THE UTILISATION OF DIFFERENT SUGARS BY THE NORMAL HEART. By HUGH MACLEAN and IDA SMEDLEY.

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ALTHOUGH many indirect observations supported the assumption that contracting muscle utilises sugar, it is only within recent years that direct proof of the consumption of sugar by muscle has been furnished. The experimental work carried out on voluntary muscle yielded on the whole unsatisfactory results, but an investigation into the action of the heart on sugars furnished conclusive proof that contracting cardiac muscle was capable of consuming glucose. In 1907, Locke and Rosenheim(1) perfused an isolated rabbit's heart with saline solution to which some glucose had been added and by quantitative determination of the amount of sugar present before and after the experiment furnished a satisfactory demonstration of the utilisation of sugar by the heart muscle. Confirmation of these results has been furnished by such observers as Rohde(2) and Gayda(3) and in a recent interesting publication, Knowlton and Starling(4) showed that the heart of the dog when fed with normal blood *in situ* was capable of consuming a considerable quantity of glucose.

In the light of these experiments there remains no further doubt that the contracting cardiac muscle is capable of utilising glucose, and all controversy with regard to this point may be taken as finally closed. Since however glucose is probably the only sugar which is normally present in blood it was of interest to determine whether this action was entirely specific and limited to glucose, or whether the other sugars which are capable of utilisation as foods in the body are also capable of being assimilated by the heart.

The animals used were rabbits and dogs. In each case the heart was isolated and perfused with saline solution containing sugar in an apparatus similar to that used by Locke and Rosenheim. Locke's modification of Ringer's fluid was used for perfusion.

In experiments of this nature it is exceedingly important to guard against the introduction of bacteria, otherwise the result may be entirely vitiated. As a general rule, when the perfusion was limited to short periods no difficulty was experienced, but in many cases after five to six hours bacteria were found in considerable numbers. These results were excluded and the majority of our later experiments limited to four hours or less. Before each experiment the whole of the apparatus was sterilised in the autoclave. In several recorded cases it appears that the consumption of sugar per hour increased with the duration of the experiment. Such effects are undoubtedly the result of bacterial action, for similar results were observed in certain of our experiments but in every case where this occurred, bacteria capable of fermenting sugar were present.

Heart of rabbit. The heart was quickly removed from the pithed rabbit and washed out with normal saline solution in order to remove excess of blood. A cannula was then tied into the aorta and about 100 c.c. of the same sugar-containing solution as that used in the actual experiment was passed through, the solution having been previously oxygenated. The apparatus was washed out with a similar solution which was circulated through it for a few minutes. The heart was then put into the chamber and perfused in the manner described by Locke and Rosenheim. A sample was taken after ten to fifteen minutes and the sugar estimated. At the close of the experiment the percentage of sugar remaining in the fluid gave an indication of the amount used by the heart.

The following sugars were investigated.

Disaccharide.		Maltose.
Hexoses.	(Aldoses)	Glucose, Mannose, Galactose.
	(Ketose)	Lævulose.
Pentose.		Xylose.
Triose.		Dihydroxyacetone.

The results obtained are shown in Table I.

The sugar was estimated by Bertrand's method and the different sugars calculated as glucose. The weight of each heart was from six to ten grms.

In four hours the quantities of glucose removed varied from 16 to 29 mgrms. In three hours 14 mgrms. of mannose disappeared so that its rate of removal is somewhat similar to that of glucose. The sample of mannose was prepared in the laboratory from ivory nut. Galactose

