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THE CARBOHYDRATE METABOLISM OF THE ISOLATED HEART OF THE FROG.

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I. Changes in carbohydrate content during perfusion.

The authors noted in a previous paper [1931] that, after frogs' hearts had been perfused for 6 hours with Ringer's fluid, the average content of reducing substances was 1·57 p.c., which was higher than the average obtained with unperfused hearts (1·42 p.c.); moreover, during perfusion sugar was excreted equal to 0·14 p.c. of the heart weight. Our figures therefore showed a clear increase in reducing substances during perfusion. We repeated these observations on a fresh series of frogs, using the same methods as before. The only change was that a Barcroft apparatus was used with a large bulb in which three hearts could be placed. This represented an important saving in time. In addition to measuring the changes in carbohydrate content, etc., in hearts perfused with Ringer's fluid, we also investigated hearts perfused for 6 hours with fluids that depressed the mechanical activity of the isolated heart, *e.g.* calcium-poor Ringer's fluid and Ringer's fluid containing 0·9 molar ethyl alcohol. The carbohydrate content of the frogs' hearts varied, both as regards the season of the year and the duration of captivity, and therefore control estimations on unperfused hearts were made throughout the course of the experiments. The values obtained at different periods are shown in Table I. The effects of perfusion on the

Table I. Total reducing substances in unperfused hearts.

Period	No. of hearts	Average weight in g.	Average p.c. of reducing substances
(*a*) April, 1931–July, 1931	24	0·147	1·228
(*b*) Oct. 1931–Nov. 1931	20	0·165	1·197
(*c*) Dec. 1931–Jan. 1932	10	0·124	1·553

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hearts are shown in Table II. In the experiments with calcium deficiency and alcohol the perfusion fluid was adjusted so that the heart's activity was reduced until it just maintained a circulation. Table II shows that

TABLE II.

Date (1931)	Nature of perfusion fluid	Duration of perfusion in hours	No. of hearts	Average heart wt. in g.	Reducing substance in p.c. (a) Perfused hearts	Reducing substance in p.c. (b) Controls	Sugar excreted per heart in mg.	Nitrogen excreted per heart in mg.	Oxygen used per heart in c.c.	R.Q.	Loss or gain of reducing substance after perfusion in mg. per heart: Loss	Loss or gain of reducing substance after perfusion in mg. per heart: Gain
April–June	Normal Ringer's fluid	6	11	0·151	0·870	1·228	0·055	0·063	1·26	0·89	0·48	—
Dec.	do.	24	9	0·120	0·966	1·553	—	—	—	—	<0·70	—
May	Ringer containing about 0·004 p.c. $CaCl_2$	6	15	0·169	1·417	1·228	0·024	0·052	0·44	0·87	—	0·34
Oct.	do.	6	15	0·130	1·330	1·197	0·13	—	0·35	0·88	—	0·30
Oct.	Ringer containing ethyl alcohol 0·9 molar	6	9	0·152	0·969	1·197	0·29	0·031	0·45	0·85	0·06	—
Oct.	Ringer plus 5 p.c. inositol	6	9	0·147	0·685	1·197	0·26	0·023	0·60	0·86	0·50	—

perfusion for 6 hours with Ringer's fluid caused a loss of reducing substances. In our previous experiments, already mentioned, we found an increase under these conditions. The loss of 0·48 mg. per heart shown by the averages in the present experiments was largely due to unusually low values obtained with three hearts in one experiment. The remaining eight hearts showed an average loss of only 0·27 mg. reducing substance per heart. Hearts suffering from lack of oxygen rapidly turn carbohydrate into lactic acid, hence any error in the technique of perfusion will tend to produce a loss of reducing substance; we believe that the loss in this case was due in part to an experimental error, particularly because the 24-hour experiments showed only a slightly greater loss of reducing substances.

The experiments with inositol were made to see if this substance spared the carbohydrate content of the heart. The hearts were definitely injured by the presence of inositol as is shown by their low oxygen consumption, hence the carbohydrate loss in this case has little significance, except that it shows that inositol does not spare the carbohydrates in the heart.

