " γ " sugars is comparatively recent, it may be advisable to review briefly the essential facts regarding their structure.

The expressions α and β are normally applied to the crystalline varieties of glucose which display downward and upward mutarotation respectively, and the following structural formulae (1) and (2) which represent the compounds as butylene oxides are applied to them:

Formulae corresponding with (1) and (2) represent the isomeric crystalline methyl glucosides, but in 1914 Fischer [1914] described a third methyl glucoside which Irvine, Fyffe and Hogg [1915], who also encountered the compound simultaneously, were able to characterise as a mixture of two methyl glucosides wherein the ring is present as in an ethylene oxide.

Following Fischer's nomenclature the parent sugar was thus provisionally described as " γ -glucose" and evidence is available to show that this variety of the sugar also exists in two isomeric forms (3) and (4) which are presumably mutually related as the α - and β -glucoses.

This capacity of reducing sugars to form numerous isomerides according to the position assumed by the internal oxygen linkage is far from uncommon and what is said above seems to be of general application.

The most outstanding feature of this newly-described type of sugar is the speed and range of the reactions it undergoes. For example condensation with an alcohol to form the corresponding glucoside takes place in a few minutes and the similar reaction with acetone is practically instantaneous. By comparison, the normal forms of reducing sugars are relatively stable, inert substances and the possibility thus suggests itself that in the rapid transformations of carbohydrate in living tissues γ -sugars may play an important, if not an essential, part in both animal and vegetable metabolic processes.

In commencing the present work this possibility was not overlooked and consequently in the experiments afterwards described the amount of sugar introduced into and returned from the intestine, peritoneal cavity, etc. was estimated not only volumetrically but also polarimetrically. In this way it was possible both to determine the amount of sugar present in any solution and also to ascertain the stereochemical condition of the sugar.

It may at once be said that the original scheme of the research was realised in so far that accurate comparative measurements have been made of the rates of absorption of glucose and fructose, but this section of the work has been suspended in view of the unexpected results which indicate, with some precision, that, in contact with certain living tissues ordinary glucose (consisting of the α and β forms in equilibrium) is converted into the " γ " form in amount sufficient to be detected polarimetrically.

Incidentally it may be pointed out that quantitative estimation of glucose by reduction of cupric or other metal salts affords no indication of the presence in solution of γ -glucose. To detect this sugar use must be made either of the polarimeter or of qualitative tests involving the decolorisation of dilute solutions of KMnO_4 or of methylene blue [Armstrong and Hilditch, 1919]. These two colour tests have certain disadvantages when employed in experiments of the nature carried out in this work, as it is found that a dilute salt solution isotonic with mammalian tissues acquires after contact with these tissues the capacity to decolorise dilute KMnO_4 solutions, owing presumably to traces of organic matter. The reduction of methylene blue by living tissues is well known.

As experimental animals rabbits mostly have been used; the general results have been confirmed on rats.

The routine methods of investigation employed were:

(a) *Intestine*. The animal being fully anaesthetised¹, a convenient loop of intestine was isolated, ligatured where desired and two glass cannulae inserted. The loop was then thoroughly washed with Ringer-Tyrode's solution [Bayliss, 1915] made up without glucose, until the washings were clear. To facilitate this process it was found advisable to starve the animal for 24 hours before the experiment. The sugar solution was then run in and retained in the intestine for the length of time desired, usually five minutes. As a rule the abdomen was then temporarily closed by clips or forceps. The blood and nerve supply to the loop were kept intact and all solutions used were at body temperature.

In view of the extreme fragility of the intestine of the rabbit it is essential that it be handled as little as possible, and further that it be not abnormally distended.

When the specified time had elapsed the loop was emptied and washed out with salt solution two or three times; the united solutions were then filtered, made up to known volume with distilled water and examined polarimetrically as soon as possible.

The following parts of the alimentary canal have been investigated: mouth (human), duodenum, small intestine (all parts) and colon.

(b) *Peritoneal cavity*. In this case the sugar solutions were introduced by means of a trochar cannula and were allowed to remain in for varying periods up to 30 minutes. As it is impossible to recover all the solution run in, an amount sufficient only for polarimetric observations was withdrawn and filtered. The sugar solutions were examined as recovered and were not diluted.

Experiments have been carried out with *d*-glucose, *d*-fructose and *d*-galactose. In view however of difficulties experienced with the two latter sugars the large majority of the observations have been made with glucose. In all cases the solutions of the sugars employed were allowed to remain at the temperature of the room until the observed rotation attained a constant value. In each case this corresponded with the permanent specific rotations accepted as standard in the literature. An indication of the state of purity of the materials used may be obtained by referring to the figures given below in the experimental part for the initial rotations of the glucose and fructose in aqueous solution.

