THE RESPIRATORY EXCHANGES OF THE HEART IN THE DIABETIC ANIMAL.

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CONTENTS.

THE numerous researches on diabetes have shown that the respiratory quotient in intact diabetic animals is low compared with that of the normal animal, and this has been commonly, though not universally, referred to a decrease in carbohydrate consumption. A similar effect in isolated tissues was indicated by the experiments of Knowlton and Starling(1) on the heart-lung preparation perfused with blood, and by those of Maclean and Smedley(2) on the similar preparation perfused with saline solution. More recently doubts have been thrown on these results by the work of Patterson and Starling(3), and the question has been complicated by the observation of Cruickshank (4) confirming that of van Noorden(5), that there is more glycogen in the diabetic than in the normal heart. In this paper we give an account of experiments on the heart-lung preparation, which we have made with the object of deciding some of the debated questions, and we hope to follow them up with experiments on other isolated tissues.

The respiratory exchanges of the normal dog's heart.

It has been previously shown(6) that the total exchanges and the respiratory quotient of the heart-lung preparation of the normal dog are subject to variations due to conditions of the animal prior to the

TABLE I. *Respiratory exchange of normal hearts. Dog.*

Weight of heart grams	Duration of observation	Average exchange per hour, corrected for lung metabolism O_2 c.c.	CO_2 c.c.	C.c. oxygen per gram heart per hour	Average R.Q.
31·6	1 hr. 20 min.	90	92	2·85	1·02
68·5	2 hrs. 20 min.	154	125	2·25	·81
32·0	40 min.	155	109	4·84	·70
30·0	1 hr. 40 min.	112	124	3·74	1·11
51·5	1 hr. 40 min.	223	201	4·33	·89
51·5	20 min.	224	227	4·36	1·01
23·0	2 hrs. 40 min.	73	70	3·17	·96
23·1	1 hr.	111	99	4·82	·89
45·5	1 hr.	173	137	3·8	·79
41	40 min.	85	74	2·1	·87
47·5	2 hrs.	112	91	2·4	·81
47·5	2 hrs.	89	77	1·87	·87
20·5	3 hrs.	59	57	2·9	·97
16·2	1 hr. 30 min.	54	55	3·4	1·01
76·0	2 hrs.	131	118	1·72	·90
52·6	2 hrs. 15 min.	219	193	4·16	·88
64·5	40 min.	187	146	2·9	·78
? 37·5	30 min.	165	145	4·4	·88
—	2 hrs.	41	36·5	—	·89
57·5	1 hr.	188	155	3·26	·82
33	2 hrs.	127	111	3·85	·87
50·5	2 hrs.	116	107	2·3	·92
56	5 hrs.	142	115	2·55	·81
41	3 hrs.	96	90	2·35	·94
67	2 hrs.	173	152	2·58	·88
70	1 hr. 45 min.	122	105	1·74	·86
—	1 hr.	147	145	—	·98[1]
58·5	1 hr. 20 min.	145	118	2·47	·81
89·5	1 hr. 45 min.	210	159	2·34	·76
52	1 hr.	223	172	4·3	·77
—		261	208	—	·80[1]
57	30 min.	169	145	2·96	·86
55	20 min.	112	91	2·03	·81
68	3 hrs.	242	198	3·55	·81
50	20 min.	160	133	3·2	·83
68	1 hr.	187	192	2·75	1·03
—	1 hr.	144	112	—	·78[1]
75	3 hrs.	248	202	3·3	·81
63	40 min.	168	146	2·66	·87
39	2 hrs. 20 min.	200	166	5·1	·83
46	20 min.	134	121	2·9	·90
64	40 min.	136	133	2·12	·98
26·5	40 min.	147	135	5·53	·92
40·5	1 hr.	135	112	3·33	·83
48·5	40 min.	162	128	3·34	·79
45	40 min.	139	126	3·1	·91
60·5	40 min.	228	167	3·77	·73[2]
37·0	40 min.	159	118	4·3	·74[3]
74	30 min.	221	166	2·98	·75[3]
71	15 min.	215	185	3·02	·86[4]
57	15 min.	239	202	4·2	·84[4]
				3·24	·845 = Mean

[1] Not corrected for lung metabolism.
[2] Diet of fat for three days.
[3] Fasting.
[4] Mixed diet.

experiment, to temporary alterations in the work of the heart, and to certain other conditions. Before we can form an opinion as to any changes in the diabetic heart as compared with the normal heart, it is essential to know the extent of the variations which occur in the normal heart; this applies especially to the respiratory quotient. Table I gives the results of determinations carried out on 51 normal hearts. The conditions of experiment varied somewhat; thus some of the animals had fasted for 12 to 16 hours, others had received special diets rich in carbohydrate or in fat; in some cases glucose had been added to the circulating blood. The mechanical conditions varied less than the nutritional ones; the output of the left ventricle was such as would represent the condition of the heart in bodily rest, viz about 200 c.c. per minute[1] in the case of a heart weighing 50 grams; the temperature was 36° to 37° C. in all cases, and the arterial pressure in the neighbourhood of 100 mm. Hg.

The method used for the determination of the respiratory exchanges was that described in a former communication (6).

In the experiments of Table I, the respiratory quotient ranged from a minimum of ·70 to a maximum of 1·11.

