THE CARBOHYDRATE METABOLISM OF THE ISOLATED HEART LUNG PREPARATION.

By S. W. PATTERSON[1] and E. H. STARLING.

(*From the Institute of Physiology, University College, London.*)

In a recent paper by Knowlton and Starling(1) on the consumption of sugar in the normal and diabetic heart a marked difference was found according as the dog from which the heart was taken was normal or diabetic. Further, when an extract of pancreas was added to the circulating blood, the heart quickened its pace and the consumption of sugar was apparently increased. It was therefore concluded that the essential feature of pancreatic diabetes was a failure of the tissues, *e.g.* the heart muscle, to utilise sugar circulating in the blood. Certain aberrant results were recorded, but these were ascribed to absence of care in the cleaning of the tubes. Apparent confirmation of the inability of the diabetic heart to utilise sugar was furnished by MacLean and Smedley(2) in a research on the sugar consumption of the isolated heart perfused with Ringer's solution.

The question is however of such importance that it seemed to us worth while to repeat the experiments with greater variation of the conditions. In Knowlton and Starling's experiments an analysis of the blood serum was made every hour, and only small quantities of serum, 5 to 10 c.c., were available. Any error of determination would tend to be multiplied considerably in calculating the total sugar consumption by the heart lung preparation. We therefore resolved to use larger quantities of blood for analysis and to take samples only at the beginning and end of the experiments. We also, after a preliminary comparison of the two methods, decided to use the method of Rona and Michaelis(3) for the precipitation of the proteins of the serum, rather than the copper sulphate method employed by Knowlton in his analysis. In many cases comparative estimations were made by both methods.

[1] Beit Memorial Research Fellow.

It is rather difficult to decide whether in these cases we should determine the sugar content of the whole blood or only that of the serum. We finally decided upon the latter on the following grounds:

(1) The sugar in the serum represents that which is available by diffusion for the use of the tissues. It is the sugar content of the serum which will determine the content of the tissue fluid.

(2) It has been shown by Höber(4) and others that there is no constant relationship between the sugar content of the corpuscles and that of the serum, though the sugar content of the corpuscles is always less than that of the serum.

(3) It is much more difficult to separate the proteins from the whole blood than from the serum.

Methods. Dogs were used throughout the research; and a completely diabetic condition was obtained by total removal of the pancreas two to seven days beforehand. The diabetic animals were fed on milk for the first 36–48 hours after the operation, and then on meat, usually with the addition of ox pancreas to supply the absent external secretion of the pancreas. In some cases different carbohydrates were added to the diet. The normal dogs were fed on the usual kennel diet of dog biscuit, except where otherwise noted.

The heart lung preparation was made by the method described by Knowlton and Starling. Scrupulous care was taken to keep the tubes and apparatus clean. After each experiment they were washed out with water and strong soda solution and the whole apparatus was boiled after every two or three experiments. In many cases the apparatus was kept filled with strong soda solution, being washed out with sterile saline solution just before the experiments.

A series of experiments was also made on the consumption of sugar by the isolated lungs in normal and diabetic dogs, carried out by an adaptation of the method used by Martin and Embley(5) for perfusing the isolated intestine.

The sugar of the serum obtained by centrifugalising the blood after the removal of the proteins by Knowlton's method or by an adaptation of the Rona-Michaelis method, was estimated by Bertrand's(6) method, and frequently also by observing the rotation of a polarised beam of light produced by the solution.

Knowlton's method of removing the serum proteins with copper sulphate and subsequent neutralisation requires careful manipulation and in our hands was found less accurate and easy than the Rona-Michaelis method with colloidal iron (Liq. Ferri Dialysatus). Sodium

sulphate was used to throw the excess of iron out of solution, as it was found that a slight excess of magnesium sulphate, which is recommended, caused a colloidal precipitate of magnesium hydroxide, which interfered with the filtration in the subsequent Bertrand manipulation. The following is the exact procedure employed:—