Examination of the glucose solutions after contact for five minutes with the mucous membrane of the living intestine showed that disturbance of equilibrium had taken place; initial readings corresponding with a specific

¹ All the animal experiments were carried out by one of us (J. A. H.). The remainder of the work was in greater part done by the other. At the conclusion of each animal experiment the rabbit or rat was destroyed by an overdose of anaesthetic.

rotation much lower than $+52.5^\circ$ were obtained and the solutions then underwent upward mutarotation to a value corresponding with that of *d*-glucose in equilibrium.

As the original solution contained the α - and β -glucoses in equilibrium and as the former is highly dextrorotatory and the latter feebly so, the most obvious explanation is that the α form had been preferentially absorbed thus leaving excess of the β -isomeride in solution.

The range of mutarotation of pure β -glucose is from $+19^\circ$ to $+52.5^\circ$, and the true initial value cannot be observed by direct experiment but is obtained by extrapolation. It follows then that even were all the α form completely absorbed and the β form unabsorbed, an initial reading corresponding with a specific rotation of $+19^\circ$ could not be obtained owing to mutarotation commencing immediately the solution was withdrawn from contact with the absorbing tissue. As a certain length of time must necessarily elapse before observations can be made and as solutions on removal from intestine or peritoneal cavity cannot be used for polarimeter work without being filtered, attention was directed to minimising the time taken for this process in order to observe the mutarotation from as early a stage as possible. Even in the first experiments where no particular attention was paid to rapidity of work, extrapolation of the curves in which the specific rotations were plotted against time, indicated that mutarotation commenced from an initial specific rotation less than $+19^\circ$. Considerable difficulty was experienced in filtering the solutions rapidly owing presumably to traces of colloidal substances secreted during the experiments.

When the unavoidable delay in observing the optical changes had been reduced as far as possible, readings were obtained on the average seven minutes after removal from the intestine. On one occasion the first reading was taken in four minutes. Under these conditions extrapolation of the curves obtained indicated that mutarotation commenced at a value which was distinctly laevo. (In all cases zero time was taken as the mean of the times of commencing and finishing removal of the sugar solution from contact with the living tissue.) In the great majority of cases also, the first reading obtained corresponded with a specific rotation much too close to $+19^\circ$ to be due to the presence of β -glucose alone. Not only so, but on one occasion in which glucose was used a laevo initial value was recorded, and on several occasions the initial specific rotations actually obtained lay between 0° and $+19^\circ$.

It is clear therefore that the upward mutarotation observed on removal from the intestine cannot solely be due to a preferential absorption of α -glucose with the subsequent establishment of equilibrium *in vitro*.

One deduction from the experimental results described is that, in contact with certain living tissues, glucose solutions must undergo a downward mutarotation, and if the above explanation be accepted, this change is due in part at least to the transient formation of γ -glucose. This is supported by the additional observation that $N/100 \text{ KMnO}_4$ in neutral solution is de-

coloured appreciably more rapidly by glucose after contact with the living intestine, than by either glucose of the same concentration in aqueous solution, or by washings from the intestine, and further that the absorption of glucose takes place in the peritoneal cavity without any stereochemical changes being manifest.

A point of considerable importance is that the sugar solutions on withdrawal from the intestine are of necessity alkaline, whilst on the other hand it would appear that faintly acid conditions are most favourable to the formation of γ -sugars. The possibility cannot be excluded however, that this particular type may be formed in feebly alkaline media so long as the alkalinity is lower than that found necessary by Lobry de Bruyn to promote the aldose $\rightleftharpoons$ ketose transformation.

During the course of each experiment the sugar solutions employed were inevitably contaminated both with intestinal secretions and also with Ringer-Tyrode's solution. It became a matter of importance therefore to determine the effect, if any, of these substances on a glucose solution in equilibrium and on the rate of mutarotation of a freshly prepared solution of glucose. Further as the experiments were carried out at body-temperature (37°), and as the rotations were taken at 11° – 15° , the effect of this alteration in temperature must be considered.

It has not been found possible to effect an alteration in specific rotation of a glucose solution in equilibrium either by the addition of Ringer-Tyrode's solution, of extracts of intestinal mucous membrane or by Ringer-Tyrode washings from the intestine. While the final equilibrium of a glucose solution is not disturbed by these substances the speed of mutarotation of a freshly prepared solution of *d*-glucose is very appreciably increased. The final permanent value obtained is the same as that of α -glucose dissolved in distilled water.