The quotient was:

Between	0·70	and	0·79	in	10	cases
,,	0·80	,,	0·89	,,	26	,,
,,	0·90	,,	0·99	,,	10	,,
,,	1·00	,,	1·09	,,	4	,,
Over	1·09			,,	1	case

The average quotient agrees well with this distribution; it is ·845.

In round numbers we may express the average figures found for the normal dog's heart as follows:

Respiratory quotient	= 0·85
Oxygen per gram heart per hour in c.c.	= 3·2
Carbon dioxide per gram heart per hour in c.c.	= 2·7
Energy utilisation per gram heart per hour under conditions of moderate work, in small calories (1 c.c. O_2=4·85 cal.)	=15·6

From these average figures we are able to calculate the approximate metabolism of the heart according to the method of Zuntz (7). Table II represents the results of such a calculation, in which the oxygen used in the oxidation of protein has been assumed to be 15 % of the total.

[1] Considerable variations of this output are without great effects on the gaseous exchanges, provided that the extreme conditions are not approached.

TABLE II. *Exchanges of normal dog's heart.*

	% of energy derived from	Calories from	Weight of substance used mg.	Vol. of oxygen used c.c.	Vol. of CO_2 produced c.c.	R.Q.
Protein	15·0	2·23	0·48	0·47	0·38	·81
Carbohydrate	42·5	6·92	1·64	1·36	1·36	1·00
Fat	42·5	6·45	0·68	1·37	0·97	·71
Total	100·0	15·6		3·2	2·71	·85

The utilisation of carbohydrate by the normal heart.

The calculation made above from the respiratory exchange and quotient indicates that the heart muscle utilises about 1·6 mg. of carbohydrate per gram per hour. The carbohydrate might be in any form, but there is no reason to think that it is consumed otherwise than in the form of glucose or of glycogen. The participation of carbohydrate in the total energy supply as calculated above is greatly in excess of that calculated by the same method by Evans(8) from some experiments given in a previous paper, even though in those cases glucose had been added to the circulating blood. The experiments in the paper referred to were made with hearts of low initial respiratory quotient, due to previous dieting with protein and fat; they indicated a carbohydrate metabolism to the extent of only 10 to 11 % of the total energy supply, previous to the addition of glucose to the blood. After the addition of glucose the carbohydrate fraction for the same hearts had a mean value of 36% of the total energy. It was stated at the time, however, that higher quotients can frequently be obtained by carbohydrate feeding, or by liberal mixed diet, than by the addition of glucose to the blood of the heart-lung preparation of fat-fed or fasting animals, and the above table and calculations certainly seem to bear this out. It seems likely that for high carbohydrate metabolism, feeding with carbohydrate is essential, since, whether the initial quotient of the heart be low or moderately high, it is not usually raised beyond a certain limit by mere addition of glucose. The condition which determines the utilisation of carbohydrate beyond this limit seems to be attained only by the protracted presence of carbohydrate, such as might result in a storage of glycogen, or in a removal of much fat from the heart muscle.

In one case an experiment was made in order to ascertain if it was immaterial whether the glucose was added to the blood after the preparation had been established and isolated from the other organs,

or whether it was previously introduced intravenously into the animal's circulation, the suggestion being that some chemical change was undergone in some other organ previous to the local combustive changes. The results of the experiment are given below.

EXP. 1. Dog, heart 39 grams. 10 grams glucose introduced intravenously at 2.30 p.m. Animal partially bled at 2.50 to 2.55 p.m. Heart-lung preparation completed at 3.30. Temperature 36; arterial pressure 100 mm. Output constant at 500 c.c. per minute and gaseous metabolism observed for 2 hours. The respiratory quotient fluctuated slightly round a mean of ·81 during that time.

The results of this experiment indicate that the immediate effects of intravenous injection of the sugar are the same as those which follow its addition to the blood after the preparation has been isolated.

A comparison of these figures as calculated from the gaseous metabolism, and those obtained by the direct observation of the glucose utilisation of the heart, indicates that the former series falls within the limits of the latter. But the results of direct estimations obtained by different workers show great variations. The highest figures are those of Gayda(9) for the rabbit; he found 3·8 to 14·9 mg. glucose per gram heart per hour, which was much in excess of his observed productions of carbon dioxide (2·77 to 6·09 c.c. per gram per hour). For the same animal other workers found much smaller usages, *e.g.* Locke and Rosenheim(10) 1·2 to 1·7 mg., Mansfeld(11) 1·2 to 2·5 mg.

In carnivora (dog), Knowlton and Starling(1) found 4·0 mg., and Patterson and Starling(3) from ·8 to 3·8 mg., but some of this was used by the lungs, and the conclusion of the latter workers was that "in the absence of any previous determination of the 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." Cruickshank and Patterson(12) attempted to allow for the glycogen factor, and their results after due correction for the lungs show an average consumption of glucose of about 1·5 mg. per gram per hour for the cat. Neukirch and Rona for the cat(13) found 1·6 mg., while Maclean and Smedley for the saline fed dog's heart found ·6 to 1·5 mg. Rohde for the cat found 1—4 mg.(14).

These results are in agreement with the calculation made above on the results of gaseous metabolism experiments, viz. 1·64 mg.

