XXII. THE METABOLISM OF CARBOHYDRATES. PART III.

THE ABSORPTION OF GLUCOSE, FRUCTOSE AND GALACTOSE FROM THE SMALL INTESTINE.

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IN 1919 the author, in collaboration with Pryde [Hewitt and Pryde, 1920], initiated a systematic research on the permeability of the living intestine to various carbohydrates, particularly the biologically important hexoses and their methylated derivatives.

It was hoped in this way to gain some information as to the effect of configuration on the processes of absorption, and as the structural chemistry of the monosaccharides is known with some definiteness, it was thought that the observations would offer some insight into the initial stages of the metabolism of carbohydrates.

The work was abandoned, however, after only a few preliminary experiments, owing to the discovery of optical changes which were interpreted as showing the conversion of the stable α and β isomerides of glucose, fructose and galactose, in part at least, into the highly reactive γ -modifications.

The results of this research were published in 1920 and an extension of the work in 1921 [Hewitt and de Sousa, 1921], while so far the original scheme has not been carried out.

In this paper it is proposed to communicate experiments on the relative rates of absorption of *d*-glucose, *d*-fructose and *d*-galactose from the small intestine.

PREVIOUS WORK.

The literature on the subject of absorption from the gut is large and, as an excellent digest is given by Goldschmidt [1921], need not be mentioned in detail here.

It may suffice to indicate that, broadly speaking, the views as to the mechanism of absorption fall into two opposed categories, viz.:

(1) There are those who assume that the epithelial lining of the intestine has some special "physiological function" and that the phenomena observed cannot be explained on known physical laws such as those which hold for

diffusion and filtration. Evidence in support of this is given by Cohnheim [1898, 1899], and general agreement with these views is afforded by Reid in a series of papers [1896–1902]. That filtration owing to respective hydrostatic pressures on the two sides of the gut wall is not a determining factor was indicated by Starling [1909], while Höber [1898] and Wallace and Cushny [1898, 1899] showed that solutions of the same osmotic pressure may have differing rates of absorption. Goldschmidt [1921] concluded that the intestine “is a membrane of varying permeability depending upon the nature of the substances presented to it.”

(2) Those which postulate a purely mechanical scheme of absorption. The chief upholder of this is Hamburger [1896–1908] who emphasises the effect of intra-intestinal pressure in increasing absorption. Hamburger cites Leubuscher in suggesting that the mechanical unfolding of the folds of the intestine by the contained liquid increases the surface exposed and in this way hastens absorption.

Hamburger and his co-workers [1917–1919] carried out a series of researches in which they investigated the behaviour of isomeric sugars (pentoses, hexoses and disaccharides) towards the glomerular membrane of the kidney of the frog. These authors employed as their criterion of permeability the amounts of each sugar in the urine formed during perfusion with solutions of the various carbohydrates.

If the writer reads Hamburger rightly he attributes the complete retention of glucose by the amphibian renal mechanism to the configuration of the whole glucose molecule and not to the spatial arrangements of the terminal groups only. He suggests that in galactose, one isomeride (which, he does not say) has a molecular architecture adjusted structurally in a manner enabling it to traverse the glomerular epithelium; while with fructose presumably both the α and β isomerides can pass this membrane.

Hewitt and de Souza [1921] investigated the excretion of sugars introduced intravenously into the anaesthetised animal (rabbit) and found no preferential elimination of either α or β galactose, for these and other hexoses when injected as an equilibrated mixture were found in an unchanged condition in the urine.

PRESENT EXPERIMENTS.

These fall into two groups:

(1) Those in which estimations were made of the amount of glucose or fructose absorbed in various times in different animals from the small intestine. In this set of experiments rabbits were used, and the animals were under full anaesthesia.

(2) Those in which the amount of glucose, fructose and galactose absorbed was estimated in the same animal in the same length of time when making use of the same isolated piece of intestine. In this set of experiments the animals employed were cats.