20–25 c.c. of serum were diluted with distilled water to about 200 c.c. in a 250 c.c. measuring flask, 20–25 c.c. of colloidal iron were added slowly with constant shaking, until the precipitate produced appeared to be going up into solution again; the mixture was shaken and allowed to stand a few minutes; then a small amount (·5–1 grm.) of sodium sulphate in solution was added to the mixture which was made up to 250 c.c. and thoroughly shaken. After standing a few minutes, the mixture was filtered through a plaited filter paper and 200 c.c. of filtrate obtained. From 5 to 7 drops of dilute acetic acid were added, and the mixture evaporated to small bulk in silver dishes on a water bath, without completely drying. Then the remainder with washings and 5 drops of alumina cream was made up to 10 c.c. in a measuring flask. The solution obtained was filtered into a 200 mm. polarimeter tube, the rotation observed by means of a three-field Schmidt and Haensch polarimeter, and 5 c.c. of the filtrate taken for estimation by Bertrand's method.

TABLE I. *Control estimations.*

Modified Rona-Michaelis (Na_2SO_4 instead of $MgSO_4$) and Bertrand.

		Reading	Result	Polarimeter: rotation observed	Polarimeter: positive rotation calculated as dextrose
1.	Serum with ·4 % glucose added	(*a*) 29·4	·400 %		
		(*b*) 29·7	·407		
		(*c*) 32·3	·398		
2.	Serum		·025		
	Serum + ·2 % glucose		·228		·157 %
	,, + ·4 ,,		·440		·314
	,, + ·2 ,, + ·4 % fructose		·658		
3.	Serum		·068		0·0
	Serum + ·6 % glucose		·63		·61
	,, ,, ,,		·65		·60
	,, + ·4 % ,, + ·6 % fructose		·97	− 1°·06	·393
					·576
			·98	− 1°·05	·400
					·580
4.	Serum + glucose		·89		·77
	,, ,, (estimated by Knowlton's method)		·98		
	do. do. do.		·985		
	Serum + glucose ($CuSO_4$ added, neutralised and filtered)		·79		
	do. do. do.		·69		
	do. do. do.		·71		

After proficiency with the method had been obtained, serum, to which known amounts of sugar were added, gave results such as those reported in Table I, the experimental error being less than 20 mgms. of sugar per 100 c.c. serum.

The figures obtained by the Rona-Michaelis and Bertrand methods are given in the table of experimental data.

Experimental Results.

1. *Sugar consumption in the lungs.* Since the blood in the heart lung preparation passes through heart and lungs, both of which are alive, and our special object is to determine the sugar consumption in the heart muscle, it is necessary in the first place to determine whether any, and if so, how much, sugar is used up by the lungs themselves. Table II contains the results of a number of experiments in which an artificial circulation of defibrinated blood was carried out through the isolated lungs, the motive power being supplied by a rubber enema syringe which was rhythmically compressed, so as to maintain an average pressure of about 15 mm. Hg. in the pulmonary artery. Since the weight of the lungs is apt to be interfered with at the end of the experiment by the presence of more or less œdema, we have not weighed the lungs themselves but have weighed the heart of the same animal and give our results in mgms. per gram heart muscle.

Table II. *Isolated lungs perfused by Martin and Embley's method.*

No.	Animal	Sugar added	Sugar p.c. before	Sugar p.c. after	Amount used per hour (mgms.)	Heart weight	Amount used per gram of heart per hour (mgms.)
1	normal	0	·195	·080	66	50	1·30
2	,,	+	·466	·310	156	111	1·40
3	diabetic 3 days	0	·227	·062	110	76	1·44
4	normal	+	·345	·273	96	73	1·30

It will be seen that the lungs constantly use up a certain amount of glucose corresponding to about 1·3 to 1·5 mgms. glucose per gram of heart muscle per hour. This observation is in accordance with Evans and Starling's(7) results on the consumption of oxygen by the lungs. The oxygen consumption of the lungs amounts to about 1·0 c.c. per gram heart muscle per hour. We found no difference in this respect between the lungs of normal animals and of diabetic animals, so that there is no reason for doubting that in the latter case the tissues of the

lungs still preserve their power of consuming and presumably oxidising the sugar of the blood.