A study of the respiratory quotient of the heart should give us some indication as to whether the heart uses glucose direct from the blood, or whether it uses glycogen derived from the glucose. That

glucose is used directly or indirectly is indicated not merely by the fact that it disappears from the blood, but also by the fact that the respiratory quotient remains constant for three or four hours, and probably for longer, although its glycogen store is only capable of serving it for about three hours. Even where the total metabolism is increased by administration of adrenalin, it was found by Evans and Ogawa (15) that after three hours the respiratory quotient had not fallen, although it was clear from the experiments of Cruickshank that the glycogen would be much diminished at the end of this time. Cruickshank and Patterson, and Maclean and Smedley find that the glucose usage remains fairly constant from hour to hour; this indicates that glucose and not glycogen is used from the first since the R.Q. is unaltered, or else that the glycogen content is kept constant by continual replacement. But the replacement of glycogen would in all probability be too slow to keep pace with the rate of disappearance, and it seems more likely that the glycogen does, as generally believed, represent a store of carbohydrate material which is only used in time of need. Even then the glycogen does not contribute much if sufficient sugar is available, as is seen clearly from the following calculation made from one of the experiments of Patterson and Starling, in which adrenalin was continually added to the circulating blood. The figures given below show the amounts of glucose and of glycogen used per 100 grams of muscle per hour, and the percentage of the total carbohydrate derived from glycogen, assuming that the heart at the commencement of the experiment contained ·5 % of that substance.

TABLE III. [*From Table IV of Patterson and Starling.*]

No. of exp. in paper	5	1	2	4
Mg. carbohydrate derived from blood, per 100 gram heart per hr.	800	410	262	30
Mg. glycogen used per 100 gram heart per hr.	92	70	220	125
% of carbohydrate drawn from glycogen	10·1	14·6	45·7	80·5
Original % of sugar in blood	0·7	0·64	0·51	0·28

The experiments of Rohde are also in agreement with the view that the glycogen represents a store of carbohydrate to be called upon in time of need, although according to Camis (16) the heart in carnivora draws its carbohydrate from its own glycogen rather than from the circulating glucose.

The glucose consumption of the lungs.

In experiments on the consumption of glucose by the isolated heart-lung preparation, the removal of glucose by the lung must be taken into account. This was done by Patterson and Starling, who found that about 1·3 mg. of glucose per gram of heart was withdrawn by the lungs. The figure is almost as large as that of the heart and lungs together in some cases, and as they point out leaves a very small, or indeed a minus quantity of sugar at the disposal of the heart. For the oxidation of 1·3 mg. glucose 1 c.c. of oxygen is necessary. The respiratory exchanges of the isolated dog's lung was found by Evans and Starling (17) to be about 1 c.c. per gram of heart corresponding to it per hour, so that it might be assumed that there was an agreement between these results. But such would not be the case unless we assumed that the metabolism was an exclusively carbohydrate one, which is hardly likely to be the case. If the metabolism of the lungs is similar to that of the heart as regards the relative amounts of the different substances oxidised, the lung tissue accompanying each gram of heart would utilise in an hour only about ·5 mg. of glucose. The remainder, ·8 mg., would be lost to the circulation in other ways, such as by physical absorption by the lung tissue or by the erythrocytes, or conceivably by being converted into some non-reducing substance.

The respiratory exchanges of the diabetic heart.

The respiratory exchanges of the hearts of 17 diabetic dogs were investigated (Table IV). The diabetes was in every case produced by complete extirpation of the pancreas from one to five days previously and the technique followed for the remainder of the experiment was the same as that used in the case of the hearts of normal dogs. The glycosuria was confirmed in every case, and in some cases hyperglycæmia was also demonstrated. In some of the experiments glucose was also added to the blood, as indicated in the table. A survey of Table IV shows that the respiratory quotient of these diabetic hearts is much lower than that of the normal hearts. In one case it was as low as ·47, while the highest quotient was ·83. The distribution of the quotients was as follows:

Below ·5 in 1 case.
Between ·5 and ·59 in 1 case.
Between ·6 and ·69 in 2 cases.
Between ·7 and ·79 in 12 cases.
Over ·8 in 1 case.

TABLE IV. *Respiratory exchange of diabetic hearts. Dog.*

No. of days since pancreas removed[1]	Weight of heart grams	Duration of observation	Average exchanges per hour, corrected for lung metabolism[2] O_2 c.c.	CO_2 c.c.	C.c. oxygen per gram heart per hour	R.Q.	Remarks
2 days	33	1 hr.	140	96	4·25	·69	0·5 gram glucose added to blood. Abundance of sugar and acetone in urine
5 ,,	53	1½ hrs.	158	120	2·98	·76	0·5 gram glucose added
2 ,,	60·5	1 hr.	166	121	2·74	·73	1·0 gram glucose added. Marked lipæmia and glucosuria
2 ,,	26	1 hr.	92	73	3·53	·79	
2 ,,	73	2 hrs.	229	108	3·14	·49	
5 ,,	65	1 hr.	97	70	1·49	·72	0·8 gram glucose added to the blood (250 c.c.)
3 ,,	66	¾ hr.	143	102	2·16	·71	Dog fed on glucose, last dose of 50 grams being given 24 hours ago
3 ,,	40·5	2 hrs.	173	93	4·27	·55	Equal volume of 0·9 % NaCl added to blood
2 ,,	47	2 hrs.	167	118	3·55	·71	Equal volume of 0·9 % NaCl added to blood. Diabetes confirmed
1 day	42	¾ hr.	196	132	4·66	·67	
2 days	65	40 min.	167	125	2·57	·75	
4 ,,	49	1⅓ hrs.	118	98	2·4	·83	
3 ,,	48	1 hr.	144	101	3·0	·70	Glycosuria confirmed. Blood sugar = ·3 %. No sugar added
2 ,,	61	½ hr.	104	77	1·7	·74	
3 ,,	41	1 hr.	144	106	3·5	·74	
4 ,,	—	½ hr.	187	142	—	·76	Not corrected for lung metabolism
3 ,,	43	1 hr.	140	102	3·3	·73	
					3·08	·708 = Mean	

[1] The aseptic operations of removal of the pancreas were performed by Prof. Starling.