In view of the work of Osterhout [1916] on the two-fold action of anaesthetics (first a decrease in permeability and second an increase, irreversible and toxic) and of Lillie [1916] who considers that the essential change in anaesthesia is not retarded oxidation but increased stability of cell membranes against permeability, choice was made in this second series of the pithed animal without the administration of anaesthetics during the observations.

General technique.

In all cases the general technique was as follows. The animal was placed under deep narcosis, and, if a cat, was pithed by destruction of the medulla and brain and artificial respiration commenced. The abdomen was then opened in the median line for a short distance, and a suitable convenient length of small intestine carefully withdrawn and isolated by glass cannulae at either end. Through these the loop was thoroughly washed with mammalian salt solution at body temperature until food detritus etc. was removed and the washings no longer reduced Benedict's reagent.

A measured volume of hexose of known concentration was then run in from a pipette, and the solution filling the cannula washed in with a few cc. of salt solution. Clips were then placed on the cannula and the loop replaced in the abdomen which was then closed and kept warm by cloths wrung in saline or by cotton wool.

When the necessary time had elapsed the remaining liquid in the loop was allowed to run into a graduated flask and the loop of intestine was washed with successive quantities of warm salt solution until free from sugar, the whole made to known volume and the hexose content determined by Bertrand's method.

The sugars employed were recrystallised specimens and gave the following constants in aqueous solution:

Sugar	Initial rotation	Final rotation
<i>d</i> -Glucose 2.868 %	103.4°	52.5°
<i>d</i> -Fructose 3.215	-107.8	-92.0
<i>d</i> Galactose 5.60	—	77.0

Solutions of about 2 % of each of the three sugars were made in distilled water at least 12 and usually 24 hours before they were used. By permitting this time interval to elapse between solution and use, any possible complication due to displacement of the usual equilibrium between the α and β hexoses was obviated. In all the experiments reported in this paper the same specimens of sugars were employed.

In addition the following precautions were taken:

(a) to introduce only such volume of liquid as would distend the gut to a normal extent; mechanical stretching and abnormal excessive intra-intestinal pressure were thus avoided;

(b) to introduce equal volumes of solution in each part of an experiment to keep conditions (as regards pressure) the same throughout the separate observations.

As will be seen below on inspection of the protocols slight differences exist as to the actual concentrations of the solutions employed. These differences, however, are not sufficient to influence materially the osmotic pressure which remains virtually constant and substantially below that of blood.

Immediately before each experiment the three solutions of sugar were standardised against the Bertrand reagents.

Series 1.

Here in 18 experiments involving the use of glucose and fructose, varying lengths of intestine were employed and times of contact of solution and tissue were not kept constant. In addition solutions hyper-, iso- and hypo-tonic were used. As the last (hypo-tonic) comprised 12 of the experiments these only are reported here.

In view of the variables mentioned above, considerable differences in absolute values in individual experiments are perhaps not surprising. It is proposed therefore to give (a) the maximum and minimum figures obtained and (b) the average absorption of each monosaccharide.

Glucose.

	Loop length cm.	Time minutes	g. introduced	g. recovered
Maximum	48	5	0.261	0.171
Minimum	59	15	0.530	0.470

The amounts absorbed therefore are 34 % and 11.4 % respectively.

Fructose.

Maximum	60	7	0.332	0.255
Minimum	53	8	0.255	0.225

The amounts absorbed therefore are 23.2 % and 11.7 % respectively.

Average of 12 experiments.

Glucose. 20 % absorbed in average time of 8 minutes.

Fructose. 16 % absorbed in average time of 7 minutes.

Too great stress is not laid on the above figures in view of the complications outlined and also the condition of narcosis, but it is considered that there are indications that glucose is absorbed more rapidly from hypo-tonic solution than is fructose in approximately equal times and similar concentration when the same amount of sugar is within the lumen of the gut.

Series 2.

In this set of experiments more stringent conditions were attained and at least two, when possible all three, sugars were introduced successively into the same isolated loop. Owing to destruction of brain and medulla under deep anaesthesia no further administration of an anaesthetic was necessary. Ten minutes at least were allowed to elapse between its cessation and the commencement of the observations.