2. *The consumption of sugar in normal hearts.* At the very outset of our experiments we were met with the difficulty that we were unable to confirm the constant sugar consumption for normal hearts described in the paper of Knowlton and Starling. When we began these experiments we had not carried out any determinations of the sugar consumption in the isolated lung, but we found in all cases that the sugar consumption in mgms. per gram heart muscle was considerably less than that previously found, namely, 4 mgms. per gram heart muscle per hour. The following table contains the results of the experiments on normal heart lung preparations.

TABLE III. *Normal heart lung preparations.*

No.	Remarks	Temp.	Time	B.P.	V.P.	Rate	Sugar added	Heart weight	Sugar				Glycogen, heart (%)
									% before	% after	mgm. per g. of heart per hr.	total mgm. per hr.	
1		36	2	40+	70	150	+	50·0	·370	·315	1·03	36	
2		36	3	20+	80	125 144	0	108·0	·136	·003	0·80	93	
3*		36	2	50	60	125	+	70·0	·615	·490	1·80	115	
4	biscuits	36	2	65	60	112	+	67·5	·320	·150	1·60	113	0·85
5		36	1½	95	60	–	+	37·0	·500	·410	3·60	112	0·14
6		36	2	95	50	slow	+	61·0	·430	·330	1·20	75	
7	fasting	36	2	95	90	160	+	69·0	·540	·440	1·30	86	0·15
8		36	1	90	75	218	+	61·0	·430	·330	3·80	233	0·35
9	lactating	36	2	95	70	110	+	74·0	·550	·425	2·10	155	0·75

* "Blood before" was after this drawn at beginning of experiment, and kept at 36° C. during the experiment in order that any glycolysis which the blood might effect should take place.

It will be seen that the figures obtained for sugar consumption are extremely variable. Only in one case however do they touch the figure of 3·8 mgm. In most of the cases the consumption per gram heart muscle per hour was less than 2 mgms., and in one case it fell to as little as ·8 mgm. We have therefore no criterion of comparison by which we may judge of the presence or absence of the power of consuming sugar in the diabetic heart. Moreover if we subtract from these results the sugar consumption which can be ascribed to the lung part of the heart lung preparation, we arrive in many cases at a *minus* quantity for the sugar consumption in the heart. It is evident that there is some factor here which we have hitherto failed to record and which may introduce sugar into the system. We thought at first that

this factor might be unknown interchanges between the corpuscles and the plasma. It seems more probable however that the disturbing factor is the glycogen of the heart muscle, the lungs themselves containing only minute traces of glycogen. The normal heart may however contain ·7 or ·8 % glycogen, and perhaps even more, *i.e.* a 50 gram heart may contain 400 mgms. of carbohydrate which is not taken account of in our blood analyses. In Table III we give the glycogen content of the heart as determined by Dr Cruickshank at the end of the experiment. It is certainly difficult to make out any definite relation between the amount of glycogen present and the sugar consumption; but this is to be expected in view of the considerable variations in the glycogen content of the heart muscle, and of the fact that it is impossible to make a determination of the glycogen in the heart muscle at the beginning of the experiment. Before however we can accept the glycogen as the disturbing factor we must have some proof that the glycogen is actually available for consumption in the heart muscle. Since we could not obtain a quantitative estimate of the utilisation of the glycogen of the heart muscle during the experiment, we endeavoured to find whether by excessive work and stimulation of the heart it would be possible to induce this organ to use up not only the sugar of the circulating blood but also the whole of its glycogen. For this purpose experiments were prolonged for three or four hours, while the blood-pressure was maintained high and the heart was continually stimulated by means of adrenalin. It has already been shown by Evans(8) that the total metabolism of the heart is approximately proportional to its rate and is very largely increased by the administration of adrenalin. The following table contains the results of experiments carried out in this way.