TABLE I. *Heart of Rabbit.*

Sugar	Amount of fluid circulated after 1st sample withdrawn (c.c.)	% sugar in solution	No. of c.c. $KMnO_4$ solution required by 20 c.c. sugar solution after perfusion for				No. of milligrams of sugar removed in		Remarks Weights of hearts=6 to 10 grms.
			15 m.	2 h. 15 m.	3 h. 15 m.	4 h. 15 m.	(a) 3 hrs.	(b) 4 hrs.	
Glucose	100	·15	5·3	—	—	4·6	—	·0185	——
	100	·2	7·7	—	—	7·1	—	·016	——
	160	·2	8·3 8·3	—	—	7·6 7·7	—	·029	——
	140	·2	7·5 7·7	—	—	6·9 6·95	—	·026	——
Mannose	100	·1	6·3	—	5·8	—	·014	—	From methyl-phenyl-hydrazone.
Galactose	100	·1	3·9	—	—	3·65	—	·0065	Purified by yeast fermentation.
	—	·1	4·65 4·65	—	—	4·40 4·45	—	·006	,, ,, ,, ,,
	—	·1	3·55	—	—	3·35	—	·0045	Purified by methyl-phenyl-hydrazone.
	—	·1	4·0	—	—	3·7 3·75	—	·0075	——
	—	·15	5·65 5·55	—	—	5·45	—	·004	——
	—	·1	3·35	—	—	3·25	—	·0025	——
	—	·2	7·5	—	—	7·3	—	·0055	——
	—	·2	6·7	—	—	6·6 6·55	—	·0035	——
Lævulose	100	·2	7·05 7·05	—	—	6·95	—	·002	——
	—	·15	5·3	—	—	5·3	—	·000	Pure from inulin.
	—	·15	5·55	—	5·50	—	·001	—	,, ,, ,,
	—	·2	6·8	—	—	6·8	—	·000	,, ,, ,,
Di-oxy-acetone	100	·2	7·15 7·15	—	—	7·25	—	+·0025	——
	—	·15	5·4	—	—	5·5 5·5	—	+·002	——
	—	·15	5·6 5·7	—	—	5·6 5·7	—	·000	——
Xylose	100	—	6·6	—	—	6·6	—	·000	——
Maltose	100	—	3·0	—	—	2·95	—	·0005	——
	100	—	3·6 3·6	—	—	3·55	—	·001	——

is apparently utilised to a much less extent—from three to eight mgrms. in four hours. In this case the quantities which disappeared were so small that at first we were inclined to ascribe them to traces of glucose present in the sample. Two methods of purification were therefore used, the material worked with being Kahlbaum's pure galactose. The galactose was converted into its methyl-phenyl-hydrazone and the sugar again liberated by boiling with benzaldehyde according to Fischer's directions. In the second method the galactose was fermented by yeast and recrystallised from alcohol several times. In both cases the rate of disappearance was similar. From these results it would appear that the cardiac muscle is capable of utilising pure galactose though to a much less extent than glucose or mannose.

The sample of lævulose was Kahlbaum's "pure from inulin." Here no disappearance was observed even after four hours, so that apparently the rabbit's heart is incapable of dealing with lævulose. In view of certain results obtained with the dog's heart and described later, it is possible that a very slight effect may be produced but this is so small as to be within the region of experimental error. If any disappearance does take place it is so exceedingly minute as to be incapable of estimation by the method used. It was thought of interest to try another ketone besides lævulose, and di-oxy-acetone was investigated. Here again there was no disappearance, so it would appear that the rabbit's heart is incapable of dealing with keto-sugars. In the case of xylose and maltose no disappearance took place.

In conducting experiments with different sugars it is of very great importance to have pure samples. Thus commercial galactose may contain glucose and the same holds good with regard to lævulose. In the case of the latter the difficulty is avoided by using lævulose prepared from inulin. Some of our earlier experiments yielded anomalous results which we ascribed to the presence of impurities. When pure samples were taken, as in the experiments described above consistent results were generally obtained. Unless the purity of the sugar used is assured no importance can be attached to the quantitative determination of the amount consumed.

Heart of dog. Owing to the small size of the rabbit's heart, it was thought advisable to use the heart of a larger animal and thus magnify the amount of sugar which disappeared. For this purpose we used the dog's heart. The method of procedure was exactly the same as that described above for the heart of the rabbit. The dog was killed and the organ isolated as quickly as possible; it was then washed and perfused as before.

TABLE II.

Heart of dog.

	Weight of heart in grms.	Sugar used	Amt. of fluid circulated after withdrawal of 1st sample (c.c.)	% sugar (approx.)	No. of c.c. $KMnO_4$ required by 20 c.c. sugar solution (Bertrand's method) after circulation for				No. of mgrms. of sugar removed during			Remarks
					15 m.	1 h. 15 m.	2 h. 15 m.	3 h. 15 m.	1 hr.	2 hrs.	3 hrs.	
(1)	80	Dextrose	200	·2	7·35	6·25	5·40	4·40	60	100·5	142·9	——
(2)	70	,,	200	·2	7·65 7·70	—	—	3·7 3·6	—	—	312	——
(3)	11	,,	125	·3	11·0	—	—	9·8	—	—	38·1	Pup 7 weeks old.
(4)	58	Mannose	225	·2	7·35 7·30	6·65	6·05	5·20	41	73	114	——
(5)	88	Galactose	275	·2	7·3	6·7	6·3 6·4	—	36	55	—	——
(6)	11	,,	125	·2	7·0	—	—	6·65	—	—	13	Pup 7 weeks old.

