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THE EFFECT OF INSULIN ON THE RESPIRATORY QUOTIENT, OXYGEN CONSUMPTION, SUGAR UTILIZATION, AND GLYCOGEN SYNTHESIS IN THE NORMAL MAMMALIAN HEART IN HYPER- AND HYPOGLYCÆMIA.

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While attention has been largely focused on the action of insulin on the mammalian organism as a whole under conditions of hyperglycæmia or maintained blood-sugar levels, comparatively little attention has been directed to the action of insulin under hypoglycæmia conditions. The many problems of carbohydrate metabolism which have been opened up by the discovery of insulin still demand that the functions of the organs of the mammalian body be individually investigated as a logical step towards the elucidation of the action of this hormone upon the body considered as a whole.

The results to be presented and discussed here are the outcome of previous work upon the action of insulin on the normal and diabetic heart. In certain respects the diabetic heart does not apparently behave as does skeletal muscle and whether or not the normal heart should be expected to conform in all functional respects to skeletal muscle is still an open question. That one of the best means by which to determine the reaction of skeletal muscle to insulin is the use of the eviscerated spinal preparation as suggested by Burn and Dale [1924] and others few will deny; but that it is a good standard by which to compare the results of similar investigations upon cardiac muscle is a moot point. And further, it is essential that, in assessing the value of results obtained on cardiac muscle, one should not be obsessed by the findings recorded for skeletal muscle. Such investigation demands that the heart be allowed to function in as normal a condition as possible: to remove the heart from the body of an animal, wash it in saline or Locke-Ringer solution, remove adherent tissue from it, attach it to a perfusion apparatus and then perfuse it with Locke-Ringer solution or diluted blood is certainly not the best means of securing figures indicative of the normal reactions of that organ. Despite its disabilities the heart-lung preparation of Starling

is still of value; the preparation can be completed in 15 minutes, the heart suffers no handling, is supplied with its own blood, and the lungs assure an efficient oxygenation of the blood, and where the gaseous metabolism of the heart-lung preparation must be determined both the circulatory system and the respiratory system can be effectively closed.

METHODS.

To arrive at an accurate idea as to the gaseous metabolism of the heart, oxygen and CO_2 determinations were carried out in a manner which will be briefly described.

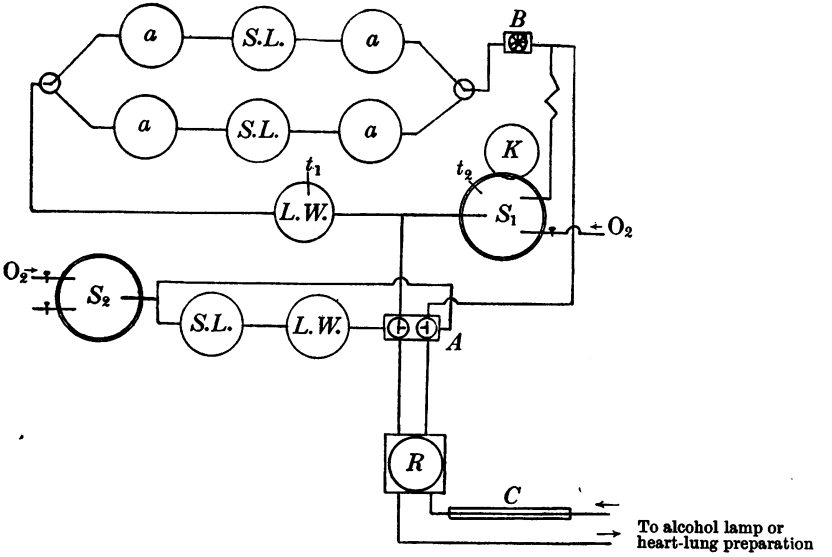

Fig. 1. Diagram of apparatus. *A*, stopcocks for connecting or disconnecting alcohol lamp or isolated heart-lung preparation from the recording system; *B*, rotary blower; S_1, spirometer with *K*, kymograph for record of oxygen consumption; S_2, reserve spirometer; *R*, respiration pump; *SL*, soda lime bottle; *a*, acid bottle; *LW*, lime water bottle; *C*, condenser; t_1, t_2, thermometers reading to 0·1° C.

Description of apparatus. An examination of Fig. 1 and its legend will show the general arrangement of apparatus. It will be seen that there are two spirometers each with a CO_2 absorbing system, with either of which the lungs of the animal may be connected by stopcocks *A*. In the case of a mammalian experiment this permits of efficient oxygenation of the blood with a removal of CO_2, thus allowing the blood CO_2 content to fall to a steady low level before commencing the experiment. It will be noted that when the animal is turned into the system associated with

spirometer S_1, no expired air can enter the spirometer. In order to ensure this, the tube marked *D*, leading from the spirometer to the rotary blower, is of 300 c.c. capacity. A large portion of the expired air passes into this tube before being passed on by the rotary blower *B*, simply because the velocity of the expiration due to the elasticity of the lungs with a respiration rate of eighteen per minute and a tidal air volume of 150 c.c. may be as high as 200 c.c. per sec. It is unnecessary to circulate the air at a rate of 28 to 30 litres per minute; 7 to 12 litres per minute is ample and allows of a complete absorption of CO_2.

The temperature is noted by thermometers reading to 0·1° C. in Williams's bottles, t_1, t_2, and in the cannula which passes the blood to the heart. Soda-lime is kept in Williams's bottles, not in the usual soda-lime bottles, which have been found unsatisfactory. When the Williams's bottles with soda lime and acid have been weighed and placed in position the rotary blower is started, and the air in the system with spirometer S_1 circulated for 15 minutes to bring it to a constant temperature. Meanwhile the lungs are being artificially inflated by a Starling Ideal pump placed in oil, the air being circulated through a CO_2 absorbing system and spirometer S_2 which has previously been filled with oxygen. When the temperature at t_1 is steady the lungs are switched into connection with S_1 by means of the stopcocks *A*, the kymograph and stop-watch are started and the temperatures noted. At any time the circulating air can be turned into the parallel absorbing system and a new period started.