TABLE IV. *Normal heart lung preparation stimulated by adrenalin.*

No.	Remarks	Temperature	Time	B.P.	V.P.	Rate	Sugar added	Heart weight	Sugar				Glycogen		
									% before	% after	mg. per g. of heart per hr.	total mgms. per hr.	heart %	liver %	
1	levulose	36	2	95	60	200	+	56·5	·640	·330	4·10	233	·36	5·75	adrenalin
2	fasting	36	2	95	100	185	+	67·0	·510	·355	2·62	175	·06		adrenalin
3		36	4	90	–	200+	0	122·0	·050	·009			·05		adrenalin
4		36	4	95	–	–	+	93·0	·280	·000	0·30	–	·00		adrenalin
5		36	4	90		200	+	78·0		·165	8·00		·13		2·5 grms. glucose + 9 c.c. of 1 in 10,000 adrenalin

In these experiments the result of the over-stimulation was either to cause a complete consumption of the sugar in the circulating blood, or if this were kept high by the continued addition of sugar the sugar consumption in the muscle rose to as much as 8 mgms. per gram per hour. When no sugar was added the glycogen disappeared completely from the heart, and even when considerable quantities of sugar were added the glycogen of the heart muscle almost completely disappeared. We may therefore conclude that the heart in our preparation is in a position to draw upon its glycogen store, and that in the absence of any previous determination of glycogen of the heart muscle it is impossible by analysis of the blood or blood serum at the beginning and end of the experiment to find with any accuracy what is the total carbohydrate metabolism of the heart.

3. *Experiments on diabetic hearts.* The following table gives the results obtained in experiments on heart lung preparations from dogs which had been rendered diabetic by removal of the pancreas two

TABLE V. *Heart lung preparations from depancreatised animals.*

No.	Remarks	Temperature	Time	B.P.	V.P.	Rate	Sugar added	Heart weight	Sugar: % before	Sugar: % after	Sugar: mg. per g. of heart per hr.	Sugar: total mgms. per hr.	Glycogen: heart %	Glycogen: liver %
1	Diabetic 2 days	36	2½	44+	100	130	0	68·5	·360	·205	1·30	93		
	"Blood before" glycolysed after this													
2	,, 2 ,,	36	2	40+	60	130	0	61·5	·220	·050	2·00	–	·44	·26
3	,, 4 ,,	36	2	20+	80	140	0	64·0	·220	·040	2·20	–		
4	,, 2 ,,	36	2	55	60	118	+	43·5	·560	·520	0·70	30	·89	
5	,, 3 ,, (meat)	36	2	70	50	106	+	70·0	·720	·555	1·70	117	·26	
6	,, 6 ,, (meat)	36	2	70	–	–	+	49·0	·450	·290	2·30	112	·26	
7	,, 6 ,, (meat)	36	2	–	–	–	+	–	·360	·290	–	–	·73	
8	,, 6 ,, (meat)	36	2	60	65	86	+	45·0	·480	·430	0·88	37·5	·875	
9	,, 5 ,, (levulose)	36	2	95	55	–	0	37·0	·320	·250	1·50	60	1·60	
10	,, 5 ,,	36	2	75	50	–	+	55·0	·460	·420	0·50	28	·59	
11	,, 6 ,, (levulose)	36	2	100	90	–	+	45·0	·515	·445	0·94	52	1·30	
12	,, 2 ,, (levulose)	36	1½	100	85	130	+	124·0	·490	·380	1·18	146	·675	·075
13	,, 5 ,,	36	2	90	–	–	+	54·0	·580	·500	1·40	–	·80	
14	,, 5 ,, (pancreas and meat)	36	2	95	–	110	0	52·0	·320	·188	1·60	–	·10	

to six days beforehand. As might be expected from the conclusions arrived at in the last section, the results do not serve either to confirm or to confute the conclusions arrived at previously by Knowlton and Starling and by MacLean and Smedley. It is remarkable however that there is no marked difference between these results taken as a whole and those obtained on normal dogs. It is true that in most cases the consumption of sugar in the lungs might account for the whole of the sugar used, but in one or two experiments there is a certain excess of sugar consumption over that which might have taken place in the lungs. In evaluating these results it is necessary to take into account the fact to which Cruickshank(9) in a recent paper has drawn attention, namely, that the diabetic heart contains considerably more glycogen than the heart of normal animals. The results obtained by Cruickshank are given in the final columns of the table.