TABLE III.

Action of Dog's Heart on Laevulose.

	Weight of heart in grms.	Sugar used	Amount of fluid circulated after withdrawal of 1st sample (c.c.)	% sugar (approx.)	No. of c.c. $KMnO_4$ required by 20 c.c. sugar solution (Bertrand's method of estimation) after circulation for				No. of mgrms. of sugar removed during			Remarks
					15 m.	1 h. 15 m.	2 h. 15 m.	3 h. 15 m.	1 hr.	2 hrs.	3 hrs.	
(1)	80	Lævulose	200	·2	7·1 7·1	—	—	5·5 5·5	—	—	87	——
(2)	70	,,	200	·2	7·1 7·3	—	6·6 6·8	—	—	27	—	——
(3)	72	,,	200	·2	7·4 7·3	6·3	5·5	4·6	61	100	138	——
(4)	110	,,	200	·2	5·9 5·95	5·6	5·45	—	16	23	—	——
(5)	137	,,	220	·2	6·95	6·0	5·25 5·35	—	56	93	—	——
(6)	100	,,	250	·1	3·6	—	—	2·25	—	69	—	Solutions were cleared with dialysed iron before estimating the sugar.
Control with frozen heart.												
(7)	64	Lævulose	250	·2	6·5	—	6·45	—	—	2	—	

With glucose, mannose and galactose results corresponding in general with those described under the rabbit's heart were obtained, the total quantities disappearing being of course greater in proportion to the greater size of the heart.

The results are shown in Table II.

Lævulose, which was unacted upon by the rabbit's heart, invariably disappeared in the heart of the dog, but the amount varied considerably. This is seen in Table III. It was thought possible that this might be due to a mechanical retention of lævulose by the heart. *A priori* we might expect some slight retention since lævulose is not normally present in the heart muscle and a certain amount might therefore be retained in accordance with the ordinary laws of diffusion. In order to test this the following experiment was devised.

A dog's heart immediately after its removal from the body was washed free from blood, placed in ice, and allowed to remain there during an hour. It was then placed in the apparatus and perfused with a 0·2 % solution of lævulose in the usual manner. After two hours only two mgrms. of lævulose had disappeared. The amounts removed by normal hearts in the corresponding period were from 23 to 100 mgrms. We conclude therefore that the disappearance observed is not caused by a mechanical retention of the sugar.

General remarks.

From the above results it appears that the utilisation of sugar by the heart is not confined to glucose: other sugars are also consumed but apparently not to the same extent except in the case of mannose. Again in the dog and rabbit the results are not quite parallel. Lævulose, which is apparently unattacked by the heart of the rabbit, is removed by the dog's heart in some cases almost as well as glucose. After we had completed the above investigation, a paper by Neukirch and Rona(5) appeared dealing with the utilisation of sugar by the heart of the rabbit and cat. Their results with rabbits are in general similar to those we have obtained: lævulose and maltose were not attacked nor were lactose and cane sugar which they also investigated. In their research with galactose, this sugar seems to have been used up to a greater extent than was the case in our experiments: otherwise our results are in accordance. In the case of the cat's heart two experiments are described lasting respectively for nine and six hours; in both cases during the first three hours very little disappearance of sugar was observed whilst in the subsequent periods considerable disappearance

in the amounts of sugar took place. From this it is concluded that a reserve substance present in the cat's heart is first consumed and that subsequently sugar is destroyed. It seems to us however that no definite conclusion can be drawn unless bacterial infection has been rigidly excluded, for the difficulty experienced in obtaining sterile conditions after several hours is very great (cp. Harden and MacLean(6)). In our heart experiments bacteria were often in evidence after five to six hours although the ordinary precautions to ensure asepsis were observed.

REFERENCES.

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