Care was taken to vary the order in which the hexoses were tested, and only those observations were taken as trustworthy in which the fitness of the animal, as judged by the strength and rate of heart beat, was manifest throughout the whole of the experiment.

Control experiments were carried out to determine within what limits recovery of sugar could be effected under the conditions of the experiment and the maximum error obtained was a loss of 10 mgm. sugar, that is 2.6 %.

The protocol of one such control is appended.

1. Cat, died after pithing. All blood vessels to isolated loop were ligatured, the loop washed with 0.9 % sodium chloride as described. When free from reducing substances 20 cm. of 1.68 % glucose were introduced into and retained in the intestine for five minutes. After this it was recovered by salt solution, made up to known volume and estimated volumetrically.

Introduced	375 mgm. glucose	} Loss 10 mgm.
Recovered	365 ,,	

When one remembers that a certain small disappearance of soluble substances may still occur when circulation has ceased, it is seen that recovery can be effected on a basis which is practically quantitative.

Protocols are now given of two experiments where the rate of absorption of all three hexoses was observed when the same piece of intestine was employed.

Emphasis is laid on the fact that in these experiments all conditions were kept constant and equal volumes of salt solution were employed to recover the sugar. In this way the condition of the intestine was kept as far as possible identical before the introduction of each individual sugar.

Experiments are also given where the absorption of two of the three hexoses was estimated under similar conditions.

Sugar in order of examination	Concentration %	g. introduced	g. recovered	Time minutes	Absorbed %
2. Glucose	2.12	0.42	0.21	10	50.0
Fructose	1.87	0.35	0.31	10	10.7
Galactose	2.03	0.41	0.35	10	15.0
Length of loop 40 cm.					
3. Galactose	1.86	0.38	0.28	10	26.2
Glucose	1.99	0.40	0.24	10	40.0
Fructose	1.86	0.37	0.32	10	14.9
Length of loop 48 cm.					
4. Glucose	1.90	0.38	0.28	10	26.3
Fructose	1.87	0.37	0.36	10	2.4
Length of loop 50 cm.					
5. Fructose	1.87	0.28	0.26	10	7.1
Galactose	1.93	0.31	0.23	10	27.5
Length of loop 37 cm.					
6. Galactose	1.89	0.38	0.26	10	31.2
Fructose	1.86	0.37	0.31	10	17.0

Some time after recovery of the fructose, the heart was noted to be very slow and weak as shown by pulsations in the mesenteric blood vessels. Glucose was then introduced with the following results.

Glucose	1.99	0.40	0.33	10	17.0
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Summary of above experiments.

No. of experiment	Amounts of hexose absorbed in 10 minutes		
	Glucose %	Fructose %	Galactose %
2	30	10.7	15
3	40	14.9	26.2
4	26.3	2.4	—
5	—	7.1	27.5
6	(17) ¹	17.0	31.2

¹ See note p. 165.

The salient features of these experiments are:

- (a) The rapid and large absorption of glucose.
- (b) The slow absorption of fructose.
- (c) The intermediate position in this respect occupied by galactose.
- (d) The individual variations exhibited in different experiments regarding the actual amount of sugar absorbed.

It should be pointed out that the maximum and minimum figures for each hexose are included in the above tables.

In view of the above results, and as a further check of the technique, experiments were carried out in which the epithelial lining of the intestine was impaired. To effect this, two methods were employed: (1) hot salt solution (51°) and (2) dilute sodium fluoride in aqueous solution. The temperature indicated was selected in view of certain work in this laboratory (unpublished) in which it is shown that at or near 47° profound changes occur in living tissues such as blood. The action of sodium fluoride in destroying living tissue is well known, and was first utilised by Reid [1900].