Although in the diabetic heart, as in the normal heart, we are unable by the method of comparative blood analysis to arrive at any definite decision as to the consumption of sugar by the heart muscle, we can use the method of over-stimulation already described (p. 142) to determine whether under any circumstances the diabetic heart can utilise sugar.

The following table gives the results of experiments in which the diabetic heart was continually stimulated for two to four hours by adrenalin, either with or without the continual addition of sugar to the circulating blood.

TABLE VI. *Diabetic heart lung preparation stimulated by adrenalin.*

No.	Remarks	Temperature	Time	B.P.	V.P.	Rate	Sugar added	Heart weight	Sugar				Glycogen, heart (%)	
									% before	% after	mg. per g. of heart per hr.	total mgms. per hr.		
1	Diabetic 5 days (meat & pancreas)	36	2	95	70	200+	+	74	·625	·515	1·46	99	·75	
2	Diabetic 2 days (meat)	36	4	95	–	200+	0	–	–	·095	–	–	·02	
3	Diabetic 6 days	36	3	100	–	200	+	54	–	·539	8+		·02	2 grms. glucose + 10 c.c. of 1 in 10,000 adrenalin

In the third experiment the sugar consumption was largely increased, the sugar content of the circulating blood being very much diminished in

spite of the continual addition of sugar. Moreover in the prolonged experiments the glycogen of the heart muscle was reduced to a minimum. We are therefore certain that in these cases the diabetic heart has used up not only the sugar of the circulating blood plus that added to it but also the increased amount of glycogen previously stored in its substance. The increased utilisation of sugar cannot be ascribed to the lungs. In four experiments adrenalin was added to the blood perfused through the isolated lung, and in each case the sugar consumption was little if at all above that observed in normal lungs. The results obtained are given in the following table.

TABLE VII. *Isolated lung treated with adrenalin.*

No.	Animal	Sugar added	% before	% after	Amount used per hour (mgms.)	Heart weight (grms.)	Amount used per gram of heart per hour (mgms.)
1	Diabetic 3 days	+	·515	·386 (2 hrs.)	182	77	2·36
	Same experiment		·386	·292 (1¼ hrs.)	185	77	2·40
2	Diabetic 5 days	+	·467	·400 (2 hrs.)	80	54	1·50
3	Normal	+	·362	·235 (2 hrs.)	118	62	1·90
4	Normal	+	·290	·140 (2 hrs.)	163	125	1·31

The repetition under more exact conditions of the experiments previously carried out in this laboratory by Knowlton and Starling have thus failed to confirm the conclusions then arrived at. In fact we have definite evidence now that the lungs of a diabetic animal use as much sugar as those of a normal animal, and that while there is no marked difference between normal and diabetic hearts in the fate of the sugar added to the blood circulating through a heart lung preparation, the diabetic heart, just as the normal heart, can be made to consume sugar at an increased rate under the effects of stimulation by adrenalin. Under these conditions not only does the heart use the sugar of the blood but it may also completely consume the glycogen stored up in its substance. The conclusions drawn previously as to the beneficial effects of extracts of pancreas on the diabetic heart must also be abandoned, or at any rate are susceptible of another explanation. Attention was drawn to the fact that the addition of pancreatic extract to the blood caused a quickening of the beat of the diabetic heart and at the same time increased the consumption

of sugar in the heart lung preparation. Later experiments have shown that the accelerating effect of pancreatic extract is not peculiar to the diabetic heart but is also to be observed on normal hearts. Quickening of the heart by means of adrenalin increases the rate of consumption of sugar, and any increased consumption observed therefore under the influence of pancreatic extract may justifiably be attributed to the increased metabolism of the heart consequent on the acceleration of its rate. We have therefore not troubled to carry out many experiments on the influence of pancreatic extract. The following experiment represents one in which pancreatic extract was added. The consumption of sugar was rather greater than that observed in most of the other experiments on diabetic hearts but in view of the acceleration caused by the extract the difference is probably devoid of significance.