The hearts perfused with solutions that reduced their mechanical activity and oxygen consumption show either a gain or no significant loss of carbohydrate as a result of perfusion. A gain in reducing substance during 6 hours' perfusion is shown even if variations in the

carbohydrate content of the controls be ignored and general averages of all our experiments be taken. From Jan. 1930 to Jan. 1932, 90 estimations were made of the total reducing substance in fresh hearts, and the average value of these estimations was 1·32 p.c. total reducing substance. In the same period 28 hearts perfused with normal Ringer's fluid for 6 hours gave an average total reducing substance content of 1·30 p.c., and 39 hearts perfused for 6 hours with Ringer's fluid containing depressants gave an average of 1·28 p.c. The average sugar excretion in both cases was equivalent to about 0·1 p.c. of the heart weight, and hence the reducing substance in the heart and fluid after perfusion in the two cases averaged 1·40 and 1·38 p.c. values, which are definitely above the control value of 1·32 p.c.

Another method of showing our results is to calculate the reducing substance recovered as the percentage of the reducing substance present in the controls. The two general averages just given for 6 hours' perfusion work out at 105 and 106 p.c. respectively. Table III shows a

TABLE III. Carbohydrate and oxygen usage of hearts.

Experimental conditions	Duration of perfusion in hours	Total reducing substance as mg. per g.		Sugar excreted mg. per g. heart	Total reducing substance recovered expressed as p.c. of that present in controls	Oxygen used c.c. per g. heart
		Present in controls	Present in perfused heart			
II. Previous exps. [Clark *et al.*, 1931]:						
Perfused Ringer's fluid	6	14·2	15·7	1·35	120	7·6
,, ,, ,,	24	14·2	7·3	3·0	72·5	18·0
Perfused Ringer-serum mixture	6	14·2	10·5	0	74	11·4
	24	14·2	4·8	0	34	22·8
II. Present exps.:						
Perfused Ringer's fluid	6	12·28	8·70	0·36	74	8·3
,, ,, ,,	24	15·53	9·66	—	62	—
Perfused calcium-poor Ringer's fluid						
1st series	6	12·28	14·17	0·14	116	2·62
2nd series		11·97	13·30	1·0	119	2·69
Perfused ethyl alcohol 0·9 molar	6	11·97	9·69	1·9	96	2·96

series of values of this kind calculated for both the present experiments and certain experiments recorded in our previous paper. In order to make the figures comparable they have all been calculated on the basis of carbohydrate and oxygen used per g. of heart.

The results recorded in Table III are shown as a graph in Fig. 1. The figures show a considerable scatter, but taken as a whole they indicate that during perfusion a quantity of reducing substance appears from some unknown source which corresponds to 20 p.c. of the quantity present in the controls or to 0·4 mg. in a heart weighing 0·15 g.

Similar conclusions have been made by other workers. Wertheimer [1930] found no certain change in either the glycogen or total carbohydrate contents of heart strips of the frog after isolation for many hours. Hines, Katz and Long [1925] showed that the normal glycogen content

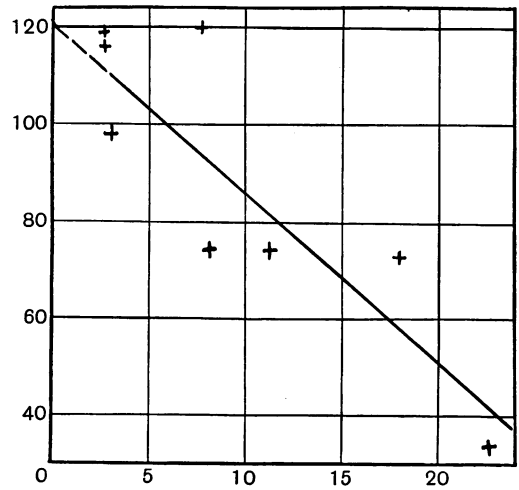

Fig. 1. Relation between oxygen consumption and carbohydrate loss in the perfused frog heart. Figures taken from Table III. Abscissa: total oxygen consumption in c.c. per g. Ordinate: total reducing substance recovered expressed as percentage of values found in appropriate controls.