[2] The values are corrected for the lung metabolism.

The following mean figures, calculated from the table, may be taken as representative for the diabetic heart:

Respiratory quotient = 0·708.
Oxygen per gram per hr. = 3·08 c.c.
Carbon dioxide per gram per hr. = 2·18 c.c.
Energy utilisation per gram per hr. (in small cal.) 14·4 (cal. value of 1 L. O_2 = 4·68).

The low respiratory quotient of the diabetic heart indicates that the consumption of carbohydrate is much less than that of the normal heart, or else that the metabolism of some other constituent is greatly deranged. These two alternatives must now be briefly discussed.

Decreased sugar usage. The experiments of Knowlton and Starling, Maclean and Smedley, and Cruickshank and Patterson all indicate a reduced power on the part of the tissues to utilise carbohydrate. But McLeod and Pearce(18) and Patterson and Starling find that the reduction of sugar utilisation is only small in some cases, and Patterson and Starling suggest that even where there is an apparent reduction of the utilisation, it is really due to the fact that glycogen is being used up and not replaced; the large amount of glycogen present in the heart would add weight to this view, though it is not easy to see why glycogen is replaced by the normal heart and not by the diabetic, where the concentration of glucose in the blood is higher. An alternative explanation would be that the diabetic heart has retained its power of storing glycogen, and, as found by Cruickshank, of converting this again into glucose, but that its power of oxidising either the glycogen or the glucose is impaired.

But even if we grant that the isolated diabetic heart can break down its glycogen irreversibly into glucose, which it then oxidises, and which it does not replace, while the diabetic heart *in situ* either does not break down its glycogen, or else replaces it as fast as it does so, we are still faced with the fact that the respiratory quotient of the heart is so low that the utilisation of sugar by the diabetic heart must be very small indeed. There is of course the alternative possibility that the tissue may still be able to carry out the oxidation of the glucose to a certain stage, but that the complete oxidation is no longer possible, but this hypothesis cannot be accepted since we have no evidence of the existence in the circulating blood, or of the excretion in the urine, of such partial products of the oxidation of carbohydrate; moreover, most of the carbohydrate, or all of it, can be recovered unchanged from the urine, after due allowance has been made for glucose derived from protein.

The conclusion of Patterson and Starling that there is often only

a diminution in the power to oxidise carbohydrates, and not total loss of this power, is supported by the observation of Stiles and Lusk(19) and of Moorhouse (unpublished), the former for phloridzinised animals, the latter for depancreatised animals, that a rise of respiratory quotient may be obtained for a short time if a diabetic animal is suddenly flooded with sugar, as by the administration of glucose solutions in large amount. The rise studied by Moorhouse was, however, quite a small and transitory one, and frequently did not appear. It might be ascribed to the results of a mass effect of the sugar accelerating for a time the tardy oxidation of carbohydrate.

The effect of adrenalin on the diabetic heart. The work of Patterson and Starling is largely based on the fact that when the work of the heart is augmented by the administration of adrenalin, not only does much sugar disappear from the circulating blood, but that its glycogen also almost disappears. If the carbohydrate which thus disappears from the system is fully oxidised, there should be a distinct and sustained rise in the respiratory quotient as long as the administration of adrenalin is continued, or until all the carbohydrate is exhausted. Some experiments were therefore made in which the respiratory quotient of a diabetic heart was determined, and then adrenalin was added to the circulating blood in small amounts at a time in order to maintain a definite acceleration in the rate of the heart, and the respiratory quotient during the addition was studied. The experiments of Evans and Ogawa(15) on the effect of adrenalin on the gaseous metabolism of the normal heart have shown that the increase of carbohydrate consumption under these conditions is only part of the general increase in catabolism, the respiratory quotient remaining unaltered. Those experiments also indicated that in order to judge of the action exercised by the adrenalin on the tissue metabolism, it was necessary to observe the respiratory exchanges of the heart over a considerable time during which adrenalin was being added, and to take the mean respiratory quotient for the whole period during which the adrenalin had been acting. By proceeding in this manner we eliminate the variations in the quotient which are incidental to the action of the drug, and the causes of which have been previously discussed. If, however, only a single dose of adrenalin were given, and the R.Q. determined, either a rise or a fall could be obtained according to the time relations of the administration and the determination.

The following experiments show the effect of the adrenalin on the diabetic heart.

Exp. 2. Dog, heart = 48 grams. Pancreas extirpated three days previously. Glycosuria confirmed. Arterial pressure kept constant at 100 mm. Temp. of blood entering heart 36°.