Alcohol lamp check experiments on the apparatus. Absolute ethyl alcohol of 99·95 p.c. purity and having a sp.gr. of 0·794 was used. The oxygen equivalent of the alcohol was, 1 c.c. = 1159·60 c.c. of O_2. The factor for the large spirometer bell is calculated from the steel mould from which it is made. This mould had a diameter of 16·25 cm., which gives a factor of 20·73 c.c. per mm. excursion of the bell. For the estimation of small amounts of oxygen consumed by the heart we had made for us a very finely balanced spirometer, the bell of which is 7·55 cm. diameter and 34 cm. in height, having a factor of 4·461 c.c. per mm.

The system as described, and as shown in Fig. 1, was tested for leaks by recording movements of the spirometer bell for 15 minutes, in which time the recording kymograph drum made one complete revolution.

Respiratory quotients with the alcohol lamp. Reference to Table I will show the details of ten check experiments. The experimental error for the estimation of oxygen consumed averages 0·667 p.c., that for R.Q.'s averages 0·225 p.c. In the case of organs where the amounts of oxygen utilized per minute is very small it is possible to secure, with the smaller

spirometer, a continuous and very accurate record of oxygen utilization over periods extending from 1, 2 or more hours, depending upon the gradient of the curve of oxygen consumption.

TABLE I. R.Q. check experiments with alcohol lamp.

No.	Alcohol c.c.	Spirometer mm.	t_2 change ° C.	t_1 change ° C.	Theoretical O_2 c.c.	Exp. O_2 c.c.	Percentage O_2 rec.	CO_2 c.c.	R.Q. exp.
1	1·42	85	0·20	0·20	205·90	205·53	99·71	136·82	0·665
2	1·76	107	0·20	0·00	132·10	131·65	99·65	87·94	0·668
3	2·45	154	0·04	0·10	189·46	193·01	101·87	130·85	0·677
4	2·78	172	0·00	0·12	214·98	214·65	99·89	145·88	0·678
5	1·75	111	0·10	0·05	203·00	205·48	101·02	136·38	0·664
6	1·42	90	0·05	0·10	205·90	210·60	102·27	143·10	0·675
7	2·20	157	0·00	0·02	216·92	217·15	100·10	144·08	0·664
8	1·90	118	0·20	0·03	220·40	220·89	100·22	146·05	0·661
9	2·03	129	0·03	0·00	235·48	239·35	101·64	158·77	0·663
10	1·85	115	0·00	0·00	214·61	215·28	100·31	143·50	0·666

Time of experiments 10 min. by stop watch, except Nos. 1 and 6 = 8 min.
Nos. 2 and 3 = 15 „

Average R.Q. = 0·6681
Theoretical = 0·6666
Average p.c. O_2 recovered = 100·66

The isolated heart-lung preparation needs no description, suffice it to add that the reservoir, in which the blood is usually exposed to the external air, was effectively closed by being connected to a small spirometer filled with nitrogen. The fall in the bell of this spirometer indicated with extreme accuracy any alteration in the blood volume of the preparation.

Sugar estimations were made by the Shaffer and Hartmann [1920] method, lactic acid by West's modification of the method described by Friedemann, Cotonio and Shaffer [1927]. Numerous experiments have been carried out and, as these entail the presentation of many figures, only two or three typical examples of each will be given. For convenience in discussing the subject-matter the work will be presented under the following divisions.

Part I. *Hyperglycæmia with and without insulin.*

(1) The utilization of sugar.
(2) The respiratory quotient and oxygen consumption.
(3) The synthesis of glycogen.

Part II. *Hypoglycæmia with and without insulin.*

(1) The utilization of sugar.
(2) The respiratory quotient and oxygen consumption.
(3) The utilization or synthesis of glycogen.

Part I. Hyperglycæmia.

(1) *The utilization of sugar.*

In discussing the question of sugar utilization it is essential not to confuse sugar utilization by the heart with the amount of sugar disappearing from the circulating blood. The early experiments of Locke and Rosenheim [1907], Knowlton and Starling [1912*a*], Mansfeld [1914], Patterson and Starling [1913], and later experiments of Hepburn and Latchford [1922], Burn and Dale [1924], of Cruickshank and Shrivastava [1930] and of many others have shown that the mammalian heart removes sugar from the blood, the figures, under varied experimental conditions, ranging from 2 to 6 mg. per g. of heart muscle per hour. In using the isolated heart-lung preparation to determine sugar utilization by the heart, Starling and Evans [1914] allowed 1·3 mg. per g. of heart per hour for the sugar consumption of the lungs and the blood. Cruickshank and Startup [1930] place the sugar consumption of the dog's lungs at 0·7 to 0·8 mg. per g. of heart muscle per hour. The rate of disappearance of sugar from circulating well-oxygenated blood has been investigated by Cruickshank and Startup [1932] and they have shown that a progressive oxidation of sugar takes place amounting to approximately 10 p.c. of the total blood sugar in the first hour and about 14 p.c. in the second hour. No glycolysis takes place in this time, provided oxygenation is good. It would seem, therefore, from these facts that a correction of 1 mg. per g. of heart muscle per hour should be made for utilization of the lungs in determining absolute figures for the sugar usage of the heart. This correction has not been made in the tables presented in this paper.

In hyperglycæmia without insulin the utilization of sugar is increased from an average normal of 5·28 to 5·84 mg. per g. of heart muscle per hour. The addition of insulin increases the sugar consumption to the average figure of 6·06 mg. per g. per hour. In these experiments the blood-sugar level was never in excess of 0·218 g. p.c. Where the sugar content is increased to 0·40 g. p.c. or more there is not a proportional increase in the utilization under insulin. The addition of sugar to the blood whereby a moderate condition of hyperglycæmia is maintained raises sugar oxidation 10·5 p.c.