of the heart muscle of cats was 0·138 p.c., whereas the lactic acid maximum in rigor mortis was 0·32 p.c. Boyland [1928] studied the production of lactic acid in minced heart muscle of the tortoise and of the frog incubated with alkaline phosphate mixture. He concluded that a quantity of lactic acid corresponding to between 0·1 and 0·2 p.c. of the muscle weight must be produced from a source other than the reducing carbohydrates. This agrees fairly well with the excess of reducing substances found by us, which corresponded to about 0·3 p.c. of the heart weight. Boyland considered that inositol was a likely source of the excess of lactic acid. He found 0·37 p.c. inositol in pig's heart. He found that the inositol in the heart decreased on incubation with phos-

phate mixture, and that addition of inositol to such a system increased the amount of lactic acid formed.

Our conclusion that a certain quantity of reducing substance is formed from some non-reducing source agrees therefore with the results obtained by other workers. One obvious possible source of error was the incomplete hydrolysis in the estimation of total reducing substances. The hearts were hydrolysed for 3–4 hours, and the following experiment showed that prolongation of the time of hydrolysis did not increase the total reducing substances.

Exp. Eight frog hearts, total weight 0·626 g., were hydrolysed for 24 hours and the total reducing substances were estimated in fractions at varying times. (The amount found was twice the usual value, but the frogs used were from a different source.)

Duration of hydrolysis in hours	3	5	7	24
Total reducing substance found as p.c. of moist weight	2·86	2·83	2·83	2·44

Exclusion of this source of error left the following problems to be investigated:

(*a*) The possibility of the presence of non-carbohydrate reducing substances which alter in amount during perfusion.

(*b*) Possible variation in the amount of lactic acid formed.

(*c*) The possibility of inositol being converted into a reducing substance.

(*a*) *The nature of the total reducing substance.*

We have previously shown [1931] that the substances included in our estimate of total reducing substance or total carbohydrate do not include creatine, the most obvious non-sugar likely to be included. Our results showing the apparent production of reducing substance during perfusion of the heart, however, rendered it necessary to examine more closely the nature of the substances estimated. For this purpose we used the fermentation method described by Somogyi [1927]. It was found that in fresh non-perfused hearts about 90 p.c. of the total reducing substance was fermentable, whereas pure glucose under similar conditions was completely fermented. In other words, of the 1·50 g. total reducing substance normally present in 100 g. of heart, 0·150 g. was non-fermentable. After perfusion for 24 hours the heart still contained 0·144 p.c. of non-fermentable reducing substance. Hence, if we are to consider only fermentable reducing substances, the figures for total carbohydrate must be reduced by an average amount of 0·150 p.c., but as the correction is the same for perfused and non-perfused hearts, all conclusions founded on the original figures are unaffected.

Although the presence of this non-fermentable reducing substance in the heart does not affect our main conclusions, it may possibly help to explain the curious fact that we rarely found a perfused heart to contain much less than 0·40 p.c. of total reducing substance, even under conditions such as anaerobiosis with alkaline perfusing fluid, which ought to produce complete exhaustion.

Rimington [1931] found that a number of proteins, on hydrolysis, yielded 3–4 p.c. of a trisaccharide which on further hydrolysis gave 2 mol. mannose (fermentable) and 1 mol. of glucosamine (non-fermentable). Our method of estimating total reducing substance involves a fairly prolonged preliminary hydrolysis, which might well split off and hydrolyse most, if not all, of this carbohydrate. Now the heart contains approximately 8 p.c. of protein which, on the basis of a 3·7 p.c. content of trisaccharide, would account for 0·296 g. reducing substance (calculated as glucose) per 100 g. of heart, and of this one-third or 0·10 g. would be non-fermentable.

The figures show that the residual reducing substance not fermented with yeast forms a small fraction of the total reducing substance, and that it remains unchanged during prolonged perfusion. The presence of this unknown material cannot account for the rise observed in total reducing substance during short periods of perfusion.