Period min.	Rate of heart per min.	Output per min.	Per hour c.c. O_2	Per hour c.c. CO_2	R.Q.		Remarks
20	138	300	189	132	·70	Mean = ·707	
30	134	293	205	143	·70		
30	125	285	182	132	·72		
20	220—240	290	342	214	·62	Mean = ·607	Adrenalin administration commenced at middle of first period and was continued throughout, about 1½ to 2 c.c. of the 1 : 10,000 solution being added to the circulating blood during each period in order to keep the pulse rate up to about 220
20	220—240	273	537	291	·54		
20	217—220	255	510	316	·62		
20	200—220	227	582	396	·68		
20	220	230	639	411	·64		
20	215	240	621	335	·54		

Sample of blood at commencement contained 0·3 % of glucose in serum
,, ,, ,, ,, end ,, 0·1 % ,, ,,
Heart glycogen at end of experiment 0·13 %

(The blood sugar determinations were kindly made by Dr Patterson and those of the glycogen in the heart by Dr Cruickshank.)

The results of the respiratory experiment show that the gaseous metabolism of the heart is increased from 2½ to 3 times as a result of the addition of adrenalin, and that during the 3 hours 20 minutes of the experiment the blood sugar diminished by ·2 % and the heart glycogen fell to the low value of ·13 %. If we assume that the glycogen in the heart at the commencement was 1 %, the total disappearance of carbohydrate from the system would be not less than 1·0 gram, or about 6·3 mg. per gram heart per hour, as an average for the whole experiment. Allowing for the different intensities of the metabolism as a whole before and after adrenalin, we could allocate 200 mg. of glucose as utilised during the period before adrenalin and 800 mg. used in the two hours after adrenalin administration was commenced. This amount of sugar would require 600 c.c. of oxygen approximately for its oxidation, *i.e.* an increase of 300 c.c. per hour, and a like increase in the CO_2 formed, and if the increased metabolism affected carbohydrates only, the oxygen used per hour by the heart should be about 500 c.c. and the CO_2 about 435 c.c., the respiratory quotient being raised thereby to ·85.

The actual results show a great deficiency in the carbon dioxide, and a lowering of the R.Q. from which we are led to the conclusion that there is some considerable discrepancy between the observed disappearance of sugar and the accomplished combustion of it.

The following experiments were made in a similar manner.

Exp. 3. Dog, heart 49 grams. Pancreas extirpated four days previously. Diabetes confirmed. Blood from two other diabetic dogs added to circulation in order to obtain sufficient volume. Arterial pressure 92 mm. Hg. Temp. 37° C.

Period min.	Rate of heart per min.	Output c.c. per min.	Gaseous exchange of preparation per hour c.c. O_2	c.c. CO_2	R.Q.		Remarks
90	118—127	265—280	167	137	·82		No adrenalin
30	170	300	250	174	·695		Adrenalin added to keep
20	170	223	238	193	·81	Mean	rate to 170 per min.
20	170	217	269	229	·85	=·791	(about 1 c.c. of 1 : 10,000
20	170	200	265	213	·81		solution each 15 min.)

% glucose in serum at end of experiment ·005
% glycogen in heart at end of experiment ·96

Exp. 4. Dog, heart 61 grams. Pancreas extirpated two days previously. Art. press. 100 mm. Temp. 36°.

Period min.	Rate of heart per min.	Output c.c. per min.	Gaseous exchange of preparation per hour c.c. O_2	c.c. CO_2	R.Q.		Remarks
30	109	—	165	122	·74		No adrenalin
30	184	—	382	219	·57	Mean	4 c.c. 1 : 10,000 solution of adrenalin
15	200	—	528	388	·73	=·65	added in small quantities during the 45 min.

Exp. 5. Dog, heart 41 grams. Pancreas extirpated three days previously. Blood from two other diabetic dogs added. Also 1 gram of glucose in 10 c.c. saline added at commencement of experiment to ensure adequate carbohydrate supply. Art. press. 100 mm. Temp. 36°.

Period min.	Rate of heart per min.	Output c.c. per min.	Gaseous exchange of preparation per hour c.c. O_2	c.c. CO_2	R.Q.		Remarks
20	162	223	184	135	·73		No adrenalin
20	153	223	186	135	·73	Mean	
20	150	207	177	136	·77	=·74	
20	200	218	519	291	·56		Adrenalin 1·9 c.c. 1 : 10,000
20	200	232	558	372	·67		,, 1·3 ,, ,,
20	200	214	537	360	·67		,, 1·5 ,, ,,
20	200	217	567	396	·70		,, 1·7 ,, ,,
20	200	217	552	375	·68	Mean	,, 1·5 ,, ,,
20	200	214	582	465	·80	=·71	,, 1·7 ,, ,,
20	200	216	555	423	·76		,, 2·0 ,, ,,
20	200	240	560	447	·80		,, 1·8 ,, ,,
20	200	220	510	392	·77		,, 1·8 ,, ,,

The results of all these experiments point in the same direction, and the results of the addition of adrenalin to the blood of the diabetic preparation do not seem to differ in any way from those obtained with the normal heart. There is no indication of a marked rise in the quotient such as we should expect if there had been any relative increase in the amount of glucose or glycogen usage. The mean quotients for the adrenalin periods indeed show a slight fall in most

cases, which is explainable in the same manner as that seen in the case of the normal heart studied by Evans and Ogawa; since the administration of adrenalin was continued to the end of the experiment, the compensatory rise had not appeared. But if we also compare the maximum quotient reached during the action of adrenalin with that before the addition, there is still no evidence of any distinct rise, such fluctuations as are seen being attributable to the difficulty of exactly adjusting the additions of the adrenalin.