The effect of insulin. The addition of ten units of insulin per hour has a much less effect than excess sugar in stimulating sugar oxidation (Table II); the average percentage increase being 3·62. In any discussion of sugar utilization, when the disappearance of blood sugar is in question,

TABLE II. Hyperglycæmia.

The effect of hyperglycæmia and of added insulin upon the R.Q. oxygen consumption and sugar oxidation.

Hour of exp.	Blood sugar g. p.c. Begin	Blood sugar g. p.c. End	CO_2 c.c.	O_2 c.c.	R.Q.	Oxygen c.c. per g. per hr.	Sugar oxidized mg. per g. per hr.	Lactic acid mg. p.c. Begin	Lactic acid mg. p.c. End	
1	0·1128	0·0859	362·29	357·71	1·012	4·47	5·97	31·5	28·8	Normal
2	0·1701	0·1419	381·68	375·67	1·016	4·70	6·25	31·5	27·0	Insulin
1	0·1432	0·1008	302·51	273·40	1·106	4·43	5·78	54·9	55·8	Normal
2	0·1863	0·1244	279·89	283·50	0·980	4·50	6·00	55·8	58·5	Insulin
1	0·1708	0·1206	361·31	363·45	0·999	4·33	5·78	27·2	28·8	Normal
2	0·2218	0·1244	369·99	375·40	1·018	4·46	5·93	31·5	27·9	Insulin
Average										
	—	—	—	336·60	—	3·96	5·28	Normal, 6 exps.		
	0·1422	0·1039	342·04	331·55	1·039	4·376	5·84	With sugar		
	0·1927	0·1319	343·85	344·85	1·004	4·553	6·06	With insulin		
	Increase due to sugar					10·50	10·50			
	Increase due to insulin					3·62	3·62			

one must clearly discern between oxidation and synthesis. As this involves a discussion of both sugar and oxygen consumption in the presence of insulin and also of the direct action of insulin on the heart muscle, the matter will be fully referred to under the section dealing with the effect of insulin upon the oxygen consumption of the heart in hypoglycæmia.

(2) *The respiratory quotient and oxygen consumption.*

The normal isolated heart in the presence of an adequate supply of blood sugar has always given a R.Q. which is unity. Bayliss, Müller and Starling [1928] find a R.Q. for the isolated heart of 0·90, a figure determined after 3 hours have been spent upon the operation and on the setting up of the apparatus. In our experiments the preparation is completed in 15 to 20 minutes and runs for periods of 1, 2 or 3 hours. The average figures for the utilization of oxygen (Table II) show how small is the effect, first of added sugar and second of sugar plus insulin. With a maintained blood sugar the heart consumes approximately 4 c.c. O_2 per g. of heart muscle per hour. Upon the addition of sugar, raising the percentage to an approximate level of 0·15 g., the oxygen consumption per g. per hour rises to an average of 4·376 c.c. while the subsequent addition of ten units of insulin raises the figure to 4·553 c.c. per g. per hour, an increase of 3·62 p.c. As with added sugar so with insulin; the increase in oxygen consumed is determined by the amount

of sugar utilized. Figures for the lactic-acid content of the blood show that changes in the production of lactic acid are of such a small order in the first experimental hour that they cannot materially alter the results obtained on the oxygen consumption and sugar utilization by the isolated heart in hyperglycæmia.

(3) *Glycogen synthesis.*

This was determined by correlating the blood sugar lost with the sugar equivalent of the oxygen consumed.

With the maintenance of the blood-sugar level above the normal there is a slight increase in the deposition of glycogen in the heart. Upon the addition of insulin there is a marked increase in the amount of sugar deposited as glycogen in the heart. Table III shows that of the

TABLE III. Hyperglycæmia.

Effect of insulin on the absolute and percentage amounts of blood sugar oxidized and stored by the mammalian heart.

(Blood-sugar percentages and R.Q.'s are shown in Table II.)

Exp.	Total sugar lost g.	Sugar oxidized g.	Sugar stored g.	Percentage blood sugar oxidized g.	Percentage blood sugar stored g.	Wt. of heart g.	Possible glycogen in heart g.	Percentage increased in heart glycogen	
I	0·4907	0·4769	0·0138	97·2	2·8	80	0·400	3·5	Normal
	0·6405	0·5008	0·1397	78·2	21·8		0·414	35·0	Insulin
							0·553		
II	0·3752	0·3644	0·0108	97·2	2·8	63	0·315	3·1	Normal
	0·5054	0·3780	0·1274	74·8	25·2		0·325	39·1	Insulin
							0·452		
III	0·5004	0·4858	0·0146	97·1	2·9	84	0·420	3·3	Normal
	0·6564	0·5005	0·1560	76·4	23·6		0·434	36·2	Insulin
							0·590		

The percentage increase in the oxidation of sugar and the deposition of glycogen in the heart in hyperglycæmia with and without insulin (Tables II and III).

Hyperglycæmia alone		Hyperglycæmia with insulin	
Oxidation	Glycogen	Oxidation	Glycogen
10·5	3·30	3·62	36·70

The effect of hyperglycæmia with and without insulin upon the balance between the oxidation and synthesis of 100 parts of blood sugar.

Hyperglycæmia alone		Hyperglycæmia with insulin	
Oxidation	Synthesis	Oxidation	Synthesis
97·2	2·8	76·1	23·5

total amount of sugar which has disappeared from the blood, the amount deposited has increased from 2·8 to 23·5 p.c. These results are even more striking when we consider the relation between the total amount of blood sugar used for oxidation and that used for the synthesis of glycogen. Taking average figures; the amount of sugar oxidized increases from 0·4433 to 0·4736 g., an increase of 0·0313 g., that used for the synthesis of glycogen increases from 0·0131 to 0·1442 g., an increase of 0·1311 g. The former figure shows a negligible increase, the latter a tenfold increase.