(*b*) *The formation of lactic acid.*

The authors have studied this subject and it will be dealt with in a later paper. The facts essential to the present discussion are that hearts, when perfused with good oxygenation, form scarcely any lactic acid, but that interference with the oxygen supply at once causes the breakdown of carbohydrate and the production of lactic acid. Any interference with the circulation of fluid through the heart may therefore cause a loss of carbohydrates. Hence the most probable error in our experiments is a conversion of carbohydrate to lactic acid during perfusion, and this would tend to increase the apparent loss of carbohydrate without increasing the oxygen consumption. If chemical treatment caused lactic acid formation more readily in the fresh heart than in the heart after perfusion this might explain the increase in carbohydrate content that we observed.

We found that the lactic acid content of fresh hearts frozen with carbon dioxide snow was 0·06 p.c., and treatment with acid at room temperature raised this value to 0·09 p.c. This value was the same in fresh hearts and in hearts after perfusion, and leaving hearts lying in

Ringer's fluid without oxygenation did not raise this value. We have therefore no reason to suppose that lactic acid formation during chemical treatment would alter the relative values of the carbohydrate content of the fresh and of the perfused hearts.

(*c*) *Inositol as a source of reducing material.*

Boyland [1928] suggested that inositol might act as a source of reducing substances. We found that it was present in the hearts both of mammals and skates.

The method used to estimate inositol in heart muscle was practically that described by Needham [1926]. Minced tissue was extracted for 24 hours with acetone, sufficient being used to give, with the water already present, a concentration of 70 p.c. acetone. The acetone was then removed *in vacuo* and the aqueous residue precipitated with lead acetate, and finally with basic lead acetate and a little ammonia (freshly prepared). The former precipitate was discarded, and the latter, which contained inositol, thoroughly decomposed with hydrogen sulphide. The clear filtrate obtained after removal of lead sulphide was concentrated to a few c.c. on the water bath. Inositol was precipitated by alcohol and ether, filtered off, dried, and weighed.

This method was applied to bullock's heart and to the heart of the skate, and their inositol contents were found to be respectively 0·10 p.c. and 0·073 p.c. of wet heart weight. We were unable to get any proof either direct or indirect of the conversion of inositol to hexoses. Various workers have described a stimulant action of inositol on the heart [Chevalier and Brissemoret (1908, rabbit's heart); Sachs, 1906; Hewitt and de Souza, 1921]. The effects recorded are, however, only slight.

Inositol added to perfusion fluid did not appear to have any carbohydrate sparing action on the heart in presence of oxygen (cf. Table I). This is inconclusive, because glucose has little action under such conditions, but the figures also show that no measurable conversion of inositol to sugar occurred. We found that glucose had a strong specific stimulant action on the heart of the frog exhausted by perfusion with alkaline fluid in the absence of oxygen, but inositol produced no benefit under these conditions. We cannot therefore produce any evidence in support of Boyland's suggestion.

II. The metabolism of the isolated frog's heart.

Table II shows the carbohydrate balance and oxygen consumption in five sets of experiments. In three of these no carbohydrate loss appeared, and in the other two cases the carbohydrate loss was 0·48 and

0·50 mg. This last figure corresponds to an oxygen consumption of $0{\cdot}5 \times 0{\cdot}75 = 0{\cdot}38$ c.c., which is about 30 p.c. of the oxygen consumption recorded with Ringer's fluid. The figures with inositol are scarcely relevant as the hearts were partially poisoned. The respiratory quotients obtained all lay between 0·85 and 0·9, and therefore definitely indicated a considerable carbohydrate consumption. On the other hand the nitrogen excretion was in no case sufficient to account for more than a fraction of the oxygen consumption. Fig. 2 shows the general relation between nitrogen excretion and oxygen usage found in our present and previous experiments [Clark *et al.* 1931, Table III]. This indicates that the excretion of 0·6 mg. nitrogen is associated with 10 c.c. oxygen usage;