The results of these observations were:

Sugars in order of examination	Concentration %	g. introduced	g. recovered	Time minutes	Absorbed %
7. Fructose	1.86	0.297	0.24	6	19
Glucose	1.99	0.318	0.28	6	15
Intestine washed with 0.9 % sodium chloride at 51°.					
8. Glucose	1.99	0.38	0.34	10	13
Fructose	1.86	0.35	0.31	10	13.6
Galactose	1.87	0.40	0.35	10	12.6
Intestine washed as in (7).					
9. Glucose	1.99	0.40	0.32	10	21
Sodium fluoride run in.					
Glucose	1.99	0.40 ¹	0.35	10	12
Fructose	1.86	0.37	0.31	10	17
Galactose	1.89	0.38	0.32	10	17

In this experiment the loop was filled for 3 minutes with the sodium fluoride solution after one observation had been made with glucose.

¹ When the second sample of glucose was introduced the gut was in partial tonus due to the fluoride. A smaller area of membrane therefore was available for absorption.

Summary of above experiments.

No. of experiment	Amounts of hexose absorbed		
	Glucose %	Fructose %	Galactose %
7. Heat	—	19	15
8. „	13.0	13.6	12.6
9. NaF (21)	12.1	17.0	17.0

DISCUSSION AND SUMMARY.

The work of numerous observers makes it probable that the mechanism of absorption is the same throughout the whole length of the intestine. Differences undoubtedly exist but these apparently are quantitative only and not qualitative in nature; if this be so it follows that results obtained with the small intestine will hold with but slight differences in other parts of the absorbing alimentary canal.

The experiments reported above show also that the difference between the cat (series 2) and the rabbit (series 1) is not a fundamental one.

Furthermore, it is stated that in hypotonic solutions of diffusible absorbable substances water passes more rapidly than the solute, but that the latter even in dilute solution is also absorbed. This is confirmed as to the absorption of the crystalloid hexoses in the present observations when they are in concentrations with osmotic pressure considerably less than that exerted by blood.

The hexoses employed have similar physical properties, the same molecular weight, the same osmotic pressure, presumably the same rate of diffusion through non-living membranes, and according to Treube [cited from Hamburger, 1904] the same surface tension in solution.

One would, perhaps, thus expect that if absorption was due to osmosis or diffusion alone, with the intestine acting as a dead membrane, all three hexoses would pass through at the same rate.

This does occur when the epithelial lining is destroyed by heat or by toxic agents. Under these conditions the gut appears to act as a permeable membrane and establishes osmotic equilibrium between plasma components and intestinal contents as it does in the case of such substances as magnesium sulphate, sodium sulphate or even lactose, which are absorbed only very slowly.

Starling [1909] notes that monosaccharides are absorbed twice as rapidly as disaccharides, and Bywaters and Short [1913] record glucose as being better absorbed than lactose.

It is however not clear whether the establishment of such osmotic equilibria is entirely due to absorption, in the case of hypotonic glucose, of the solvent, or with hypertonic solutions, of the solute. The possibility of excretion of substances from the blood has to be considered especially in view of the experiments of Fisher and Moore referred to below.

It may be concluded that under the abnormal conditions described the three hexoses are absorbed from the gut at equal rates in hypotonic solution.

With the "normal" intestine a different condition of affairs is experienced and here widely varying rates of absorption exist for the three carbohydrates investigated, the order of rapidity of absorption being (1) glucose, (2) galactose, (3) fructose¹.

¹ Only one discordant result was obtained. Inspection of the notes made at the time reveals no explanation.

It is difficult to attribute the results obtained to any of the following for the reasons given.

(a) *Blood flow varying in the experiment.* The duration of the whole experiment was short and those are rejected in which any diminution of the efficiency of the heart was observed.

(b) *The order of introduction of the sugars into the intestine.* As already indicated the order was altered as far as possible.

(c) *Alteration in intra-intestinal pressure.* The same volumes of solution were run in, in each part of any experiment.

(d) *Differing osmotic pressure of the solutions.* The percentage of these varied so little in each experiment that this is virtually constant.

(e) *State of metabolism, age, sex, etc. of animal.* Animals were selected promiscuously at various times of the day; the rabbits were not fed for 24 hours immediately before the experiment.

Moore, Roaf and Webster [1911] state that substances in physiological systems will always pass against an osmotic pressure gradient, as the cell contents in the colloidal state possess an attraction for crystalloids.