If we assume that the heart muscle has an initial glycogen content of 0·500 g. p.c., estimated as sugar, we see from Table III that there has been an average increase from 3·3 to 36·7 p.c. These figures for glycogen deposition, which are in terms of the original amount of glycogen in the heart, are in remarkable agreement with the findings of Cruickshank and Shrivastava [1930] who found, by numerous direct estimations of heart glycogen in dogs, that at the end of 2 hours' insulin administration with a blood sugar not greater than 0·222 g. p.c., there was an average change from 0·535 to 0·719 g., a 34·3 p.c. increase.

From a consideration of such results it is fair to conclude that insulin has, as far as cardiac muscle is concerned in the presence of hyperglycæmia, a very marked stimulating effect upon glycogen synthesis, and plays but a small part in increasing sugar oxidation. It is also of interest under these conditions to note the effect of insulin on the percentage distribution of blood sugar between oxidation and synthesis. Normally the ratio is 97·2 to 2·8; with insulin it becomes 76·1 to 23·5, a ratio which is noteworthy, as will appear later.

Part II. Hypoglycæmia.

(1) *Utilization of sugar.*

It may be inferred that the heart is faced with a condition of hypoglycæmia when the blood sugar is not sufficient to supply fully the energy requirements of the organ, and as a result thereof a certain amount of glycogen has been utilized. This condition of affairs invariably happens when the blood sugar has been reduced to 0·05 g. p.c. In an isolated heart-lung preparation with a blood sugar of 0·0935 g. p.c. at the beginning and 0·0546 g. p.c. at the end of 1 hour's experiment, the amount of sugar utilized averages 4·55 mg. per g. of heart muscle per hour (Table IV). But the amount of sugar disappearing from the blood is not sufficient for the energy needs of the heart, with the result that there is a call upon the glycogen content of the heart. It can be seen from Table V that the

Table IV. Hypoglycæmia.

Effect of hypoglycæmia upon the R.Q., the oxygen consumption and sugar oxidation in the mammalian heart. Time = 1 hour.

Blood sugar g. p.c.		CO_2	O_2		Oxygen c.c. per g.	Sugar oxidized mg. per g. per hr.	Lactic acid mg. p.c.	
Begin	End	c.c.	c.c.	R.Q.	per hr.	g.	Begin	End
0·0979	0·0572	278·89	280·35	0·992	3·50	4·67	40·5	43·6
0·0928	0·0599	336·07	339·12	0·991	3·02	4·04	45·0	49·5
0·1121	0·0620	473·14	468·00	1·011	3·60	4·80	36·0	41·4
0·1009	0·0712	549·60	554·41	0·996	3·46	4·62	37·8	42·3
0·1019	0·0488	509·79	524·37	0·972	3·49	4·66	57·6	59·4
Average								
0·0935	0·0546	434·10	425·61	0·992	3·42	4·55		
Normal of 6 exps.		—	336·60	—	3·96	5·28		
Percentage fall below normal			—	—	13·90	13·90		

TABLE V. Hypoglycæmia.

Effect of hypoglycæmia on the glycogen content of the heart. (Blood-sugar percentages and R.Q.'s are shown in Table IV.)

Exp.	Blood sugar lost g.	Total sugar oxidized g.	Percentage sugar added to blood sugar for oxidation	Glycogen lost g.	Weight of heart g.	Possible heart glycogen g.	Percentage glycogen lost
I	0·2584	0·3738	44·6	0·1154	80	0·400	28·8
II	0·3342	0·4521	30·9	0·1184	112	0·560	21·1
III	0·5608	0·6240	11·3	0·0636	130	0·650	9·7
IV	0·5961	0·7392	23·9	0·1431	160	0·800	17·8
V	0·4828	0·6858	42·0	0·2030	150	0·750	27·0

Sugar added to blood sugar from heart reserves = 30·54 p.c.
Average loss of glycogen in first hour = 20·88 p.c.

blood sugar used has been augmented 11·3 to 44·6 p.c. by a call upon the carbohydrate reserve of the heart muscle. It is, of course, impossible to determine the glycogen content of the heart at the commencement of the experiment, but it is of interest to note the loss or gain of glycogen assessing the original heart glycogen from the assumption that the normal heart contains 0·500 g. p.c. of its weight as glycogen. It is an assumption based on the average results of numerous experiments dating from the year 1912; reference has already been made to the close parallel between these figures and those obtained from direct estimation of heart glycogen. On this assumption it is seen that there is a glycogen loss varying in five experiments from 9·7 to 28·8 p.c., with an average loss of 20·88 p.c. From such results one is led to the conclusion that the heart, when faced with a slight hypoglycæmia, draws upon its carbohydrate reserves

and, as will be seen later when the R.Q.'s are discussed, upon nothing else, at least not as far as can be judged from an experiment lasting only 1 hour. This is not in accord with what obtains in the eviscerated spinal cat, where a slight degree of hypoglycæmia is not accompanied by any marked loss in the glycogen of the skeletal muscle [Best, Hoet and Marks, 1926]. In the eviscerated preparation, however, liver glycogen may have been available, the blood-sugar level was never lower than 0·068 g. p.c., and no estimation of heart glycogen was made.

The effect of insulin. It has been seen that, when no sugar is added to maintain a constant blood-sugar level, the sugar utilization falls from the

TABLE VI. Hypoglycæmia.

The effect of insulin on the R.Q., oxygen consumption, and sugar utilization of the heart.