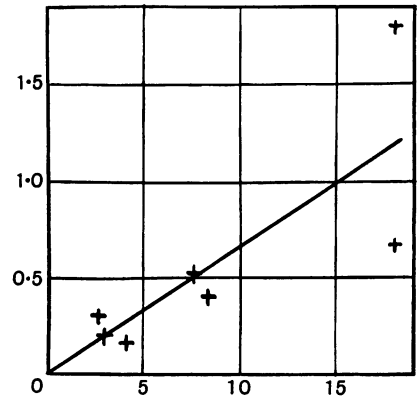

Fig. 2. Relation between oxygen consumption and nitrogen excretion. Figures from Table II and from Clark, Gaddie and Stewart [1931, Tables III and X]. Abscissa: oxygen consumption in c.c. per g. Ordinate: nitrogen excretion in mg. per g.

if the oxidation of material containing 1 mg. of nitrogen uses 6 c.c., then the oxidation of material containing 0·6 mg. uses 3·6 c.c. The nitrogen excretion observed can therefore account for about 36 p.c. of the oxygen used. Fig. 1 indicates the essential difficulty met with in our experiments, namely, that the carbohydrate content of the heart and the perfusion fluid together only falls to the level of the control values after an oxygen consumption of about 6 c.c. per g. of heart, a quantity which is used by a heart after about 4 hours' perfusion with normal Ringer's fluid. The only way to meet this difficulty and to balance the oxygen consumption with the metabolic changes is to assume the production during perfusion of reducing and fermentable substances amounting to about 20 p.c. of the initial content. If this is done an approximate balance

can be produced. Fig. 1 shows that, as a general average, the consumption of 10 c.c. of oxygen is associated with the disappearance of 40 p.c. of the carbohydrate in 1 g. of heart. The general average for our controls is 1·32 p.c. reducing substance or 13·2 mg. per g., and 40 p.c. of this is 5·28 mg. This corresponds to an oxygen consumption of 3·96 c.c. oxygen, or 39·6 p.c. of the oxygen consumed.

The nitrogenous and carbohydrate metabolism demonstrated can therefore account for 36 + 39·6 or 76 p.c. of the oxygen consumption observed, provided that we assume that during the first few hours' perfusion there is a production of reducing substance from non-reducing material. The figures thus obtained suggest an equal oxidation of protein and of carbohydrate. The figures for nitrogen excretion are, however, minimum figures, whereas any experimental errors leading to deficient oxidation would increase the carbohydrate consumption by causing lactic acid formation. Hence the evidence indicates that oxygen consumption due to protein metabolism is greater rather than less than the carbohydrate metabolism. If the metabolism were 60 p.c. protein and 40 p.c. carbohydrate, this would give a R.Q. of 0·88, and 32 experiments in which hearts were perfused with Ringer's solution for 6 hours gave an average value for the R.Q. of 0·87.

The obvious objection to the hypothesis outlined is that we have been unable to discover any probable source for the reducing substance that appears in such a mysterious manner. We believe that this will have to await further advances in our knowledge of carbohydrate metabolism, but have thought it desirable to publish these results because they indicate that the cardiac metabolism is not so completely unlike that of skeletal muscle as was suggested by our previous paper.

SUMMARY.

1. At least 90 p.c. of the total reducing substance found in the fresh heart is removed by fermentation. The quantity of non-fermentable reducing substance does not alter during perfusion.

2. After perfusion with calcium-poor Ringer the carbohydrate content of the perfused frog's heart is higher than that of the controls.

3. The evidence suggests that a quantity of carbohydrate equivalent to about 20 p.c. of the total amount found in the controls is produced during perfusion from some unknown source.

4. We have been unable to obtain any evidence to prove the consumption of inositol by the frog's heart.

5. The assumption that fermentable reducing substance is produced from a non-carbohydrate source makes it possible to account for 76 p.c. of the oxygen consumption observed. According to this hypothesis the carbohydrate consumption would amount to about 40 p.c. of the total metabolism, which would give an R.Q. of 0·88.

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