Temperature variations of from 22° – 26° may, in the case of glucose, be ignored [v. Lippmann, 1904]. Even in the case of fructose, which is notoriously sensitive in this respect, only at 87.3° does the specific rotation reach the value of -52.5° [Armstrong, 1919], and it is considered that the changes in optical activity obtained with this sugar cannot wholly be due to temperature variations. Pending further work with a constant temperature apparatus, it is not proposed to discuss either fructose or galactose in greater detail.

The fact that the final permanent specific rotation attained after removal from the intestine is the same as that given by the sugar before being introduced precludes also any possibility of these alterations in rotatory power being due to formation of disaccharides or other sugar complexes. As an additional check the concentration of glucose as estimated from the optical value was verified volumetrically by Benedict's method. Close agreements were found in each case.

It should be mentioned that in some of the experiments in which glucose solutions were employed the mutarotation effects were ill-defined and in

some cases were completely negative; it is thus advisable to describe as closely as possible the conditions adopted when positive results were obtained.

In experiments on the absorption of glucose by the small intestine, solutions have been employed hyper-, iso- and hypo-tonic to mammalian tissues. In hypo-tonic solutions only have the changes been as described. In solutions of higher concentration the range of mutarotation *in vitro* has not been so great. In the large intestine also, with dilute solutions of glucose the results obtained were in no way so well-marked as in the case of glucose of similar concentration in the small intestine.

Examination of glucose solutions retained intra-peritoneally for 15–30 minutes showed that the equilibrium of the various isomerides was not disturbed.

After being retained in the mouth (human) for five minutes polarimetric evidence was obtained in the case of glucose that stereoisomeric changes had taken place. Mutarotation was then observed similar to, but much less well-marked than, that noted with dilute solutions of glucose in the small intestine.

EXPERIMENTAL.

The general methods adopted in these experiments have been described above.

In connection with the difficulty experienced at times in obtaining solutions sufficiently clear for polarimetric observations it may be said that after many trials of various types of filter papers, shaking with silica and centrifuging, etc. the most satisfactory procedure was found in filtering directly into a graduated flask, under very slight suction, through a "filter-hat" manufactured by Messrs W. and R. Balston, Ltd. These filter-hats are thick and spongy in texture and, provided they are well washed with water before use, yield, in the great majority of cases, solutions clear enough for accurate readings in a 2 dm. tube with sodium light.

In certain very few cases, even after careful filtration, the solution was not sufficiently clear for sodium light to be used and recourse had to be made either to incandescent gas flame or to the electric arc. As a routine proceeding all three sources of light were available by rotating the polarimeter on the table to the light desired. Monochromatic sodium light was always tried first and then in order the incandescent flame and the arc. In the latter case light filters for yellow light were employed [Landolt, 1898].

Polarimetric examination of the glucose and fructose employed in these experiments gave the following initial specific rotations three minutes after commencement of solution of the sugar:

$$d\text{-glucose, } 2.868 \% [\alpha]_D^{12^\circ} = + 103.4^\circ$$

$$d\text{-fructose, } 3.215 \% [\alpha]_D^{12^\circ} = - 107.8^\circ.$$

At the end of $1\frac{1}{2}$ hours the fructose had not attained equilibrium, and after six hours the glucose gave readings corresponding with a specific rotation

On the graph given below (Fig. 1), are plotted:

I.	The velocity of mutarotation of <i>d</i> -glucose in aqueous solution.
II.	" " " filtered intestinal washings.
III.	" " " Ringer-Tyrode's solution.

Specific rotations

Time in minutes

Permanent specific rotation of α -glucose

I

II

III

Fig. 1.

- I. Glucose 2.907 % in aqueous solution.
II. „ 2.924 % in filtered intestinal washings.
III. „ 2.887 % in Ringer-Tyrode's solution.

(a) *d*-Glucose, hypo-tonic aqueous solutions; 1.571 % and 2.120 %.

As stated above, the final readings before and after the addition of the trace of alkali gave a concentration of sugar which agreed remarkably well with the concentration as determined from volumetric estimations. The

Benedict's solution employed was previously standardised against the sugars used.

(b) *d*-Glucose, iso- and hyper-tonic aqueous solution, 5.258 % and 8.040 %.

Regarding these it can be said that evidences of disturbance of equilibrium are shown, but are slight. In no case has a reading indicating a specific rotation below $+40^\circ$ been obtained under similar conditions to those already described above.

Fig. 2. *d*-Glucose.