Hour of exp.	Blood sugar g. p.c. Begin	Blood sugar g. p.c. End	CO_2 c.c.	O_2 c.c.	R.Q.	Oxygen c.c. per g. per hr.	Sugar oxidized mg. per g. per hr.	Insulin units	Lactic acid mg. p.c. Begin	Lactic acid mg. p.c. End
1	0·1024	0·0592	356·23	353·63	1·007	4·21	5·61	10	34·2	36·9
2	0·0592	0·0246	346·05	360·50	0·960	4·29	4·74	10	36·9	43·2
3	0·0246	0·0246	277·08	357·48	0·775	4·25	1·42	10	43·2	40·5
1	0·1091	0·0605	381·57	352·30	1·082	4·14	5·52	10	45·0	40·5
2	0·0605	0·0447	305·34	356·40	0·860	4·20	2·85	10	40·5	36·9
3	0·0447	0·0313	264·44	356·11	0·744	4·20	0·72	10	36·9	22·5
1	0·0977	0·0413	356·23	364·50	0·988	4·18	5·58	10	27·0	27·0
2	0·0413	0·0261	305·34	380·75	0·802	4·38	1·83	10	27·0	24·0
1	0·1009	0·0712	549·60	554·41	0·996	3·46	4·62	0	37·8	42·3
2	0·0712	0·0506	503·80	574·12	0·877	3·58	2·77	10	42·3	25·2
1	0·1019	0·0488	509·79	524·37	0·972	3·49	4·66	0	57·6	59·4
2	0·0673	0·0315	492·13	550·88	0·910	3·66	3·47	10	54·9	41·4

Note. (1) In the two last experiments insulin was given at the commencement of the second hour.

(2) The amount of sugar oxidized for R.Q. given has been determined from the tables of Zuntz and Schumberg.

Average figures.

Normal of 6 exps.	336·60	—	3·96	5·28
Average weight of heart = 85·4 g.				

Hypoglycæmia with insulin from the beginning of the experiment.

First hour:			
Commencing blood sugar normal	1·028	4·17	5·56
Second hour:			
With very low blood sugar	0·881	4·30	3·09
Third hour:	0·760	4·24	1·07

Percentage changes in the amount of oxygen used and sugar oxidized in hypoglycæmia with insulin.

	Oxygen used	Sugar oxidized
First hour	+5·30	+ 5·30
Second hour	+3·12	−44·42

normal of 5·28 to 4·55 mg. per g. per hour, a depression of 13·90 p.c. The addition of insulin at the beginning of the experiment when the blood sugar is normal results in a slight increase of sugar utilization to 5·56 mg. per g. per hour, an increase of 5·30 p.c. (Table VI). If, however, the insulin be added at the end of the first hour of the experiment, when a definite degree of hypoglycæmia has become established, the effect is a decrease in sugar consumption as shown in the last two experiments in Table VI. This is corroborated by what obtains when insulin is added hour by hour; the amount of sugar used becomes less and less, until finally little sugar if any may be utilized. Table VII shows this result; after a slight initial rise in the first hour, sugar utilization falls 44·42 p.c.

TABLE VII. Hypoglycæmia.

The effect of insulin on the oxidation and storage of blood sugar by the mammalian heart. (Blood-sugar percentages and R.Q.'s as in Table VI).

Hour of exp.	Total sugar lost g.	Blood sugar oxidized g.	Blood sugar stored g.	Percentage of sugar oxidized	Percentage of sugar deposited g.	Weight of heart g.	Possible heart glycogen g.	Percentage gain heart glycogen
1	0·5332	0·4712	0·0620	88·38	11·62	84	0·420	14·80
2	0·4482	0·3985	0·0497	88·91	11·09	—	0·482 0·532	10·31
1	0·5263	0·4696	0·0567	89·23	10·77	85	0·425	13·34
2	0·2475	0·2204	0·0271	81·52	19·48	—	0·481 0·508	5·58
1	0·5367	0·4666	0·0701	86·94	13·06	87	0·435	16·10
2	0·1788	0·1589	0·0199	88·78	11·22	—	0·506 0·524	3·54
2	0·5246	0·4436	0·0810	84·56	15·44	160	0·656 0·737	12·34
2	0·5877	0·5210	0·0667	88·65	11·35	150	0·547 0·614	12·19

The percentage changes due to hypoglycæmia with and without insulin in the oxidation processes and the deposition of glycogen in the heart (Tables VI, VII).

	Hypoglycæmia alone		Hypoglycæmia with insulin	
	Oxidation	Glycogen lost	Oxidation	Glycogen gained
First hour	−13·90	−20·88	5·30	13·75
Second hour	—	—	3·12	6·48

The effect of hypoglycæmia with and without insulin upon the ratio between the oxidation and synthesis of 100 parts of blood sugar.

	Hypoglycæmia alone		Hypoglycæmia with insulin	
	Oxidation	Synthesis	Oxidation	Synthesis
First hour	100·00	0·00	87·55	12·45
Second hour	—	—	86·17	13·93

in the second hour and 66·11 p.c. in the third hour. Third-hour results have not been included in the tables because the onset of lung œdema makes them unsatisfactory. When the blood sugar is reduced to about 0·025 g. p.c. the heart has apparently ceased to make use of sugar as its chief source of energy. That there is a condition of glucatonia is clear from the very small amount of sugar in the serum at the end of the third experimental hour. Such a diminution of sugar in the serum points to an extremely small amount of free muscle sugar, proof of a thorough removal of sugar by the heart. The question arises now as to whether the sugar so removed has been oxidized or synthesized.

(2) *The respiratory quotient and oxygen consumption.*

The respiratory metabolism of the isolated mammalian heart can best be investigated by the isolated heart-lung preparation of Starling [Knowlton and Starling, 1912*b*], for here the heart can be kept beating powerfully and efficiently for several hours with both the circulating and respiratory systems closed from the external air.

"If the heart muscle, like skeletal muscle, performs its work by oxidising preformed carbohydrate exclusively then the respiratory quotient should be unity" [MacLeod, 1928]. Lovatt Evans [1912] found great variations in the R.Q. of mammalian hearts, the average for dogs being 0·901. Starling and Evans [1914] obtained quotients as low as 0·70 and as high as 1·09 with an average of 0·85. It has been assumed, because of such results, that important metabolic differences exist between skeletal and cardiac muscle.