We may say therefore that adrenalin alters the metabolism of the diabetic, as of the normal heart, quantitatively but not qualitatively, and that there is good reason to think that the low respiratory quotients of the diabetic tissue, as of the entire diabetic animal, are due in the main to diminished combustion of carbohydrate.

Qualitative alterations in the tissue metabolism. The majority of the experiments yielded a respiratory quotient which was compatible with a metabolism of protein and fat only. Thus a metabolism of 85 to 90 % of fat, the remainder being protein, would yield a quotient of about ·72, if we assume that the protein is completely consumed, and somewhat lower if we assume that the glucose formed from the protein is not utilised (from ·613 to ·706 according to Magnus Levy).

In two of the cases investigated, however, the R.Q. was lower than this, and if these observations are to be accepted as reliable ones they are of importance, since they point to some profound disturbances of metabolism. Thus the formation of sugar from fat, which according to Pembrey (20) would have a quotient of ·28, even if occurring only to a moderate extent, would greatly lower the quotient. Similarly, the formation of β-oxybutyric acid from fatty acid (R.Q. ·53) would assist materially in lowering the quotient. Quotients below ·7 have only rarely been obtained during long periods from the normal heart, and there does not seem to be any reason to doubt the accuracy of the results of the two experiments on diabetic hearts which gave quotients as low as ·49 and ·55 respectively. There were no great fluctuations in the quotients from period to period, during 2 or 3 hours, as may be seen from the following details of the experiments.

Exp. 6. Dog, heart 73 grams. Pancreas extirpated two days before. Arterial pressure 60 mm. Hg. Temp. 36·5° C.

	c.c. O_2 per hour	c.c. CO_2 per hour	R.Q.	
1st hour	345	166	·48	
2nd hour	309	152	·50	Mean = ·49
3rd hour	387	190	·49	
Next half hour period	349	175	·50	

Exp. 7. Dog depancreatised three days previously. Blood mixed with an equal volume of saline solution. Temp. 36° C.

	c.c. O_2 per hour	c.c. CO_2 per hour	R.Q.	
1st half hour	214	120	·56	
2nd half hour	238	128	·54	Mean = ·55
3rd half hour	214	116	·54	
4th half hour	211	117	·56	

Had the low quotients been due to experimental error, such as might be occasioned by a leak in the lungs, this constancy in the respiratory exchange would not be met with, since a leak tends to alter during the course of the experiment. These experiments are of interest in view of the opinion of Magnus Levy[1] that the quotients below ·6 obtained by French workers "must be regarded as incorrect, and due to error of some sort or other."

The energy exchange in diabetic tissues.

If the calorie value of unit volume of oxygen be the same for the altered metabolism of the diabetic as for the normal tissue, then we can calculate the total energy exchange of the diabetic tissue, or of the entire diabetic animal, from the results of gaseous metabolism experiments.

It has been held by some investigators that in the case of the entire organism, the oxygen requirements are the same for the diabetic as for the normal animal, and if this is so it is highly probable that the energy exchanges are likewise equal in the two cases.

Our average results indicate a utilisation by the diabetic heart of about 14·4 small calories per gram per hour, the calorie value of oxygen being taken as somewhat less than that which obtained in the normal organ, namely 4·68 instead of 4·85, in accordance with the altered respiratory quotient. The energy of the normal heart was on an average about 15·6 cal. so that the two can be regarded as practically the same. We may conclude, therefore, that what has been held to apply to the dog as a whole, using about 4·5 cal. per gram per hour, applies to the heart which uses energy at more than three times the rate, and that the energy requirements of neither the whole nor of the part are greatly altered by the diabetic condition. As regards the heart under the conditions of our experiments, it should perhaps be mentioned that it does not regulate its own temperature as the intact

[1] Magnus Levy in van Noorden's *Metabolism and Practical Medicine*, I. p. 202. 1907. But see also III. p. 554.

animal does, but has its temperature fixed by the conditions of the experiment.

Alterations in the gaseous metabolism after extirpation of the pancreas.

It is known that sugar appears in the urine within a very short time after extirpation of the pancreas, and it has been shown by Verzár (21) that the respiratory quotient begins to fall within a few hours after the depancreatisation has been performed. In our aseptic extirpations the animals attain in a few hours a condition which remains almost unchanged, except for the progressive emaciation, until they are used for the experiments, and it is of some interest to find whether the respiratory quotient of the heart indicates that the tissues of which it is chosen as a type, are also in a stationary condition during the several days which usually intervene between the operation and the final experiment, or whether there is a progressive change in the quotient signifying a steady decrease of normal metabolism and increase of oxidations or other changes of an abnormal type.

The following table gives the results of Table II arranged according to the time which elapsed between the operation and the respiratory experiment. It will be seen that there is no regularity about the quotients to suggest any fundamental alteration in the conditions of the tissue metabolism during the first to the fifth days after operation. There is at all events no indication of progressive impairment, but rather the reverse.