In this and in all subsequent graphs (except no. 5) the values plotted represent the average of at least two readings taken in as short a time as possible. Readings were continuous on the part of one author, verified as a rule at intervals by the other. Below each graph are given the rotations from which the specific rotations were calculated. All rotations are dextro unless otherwise stated.

Observed rotations

-0.017° , 0.156° , 0.166° , 0.213° , 0.247° , 0.315° , 0.383° , 0.425° , 0.468° , 0.468° , 0.500° , 0.485° , 0.500° , 0.500° , 0.485° , 0.490° , 0.520° , 0.500° .

After addition of a trace of alkali. Mean of nine readings,

0.483° .

(c) *d*-Fructose, hypo-tonic aqueous solution, 2.01 %.

One graph is reproduced (Fig. 5). Other experiments have shown, in the initial observations, greater deviation from the equilibrium rotation, but the tracings are complicated by what appears to be the existence of two simultaneous reactions.

(d) *d*-Galactose, hypo-tonic aqueous solution, 2.317 %.

Evidence has been obtained that the specific rotation of this sugar in equilibrium is disturbed through contact with living intestine. The alteration in rotation observed is, however, small.

Fig. 3. *d*-Glucose.

Observed rotations

0.083°, 0.150°, 0.250°, 0.217°, 0.342°, 0.350°, 0.283°, 0.325°, 0.367°, 0.333°, 0.333°, 0.342°, 0.325°, 0.333°, 0.325°, 0.325°.

After addition of a trace of alkali. Mean of seven readings, 0.325°.

2. Large intestine.

In the rabbit with a 3 % solution of glucose, an initial specific rotation of +43.03° has been obtained nine minutes after removal from the intestine. After 14 minutes the value +52.31° was found. In experiments where the normal activity of the intestine was impaired, either by killing the animal previously or by interrupting the blood supply to the observed loop, no alteration of rotation was detected.

3. Mouth (human).

Dilute glucose solutions on being examined after being retained in the mouth for five minutes show upward mutarotation. The range of the mutarotation is small.

Fig. 4. *d*-Glucose.*Observed rotations*

0.033°, 0.083°, 0.117°, 0.150°, 0.167°, 0.167°, 0.183°, 0.167°, 0.167°, 0.167°, 0.150°, 0.167°, 0.183°, 0.183°, 0.183°, 0.167°, 0.167°, 0.183°, 0.167°, 0.183°, 0.183°, 0.183°, 0.183°, 0.167°, 0.183°, 0.200°, 0.183°, 0.167°, 0.183°, 0.200°, 0.183°, 0.183°, 0.167°, 0.183°.

After addition of a trace of alkali. Mean of 10 readings, 0.180°.

Fig. 5. *d*-Fructose.*Observed rotations*

-0.070°, -0.066°, -0.750°, -0.750°, -0.833°, -0.783°, -0.833°, -0.816°, -0.833°, -0.850°, -0.833°, -0.816°, -0.866°, -0.833°, -0.850°, -0.800°, -0.833°, -0.833°, -0.850°, -0.833°, -0.833°, -0.816°, -0.816°, -0.800°, -0.833°, -0.850°, -0.816°, -0.833°, -0.833°, -0.850°, -0.850°, -0.816°, -0.833°.

After addition of a trace of alkali. Mean of 14 readings, -0.831°.

Considerable difficulty was experienced in filtering the solutions owing to admixture of saliva.

4. *Peritoneal cavity.*

No evidence has been obtained from polarimetric observation that alteration of rotation of glucose in equilibrium takes place in the peritoneal cavity of either the rabbit or the rat.

SUMMARY AND CONCLUSIONS.

1. Solutions of *d*-glucose in equilibrium when introduced into the intestine of the living animal undergo stereochemical changes.

2. The change in the intestine is a rapid downward mutarotation of the sugar solution to optical values corresponding with specific rotations much below + 52.5°. Several specific rotations below + 19°, and one laevo rotation have been recorded.

3. After withdrawal from the intestine these solutions undergo slower upward mutarotation to a permanent value corresponding with the specific rotation of α - and β -glucoses in equilibrium.

4. The stereochemical changes in the intestine cannot be attributed to the production in excess of β -glucose through preferential absorption of the α form, or to the formation of sugar complexes such as disaccharides.

5. Contact with the mucous membrane of the living intestine probably effects the production of γ -glucose in excess of any amount normally present in glucose solutions which have attained a permanent specific rotation.

6. The above stereochemical changes do not occur in sugar solutions which have been introduced into the peritoneal cavity.

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