A high R.Q. may be due to the washing out of CO_2 in such experiments [Kilborn, 1928], but this factor has been controlled in the experiments discussed here by adjusting the extent of ventilation to the size of the preparation [Corkhill, Dale and Marks, 1930; Eggleton and Evans, 1930], maintaining a much more moderate degree of ventilation than is usual in such experiments and by allowing at least half an hour for complete gaseous equilibrium to be obtained before commencing the actual experiment. With a ventilation rate which was generally constant at twelve strokes per minute, the stroke volume, which varied between 50 and 100 c.c., was changed in accordance with the size of the heart and lungs. Over-ventilation was thus guarded against, and the small amount of tissue used permitted of a fairly quick arrival at gaseous equilibrium. No anoxæmia was ever present, a perfect oxygenation of the blood was always in evidence to the end. That there was no failure in oxidative processes in the preparation is also seen from the fact that there was little

increase of lactic acid in the blood, in fact, in the insulin experiments there was usually a marked diminution in the lactic acid content of the blood.

The effect of hypoglycæmia per se *on the respiratory metabolism of the heart.* Table IV shows that the R.Q. for the first experimental hour is essentially unity, the highest figure being 1·011, the lowest 0·991, with an average of 0·992. A moderate degree of hypoglycæmia has therefore had no effect upon the type of metabolic activity. The oxygen consumption is also without marked variations when reduced to c.c. per g. of heart muscle per hour. The lactic acid of the blood varied in these experiments from 36 to 59 mg. p.c. The variation in each experiment is so small as to be regarded as negligible as far as any change in oxidative processes may be concerned. Hypoglycæmia produced a diminution in the utilization of oxygen from 3·96 to 3·42 mg. per g. of muscle per hour, an average fall of 13·90 p.c.; sugar utilization has also been reduced to a similar extent from 5·28 to 4·55 mg. per g. of heart muscle per hour. The normal figures are from the records of six experiments in which the sugar level was maintained by the use of a Master's constant injection pump.

The effect of insulin. It has been shown that, with an increase in the sugar content of the blood above the normal level for the animal, there is a slight increase in oxygen consumption. The addition of insulin in hyperglycæmia effects a further slight increase in the oxidation of sugar by the heart, but the oxygen absorbed does not account for all the sugar which has disappeared; much of it has been retained as glycogen. Experiments by Burn and Dale [1924] upon eviscerated decapitated cats led them to the conclusion that the earlier stages of insulin action are accompanied by increased consumption of oxygen in a preparation, the R.Q. of which was always about unity.

In a later paper Best, Dale, Hoet and Marks [1926*a*] state that there is always a slight depression of respiratory metabolism following insulin administration which, calculated from only one experiment, amounts to 5·4 p.c. of the oxygen figure for the normal period, and they say that "the excess oxygen consumption observed by Burn and Dale [1924] can be entirely accounted for by the contribution to the blood from the carbohydrates of the liver." But a recalculation of Burn and Dale's results will show that, after allowing for the leakage of sugar, the increased amount oxidized in the presence of insulin varies from 9·1 to 43·8 p.c. with an average figure of 23·4 p.c. or, leaving out the extreme figure of 43·8 p.c., with an average figure of 16·3 p.c. Experiments of Chaikoff and MacLeod [1927] have shown that insulin causes a relatively slight increase in the oxidation of carbohydrates in the

rabbit but a definite increase in the isolated perfused muscles of the cat. They also point out that in dogs there is also a very slight increase in oxygen consumption when insulin is administered with glucose. A marked increase in oxygen consumption by animals to which insulin has been given has been thought to be due to a condition of hyper-excitability of muscle tissue which preceded the onset of convulsions [Dickson, Eadie, MacLeod and Pember, 1924]. On the other hand, Visscher and Müller [1927] have demonstrated that non-pressor samples of insulin produce no direct stimulation of oxidative metabolism in the heart.

It is clear from what has been said that one should determine whether or not insulin has any direct effect upon the preparation used, be it a whole animal or an isolated tissue. Since the discussion here is one of synthesis versus oxidation as characteristic of insulin action, pressor effects must either be eliminated or accounted for. In the isolated heart-lung preparation the heart suffers no change in rate when twenty units of insulin are added to the blood; there is, however, a slight rise in blood-pressure which never exceeds 10 mm. Hg, and which passes off within 10 to 15 minutes. Further it is seen that there is, during this time, a slight increase of 20 to 30 c.c. in the volume of blood in the reservoir, which is due to a reduction in the blood volume of the preparation, the volume returns to its pre-insulin level within 15 minutes. Here then is evidence of a slight and transient pressor effect upon isolated heart muscle. To make a correct allowance in the figure for oxygen consumption for this pressure change one must take into consideration its duration. A 10 p.c. rise in pressure disappearing in 10 minutes would necessitate a correction for the figures given of approximately 1·7 p.c. This correction has not been made in the tables.

Experiments were carried out on the whole animal (two dogs and three cats) to see to what extent oxygen consumption would be increased in the presence of forty units of insulin. In the cats the oxygen consumption increase averaged 7·69 p.c. in 1 hour, there was a very slight increase of 5 mm. Hg in blood-pressure, the increase in heart rate averaged 1·8 p.c. In the dogs there was no change in blood-pressure, an average increase in heart rate of 1·7 p.c. and an average increase in oxygen consumption of 2·7 p.c. A repetition, within half an hour of the first dose, of forty units of insulin caused a slight fall in blood-pressure. Pressor effects due to the insulin can therefore be regarded as negligible. Dr K. K. Chen of Eli Lilly and Co. very kindly tested all the samples of insulin which he sent to us and found that they caused no rise of blood pressure in the pithed cat.