TABLE V. *Respiratory quotients of hearts after depancreatisation.*

Days after depancreatisation	No. of animals	Mean R.Q.	Extremes of R.Q.
1	1	·67	—
2	7	·70	·49—·79
3	5	·686	·55—·74
4	2	·795	·76—·83
5	2	·740	·72—·76

The alleged presence of toxic bodies in the blood of diabetic animals.

An ingenious theory of auto-intoxication as the cause of pancreatic diabetes was put forward by Tuckett (22), and some experiments of Lépine and Boulud (23) made some years later seemed to support it. The basis of this theory is that the pancreas supplies to normal blood an internal secretion which neutralises a toxic body absorbed from the alimentary canal. Normal blood was believed to contain considerable

amounts of this antitoxin, and pancreatic diabetes was primarily due to its failure and thus to the unopposed action of the toxin.

The importance of applying experimental methods to the examination of such a theory is obvious, and some few experiments were made with this purpose. If the toxic theory is a correct explanation, then it should be possible

(1) To induce the diabetic condition, with a low quotient in a normal heart, by supplying it with diabetic blood instead of with normal blood.

(2) To carry out the converse experiment, of restoring the diabetic heart to the normal condition with normal blood.

(3) To diminish or remove the diabetic condition by dilution of the diabetic blood with salt solution, in order to attenuate the toxin.

(1) *Normal heart treated with diabetic blood.*

Exp. 8. Normal dog's heart, 26·5 grams. After establishment of the heart-lung circulation with the dog's own blood, half-an-hour elapsed in order to reach a steady condition. The respiratory exchange was then determined, after which the normal blood was run out of the apparatus and replaced by diabetic blood. The circulating blood now contained only about one-fifth of its volume of normal blood. After a further interval of 20 minutes periods 2 and 3 were then taken. Blood-pressure 100 mm. Temp. 36° C.

	Period min.	Pulse rate	Output c.c. per min.	Gaseous exchange per hour c.c. O_2	c.c. CO_2	R.Q.	Blood
(1)	20	191	250	177	163·5	·922	Normal
(2)	20	187	263	170	157·5	·925	Diabetic
(3)	20	179	171	146	142·5	·975	Diabetic

The result of this experiment was to slightly raise the respiratory quotient, perhaps as a result of the addition of the hyperglycæmic diabetic blood. It might of course be urged against this experiment that it was not of sufficient duration—the diabetic blood only circulated for an hour altogether.

(2) *Diabetic heart with normal blood.*

Exp. 9. Diabetic dog, heart 43 grams. Pancreas extirpated four days previously. Diabetes confirmed; the blood was also intensely lipæmic. After running for 30 min. to attain equilibrium, the respiratory exchange was observed for an hour and a half. Then the diabetic blood was run away and replaced by an equal amount of normal blood from a freshly killed dog, the blood was hirudinised and glucose added to make the sugar content to 0·5 %. After a further interval of an hour, to ensure equilibrium, the respiratory exchanges were measured for a further hour.

Duration of period min.	Mean pulse rate	Mean output c.c. per min.	Exchange per hour by preparation c.c. O_2	c.c. CO_2	R.Q.	Blood
90	117	367	183	133	·73	Diabetic
30	114	—	182	144	·79	Normal
30	118	—	204	151	·74	Normal

The addition of normal blood was without appreciable effect after two hours, so that any question of a neutralisation of a toxin seems to be out of the question. The same would apply to the presence of any specific hormone in the blood, and it may be recalled in connection with this that Hédon (24) has recently obtained some evidence to indicate that the hormone is present in appreciable amount only in the blood of the pancreatic vein, and not in arterial blood.

The following experiment is similar.

Exp. 10. Dog operated five days previously. Heart 53 grams. Circulation first with diabetic blood, to which first glucose, and then normal blood and glucose were added. Temp. 36·5° C. Art. press. 80 mm. One lung tied off. There was 25 min. interval between the addition of the normal blood and the determination of the respiratory exchange.

Period min.	Rate per min.	Output c.c. per min.	Gaseous exchange per hour c.c. O_2	c.c. CO_2	R.Q.	
30	128	176	190	151	·79	
21	125	—	182	135	·74	0·5 gram. glucose
30	118	147	181	133	·73	Normal blood + 0·5 gram glucose

In this experiment, the addition of sugar to the diabetic blood does not result in a rise of respiratory quotient, and the addition of hyperglycæmic normal blood is also without any effect.

(In Exp. 15 and also in other cases, the addition of glucose to the blood has always been without effect on the respiratory quotient of the diabetic heart.)

(3) *Diabetic heart treated with diluted blood.*

Exp. 11. Pancreas of dog extirpated four days previously. Heart-lung circulation established with diabetic blood. At the end of the first period of the respiratory experiment, 200 c.c. of 0·9 % NaCl solution at 36° C. was added to the circulating blood (200 c.c.), without disconnecting the lungs from the respiration apparatus. Arterial pressure 60 mm. Temp. 36° C.

Period min.	Rate per min.	Output c.c. per min.	Gaseous exchange per hour c.c. O_2	c.c. CO_2	R.Q.		Circulating fluid
30	143	210	187	142	·76		Diabetic blood
30	150	270	236	164	·70	Mean = ·76	Equal volume of saline solution added
15	140	220	261	214	·82		
8	—	—	237	181	·76		

Apart from a transitory disturbance of the quotient, there was no appreciable effect on adding the salt solution to the circulating blood.