TABLE VIII. *Diabetic heart lung preparation stimulated by extract of pancreas.*

									Sugar				Glycogen	
No.	Remarks	Temperature	Time	B.P.	V.P.	Rate	Sugar added	Heart weight	% before	% after	mg. per g. of heart per hr.	total mgms. per hr.	heart %	liver %
1	Diabetic 3 days (levulose)	36	2	95	50	168	+	42	·50	·42	1·6	72	·72	·05

The Consumption of Sugar in other Tissues of the Body. Macleod and Pearce(10) have recently drawn attention to the fact that the rate of disappearance of sugar from the blood circulating through an animal after cutting the liver out of the circulation is the same in diabetic animals as in normal animals. Similar experiments were performed by us at the beginning of our work. Although the conditions are too complex to allow of satisfactory analysis, the results, so far as they go, agree with those obtained by MacLeod and Pearce and tend therefore to confirm their conclusions, as well as those drawn by us from the experiments on the heart lung preparation. The experiments were as follows:

TABLE IX. *Experiments on eviscerated animals.*

1. Eviscerated, liver and kidneys ligated off; adrenals intact; urethane intravenously.

Blood sugar at outset (Rona-Michaelis & Bertrand)	·43 %
,, ,, after 2 hrs. ,, ,, ,,	·31

2. Eviscerated, liver and kidneys ligated off; adrenals intact; A.C.E. mixture.

Blood before evisceration	·25 %
,, after ,,	·23
,, 2 hrs. after evisceration	·04

3. Depancreatised 5 days; eviscerated; adrenals intact; A.C.E. mixture.

Blood before evisceration	·355 %
,, after ,,	·325
,, 2 hrs. after evisceration	·095

The question arises what significance we are to attach to the results obtained by MacLean and Smedley. It is possible that here, as in our experiments, the glycogen of the heart muscle may be a disturbing factor. Cruickshank has shown that glycogenolysis goes on in the heart after death as rapidly in the diabetic as in the normal animal. It is possible therefore that in the heart perfused with Ringer's fluid a slow glycogenolysis proceeds in the course of the experiment. Special experiments have shown that sugar produced in this way does not diffuse out into the perfusing fluid; but it may nevertheless be utilised by the muscle cells within which it is produced, so that the actual consumption of sugar deduced from the loss of this substance in the circulating fluid would not take into account the sugar derived from the glycogen and would therefore be inferior to the actual consumption of sugar by the beating heart. The apparent consumption of sugar in normal and diabetic hearts in MacLean and Smedley's experiments may therefore be occasioned by the great excess of glycogen in the diabetic hearts and by the fact that the sugar derived from this glycogen met the needs of the heart and so masked altogether the consumption of sugar by the heart muscle as judged by analysis of the perfusing fluid. As we have said we are inclined as a result of our work to abandon the view that the essential or at any rate, the primary factor in diabetes is the absence of power on the part of the tissues to consume sugar.

REFERENCES.

(1) Knowlton and Starling. This Journal, XLV. p. 146. 1212.
(2) MacLean and Smedley. This Journal, XLV. p. 463. 1913.
(3) Rona and Michaelis. Biochem. Ztsch. VII. S. 329. 1908.
(4) Höber. Biochem. Ztsch. XLV. S. 207. 1912.
(5) Martin and Embley. This Journal, XXXII. p. 147. 1905.
(6) Bertrand. Bull. d. Sci. Pharm. XIV. p. 7. 1907.
(7) Evans and Starling. This Journal, XLVI. p. 413. 1913.
(8) Evans. This Journal, XLV. p. 213. 1912.
(9) Cruickshank. This Journal, XLVII. p. 1. 1913.
(10) Macleod and Pearce. Ztrlb. f. Physiol. XXVI. S. 1311. 1913; and Amer. Journ. Physiol. XXXII. p. 184. 1913.