Insulin given in ten unit doses hourly in hypoglycæmia raises oxygen consumption 5·30 p.c. in the first hour (Table VII). In the second hour there is a continued slight rise in oxygen consumption, and in the third hour there is no appreciable change. Further, insulin addition generally produces a definite fall in the lactic-acid content of the blood which, with a slight increase in oxygen used, a decrease in CO_2 produced, and a fall in the R.Q. would indicate that the lactic acid is not oxidized but is reconverted to glycogen. The slight increased oxidation must therefore be attributed to a consumption by the heart of non-carbohydrate substances. From such results one must conclude that the stimulation of oxidative processes is not essentially a characteristic of insulin action.

The most noteworthy change, however, is that seen in the R.Q. A fall in the R.Q. from 1·00 to 0·744 (Table VI) indicates a definite change in metabolism. As no measure of the extent of protein metabolism has been obtained and as a correction at a R.Q. of 0·82 would entail approximately a 10 to 15 p.c. change which would not materially affect the results as set down, the R.Q.'s have been taken as representing the balance between carbohydrate and fat in so far as figures for the oxidation of sugar have been given. The relative proportion of the oxygen consumed by sugar has been obtained from the well-known tables of Zuntz and Schumburg. It may be taken, from a consideration of the fall in the R.Q.'s, that the energy cost of the work that is being done by the heart is greater since the calorific output as estimated by oxygen consumption is slightly raised and maintained over a period of 3 hours, and the metabolic activity of the heart muscle has changed in type, namely from a purely carbohydrate source of energy to one consisting to a large extent of non-carbohydrate material.

(3) *The utilization or synthesis of glycogen.*

It has been clearly shown by Best, *et al.* [1926*a*] and Burn and Dale, [1924], Cori, Cori and Pucher [1923], Bissinger, Lesser and Zipf [1923], Cruickshank and Shrivastava [1930] that the chief action of insulin is to cause increased glycogen formation in the presence of an adequate supply of blood sugar. The observations of Bissinger, *et al.* and of McCormick and MacLeod [1923] showed that, while glycogen is deposited as an early action of insulin, little if any excess remains after a certain period. Dudley and Marrian [1923] concluded as a result of their experiments that hypoglycæmia due to insulin produced a definite glycogenolysis in liver and muscle but an increase in glycogen within the heart muscle. It has, however, been clearly shown [Best, Hoet and

Marks, 1926*b*] that hypoglycæmia with or without insulin does not, unless convulsions supervene, produce any breakdown in the glycogen of skeletal muscles. In the eviscerated spinal preparation the blood sugar is evidently sufficient for the energy needs of the preparation, with the result that a certain small amount of glycogen is stored. Upon the addition of insulin with a blood-sugar level at the end of the experiment of 0·06 g. p.c. there is an increase in glycogen to the extent of 28 p.c. [Best, *et al.* 1926*a*].

Reference has already been made to the loss of heart glycogen occasioned by a marked hypoglycæmia, namely an augmentation of blood sugar from heart glycogen averaging 30·54 p.c. or an average of 20·88 p.c. of the calculated glycogen content of the heart being utilized. This loss is progressive and may with a continued hypoglycæmia be as much as 30 p.c. of available glycogen at the end of 2 hours. It would be of interest to determine the ultimate reaction of the isolated mammalian heart when faced with practically no blood sugar, and a 50 or 60 p.c. depletion of its glycogen.

The effect of insulin. The results shown in Table VII indicate that in insulin hypoglycæmia lasting 2 to 3 hours there is such a depletion of blood sugar that further sources of energy are necessary. It should also be remembered that the depletion of sugar may be greater than the figures would indicate, because the method of estimation of blood sugar has not excluded non-carbohydrate reducing substances [Somogyi, 1930]. In the first hour, with a R.Q. of unity, of the total amount of blood sugar which has disappeared, an average of 12·5 p.c. has been deposited in the heart muscle, in the second hour 13·93 p.c. In terms of the assumed original amount of glycogen in the heart the increase in glycogen is, in the first and second hours, 13·75 and 6·48 g. p.c. This shows that in a progressive insulin hypoglycæmia while the blood is rendered almost sugar free, and the amount of glycogen deposited in the heart has accordingly fallen, yet the ratio distribution between sugar oxidized and sugar synthesized is remarkably well maintained. It is apparent that insulin effects a balance between these two activities and that in favour of synthesis, and further, that it continues to do so when the heart exposed to an extreme degree of hypoglycæmia is forced to utilize other substances as sources of energy.

It is thus demonstrated that, what can be easily obtained by skeletal muscle under much more favourable conditions than those afforded the isolated heart, has been attempted, and that with considerable success, by cardiac muscle.

Discussion.

It would appear from such results that, while carbohydrate is available to meet the increased energy needs of the heart muscle, carbohydrate only is used, but, that with the maintained energy requirements and with a rapid depletion of sugar supplies the heart muscle is forced to fall back on other sources of energy. That in its early stages exercise may be accomplished by the metabolism of carbohydrate has been shown by Hill [1925] and his co-workers, and that strenuous and continued exercise demands that the musculature of the body fall back on fat has been shown by Furusawa [1926].

It has been suggested by Greene [1926], from observations on the glycogen content of the muscle of salmon, that carbohydrate is not the immediate fuel of muscular activity; but that carbohydrate is the chief source of the energy of muscular contraction has been demonstrated by the work of Meyerhof [1924] and Hill [1922, 1932]. Burn and Dale [1924] found that the skeletal muscle of the eviscerated preparation before and after insulin had a R.Q. close to unity, and suggested therefrom that carbohydrate is the chief source of energy of isolated muscle.

On the other hand Kilborn [1928] offers no support to the theory that carbohydrate is the only source of energy for muscle contraction, and he attributed the high R.Q. of Burn and Dale to a washing out of CO_2. Bornstein [1929] showed that, in the eviscerated dog, a R.Q. of unity was maintained provided the blood-sugar level was kept up, but that it fell with a diminution in the carbohydrate supply. Corkhill, Dale and Marks [1930] have more critically investigated the question of over-ventilation as affecting the R.Q. in the eviscerated cat, and they state that a R.Q. of unity should be regarded as correct for that preparation provided the blood sugar is maintained at the normal value, and further they affirm that such a quotient cannot be regarded as artificially produced by excessive ventilation.