The question of adsorption of hexoses has been considered by Cohnheim [1913] in connection with their excretion by the kidney. He experimented with kidney pulp and noted differences. Hamburger [1919] criticises the technique and the results obtained are therefore still in doubt. It may be concluded that the conditions obtaining in Cohnheim's experiments are far removed from those holding in the normal glomerular epithelium.

An alternative method of attacking the problem however is available, viz. investigation of resistance of erythrocytes to haemolysis, *i.e.* to alterations of permeability, after exposure to isotonic solutions of the various hexoses. Eisler [1909], by examining the inhibition of specific haemolysis by saponin, has shown that glucose modifies permeability.

The problem in its more extended form is being taken up.

One can note however that the solubilities of the three sugars in lipoidal solvents are not the same but do not vary in the order found for their absorption. The solvent action of lipoids on them is practically nil.

One observation, perhaps, is of importance when viewed in the light of the experiments of Wallace and Cushny [1898, 1899] with equimolecular solutions of inorganic salts and organic esters. These authors found that substances which form an insoluble calcium salt, such as fluorides, oxalates and phosphates, are not appreciably absorbed.

In the experiments reported in this paper, fructose is absorbed most slowly and is the one monosaccharide characterised by the capacity to form a relatively insoluble calcium derivative [Dubrunfaut, 1849, 1856, 1869; Peligot, 1880].

Osterhout [1911, 1915] among others has shown that calcium salts decrease permeability while sodium salts have the reverse action. Lillie [1921] has recently demonstrated this opposed action in star fish eggs. He suggests

that insoluble colloidal salts of calcium are formed with the structural colloids of the protoplasm and are necessary for the normal behaviour of the plasma membrane. In sodium chloride solution water-soluble compounds are formed with disintegration.

In regard to the absorption of glucose, Reid [1900, 1902] proved that glucose in sodium chloride solution was more readily absorbed than in water, while Fisher and Moore [1907] noted that, where a glycosuria exists, there may be no excretion of glucose into the intestine, whereas, if the salt content of the blood is raised by intravenous injection of sodium chloride, this sugar can be found therein.

Permeability may thus be affected from both sides of the mucous membrane of the intestine.

It is not improbable then that union may take place between the hexoses and protoplasmic substance with concomitant variations in permeability. It is indicated that factors other than osmosis and diffusion are at work in the absorption of monosaccharides from the alimentary canal of the normal animal.

While no explanation is forthcoming at present, the results are not without interest when one considers the diet of the young mammal, containing as it does the total carbohydrate supply as lactose which gives rise to the two aldoses most readily absorbed. The necessity for galactose in the formation of the cerebrosides is also significant.

It is commonly stated also that fructose is tolerated to a greater extent than glucose in the diabetic organism. This may well be due to its more gradual and slow absorption compared with glucose.

The whole problem of the metabolism of fructose and galactose is obscure and demands investigation.

CONCLUSIONS.

(1) Glucose, fructose and galactose are absorbed from hypotonic solution from the small intestine.

(2) This absorption occurs whether the epithelial lining has been destroyed or not.

(3) When the epithelium has been destroyed all three sugars are absorbed at the same rate.

(4) When the intestinal wall is normal, glucose is absorbed more rapidly than galactose, and the latter more rapidly than fructose.

(5) The results cannot be explained only by osmosis or diffusion.

(6) Attention is drawn to the influence of calcium and sodium salts on the permeability of cells to these crystalloids.

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ADDENDUM.

Since the foregoing experiments were carried out and the paper written, the author has become aware of the work of Nagano (*Pflüger's Arch.* 1902, 90, 389-404) in which a research of similar nature is reported.

Nagano employed dogs with a *Vella fistula* and was more occupied with the problem of absorption at different levels of the alimentary canal. His experiments were made with considerable time intervals between each one.

Like the present author he found fructose to be more slowly absorbed than either glucose or galactose, but he concluded that galactose was slightly more rapidly taken up by the dog's intestine than glucose.

Nagano clearly states however that in the dog "the rate of absorption of stereoisomeric sugars is different."