Meyerhof and Boyland [1931] have shown that skeletal muscle poisoned with iodoacetic acid and having a R.Q. of 0·7 to 0·8 recovers in oxygen with no lactic acid and with but little carbohydrate oxidation. The observations of Meyerhof [1931] and those of Witting, Markowitz and Mann [1930], on the isolated rabbit's heart perfused with glucose-free Ringer-Locke's solution, which show that an almost glycogen-free heart will beat as long as the normal perfused heart, would tend to strengthen the view that the work of muscular contraction may be performed by the oxidation of material other than carbohydrate. More

recent work by Clark, Gaddie and Stewart [1932] shows that frog's heart perfused for 6 hours under aerobic conditions gives a R.Q. of 0·87, from which they conclude that carbohydrate and protein would form 40 and 60 p.c. respectively of the total metabolism.

The results of our experiments on cardiac metabolism under hyperglycæmia and hypoglycæmia lead us to the conclusion that as with skeletal muscle so is it with mammalian heart muscle; given an adequate supply of carbohydrate the R.Q. will be in the region of unity, with a diminution of carbohydrate supplies the R.Q. will accordingly fall, indicating a definite change in the type of metabolism. While the question of fat and protein utilization is beyond the scope of this paper, still it would appear that when carbohydrate is not readily available as in hypoglycæmia then the heart must needs fall back on protein and fat, a view in keeping with the newer ideas as put forward by Embden and Meyerhof on the chemistry of skeletal muscle contraction.

It is further evident that, for the increase of glycogen in the heart in hyperglycæmia as for its restoration in hypoglycæmia, insulin is essential. This conclusion is in keeping with results obtained on skeletal muscle [Dale and Burn, 1924; Debois, 1930, 1931] and on dehepatized animals [Markowitz, Mann and Bollman, 1929]. It makes more difficult of explanation the fact that the diabetic heart contains more glycogen than the normal [Cruickshank, 1913] and that insulin does not lead to any increase in glycogen in the diabetic heart [Cruickshank and Shrivastava, 1930]. It is probable that glycogen synthesis is dependent upon a balance between sugar concentration and insulin activity.

The addition of insulin increases the call upon the blood sugar and, were it not for the fact that the function of synthesis is intensified by insulin, it is possible that the carbohydrate reserve of the heart would be the more readily available. While there is here no evidence for the assumption it may also be that insulin has stimulated gluconeogenesis from protein and fat within the heart muscle.

It would appear therefore from these experimental results that the immediate effect of insulin is to raise, very slightly, oxygen consumption and to increase markedly glycogen synthesis and to do so at the expense of blood sugar. This conclusion is inevitable in view of a R.Q. of unity and a definite degree of glycogen synthesis as shown in Tables VI and VII.

The continuance of insulin with the production of a definite hypoglycæmia, while it maintains oxygen consumption and glycogen synthesis, is associated with a marked fall in the amount of blood sugar utilized. With a fall in the R.Q., to an average of 0·879 and a reduction of 44 p.c.

in sugar utilization, it must be concluded that substances other than carbohydrates have been called upon to supply the energy requirements of the cardiac muscle. In the third hour blood sugar is practically depleted and glycogen continues to be stored, from which it is clear that there is a sparing of the carbohydrate supplies of the heart effected by the synthetic action of insulin. When available blood sugar has been reduced to an almost negligible quantity by insulin and the R.Q. has fallen to 0·760, the heart still endeavours to maintain an approximately normal oxygen consumption with protein and fat forming the great part of the fuel. And, further, from the fact that glycogen is synthesized it must be concluded that the synthetic action of insulin has far outweighed its action as a stimulant of oxidative processes: in fact the synthetic action has dominated the picture throughout, be it in hyperglycæmia or in hypoglycæmia.

Summary.

A method for the more accurate estimation of the R.Q. of the isolated heart has been described.

The gaseous metabolism, the sugar utilization, and glycogen synthesis of the normal heart have been investigated, with a maintained normal blood sugar, in hyperglycæmia and hypoglycæmia, with and without insulin.

Under maintained normal conditions of blood-pressure and blood sugar, the R.Q. is unity, the oxygen consumption is 3·960 c.c. per g. of heart muscle per hour, blood-sugar utilization being equivalent to the oxygen consumed.

Hyperglycæmia is always associated with an increase in oxygen consumption, sugar utilization, and glycogen synthesis.

The effect of hypoglycæmia *per se* on the normal heart has been investigated and it has been shown that, despite a definite depression in the general metabolic activity of the heart, and a progressive loss of glycogen, carbohydrates remain the only source of energy as long as carbohydrate is readily available.

The effect of insulin upon the metabolism of the heart in hyper- and hypoglycæmia has been studied, and it has been shown that, while in hyperglycæmia insulin increases oxygen consumption and sugar utilization to a very small extent, it markedly increases the synthesis of glycogen in heart muscle.

In the presence of a definite hypoglycæmia produced by insulin, the heart, with a R.Q. of unity, shows little increase in oxygen consumption,

but a marked increase in glycogen synthesis. With a progressive and marked reduction in the blood sugar the R.Q. of the heart steadily falls, indicating a progressive increase in the use of protein and fat as sources of energy, sugar utilization diminishes rapidly while glycogen synthesis is maintained. With a continued administration of insulin, the percentage relation between oxidation and synthesis tends to be changed towards the hyperglycæmic insulin ratio, namely from 97 : 3 to 86 : 14.

It has been demonstrated, in so far as the carbohydrate metabolism of cardiac muscle is concerned, that the essentially characteristic action of insulin is that of a stimulant of synthetic processes and not of oxidative metabolism.

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