These experiments, admittedly somewhat scanty, do not lend support to the toxic theory, and were not pursued further.

The effect of pancreas extracts on the gaseous metabolism of the diabetic heart.

Knowlton and Starling, and Maclean and Smedley found that the administration of pancreas extract caused a notable increase in the (much depressed) carbohydrate utilisation, which approached the normal level again. Patterson and Starling were inclined, however, to attribute the increase of sugar consumption to the fact that the extract has an accelerating effect on the heart, and thus, like adrenalin, quickens all its metabolic activities. As Patterson and Starling did not specially investigate this point, we give the results of some experiments in which the effect of the pancreatic extract on the gaseous exchanges of normal and diabetic hearts was tried.

The extracts for these experiments were all made in the same way; a piece of fresh pancreas, about five to ten grams in weight, was crushed with sand and some ·2 % HCl in a mortar, more ·2 % acid was added and the whole, now about 30 c.c., boiled, nearly neutralised and filtered.

Before making the addition of the extract thus prepared one or more periods were made with the heart in order to determine the respiratory quotient of the preparation. The pancreas extract was then added in one or more doses to the circulating blood of the preparation, and the respiratory quotient during subsequent periods was determined in like manner.

Experiments were first of all made with *normal hearts* in order to ascertain the effects of the quickening of the rate of heart beat brought about by the addition of pancreas extract, or of the extract together with adrenalin, in small amounts.

(*a*) *The effect of pancreas extract on normal hearts.*

Exp. 12. Dog, heart 33 grams. Temp. 37° C. Arterial pressure 80 mm.

Period min.	Pulse rate per min.	Output c.c. per min.	Gaseous exchange of preparation c.c. per hour c.c. O_2	c.c. CO_2	R.Q.	
60	162	200	162	139	·86 } Mean	
60	181	188	158	141	·89 } = ·87	
60	181	194	158	143	·905	1 gram. glucose added
60	300	162	212	175	·83	10 c.c. 'hormone' solution at commencement

Exp. 13. Dog, heart 50·5 grams. Arterial pressure 30 mm. Hg. (at higher pressures beats were dropped).

Period min.	Pulse rate per min.	Output c.c. per min.	Gaseous exchange of preparation c.c. per hour c.c. O_2	c.c. CO_2	R.Q.	
60	158	169	166	153	·92	
60	158	163	186	171	·92	1 gram. glucose added
60	158	154	188	177	·94	1 c.c. pancreas extract at commencement of period and 5 c.c. 30 min. after

Exp. 14. Dog, heart 67 grams. One lung tied off. Arterial pressure 55 mm. Hg. Temp. 36·5° C.

Period min.	Pulse rate per min.	Output c.c. per min.	Gaseous exchange of preparation c.c. per hour c.c. O_2	c.c. CO_2	R.Q.		
60	154	200	202	177	·88		
60	154	187	211	187	·89		1 gram. glucose added
30	285	207	288	222	·77	Mean	5 c.c. pancreas extract
30	194	200	257	242	·94	= ·855	
30	200	211	357	298	·84		0·5 c.c. 1 : 10,000 adrenalin
45	165	170	241	208	·865		2 c.c. pancreas extract

In these three experiments the effect of the extract on the respiratory quotient was only slight; on the whole, so far as there was any effect, it was a fall. In the fourth period of Exp. 14, there was a transitory rise, but this is to be explained as resembling the adrenalin effect, since it follows a short fall of quotient; the mean of the two quotients, as in the cases where adrenalin is administered to normal hearts, shows a fall. On the normal heart therefore, apart from the disturbances due to the acceleration, the extract has very little effect.

The experiments on the *diabetic hearts* were made in the same way. On the diabetic heart, the effect of the extract in causing an acceleration of the pulse rate is seen as in the normal organ, and it causes similar disturbances in the quotient. In order to judge of the effects it is therefore necessary to take the mean of periods extending over at least an hour from the first addition of the extract, and to ignore any transitory rises of the quotient for the reason already stated. Only when there has been a rise in the *mean* quotient of more than ·05 can we consider the effect to indicate an increased sugar usage. The following are some examples of these experiments.

(*b*) *The effect of pancreas extracts on diabetic hearts.*

Exp. 15. Dog. Pancreas removed two days previously. Heart 60·5 grams. Temp. 36·5° C. Arterial pressure 54 mm. Hg. One lung off. 10 c.c. 10 % glucose solution added at commencement of experiment.

Period min.	Pulse rate per min.	Output c.c. per min.	Gaseous exchange per hour c.c. O_2	c.c. CO_2	R.Q.		
30	143	197	198	143	·72	Mean	
30	141	166	194	142	·73	= ·725	
30	200	162	212	158	·75		3 c.c. extract
30	157	130	218	185	·85		
30	123	120	188	165	·88	Mean = ·855	
30	116	99	189	169	·895		
30	130	150	202	182	·90		5 c.c. extract

Exp. 16. Dog operated two days previously. Heart 65 grams. Both lungs. Arterial pressure 100 mm. Hg. Temp. 36° C.

Period min.	Pulse rate per min.	Output c.c. per min.	Gaseous exchange per hour c.c. O_2	c.c. CO_2	R.Q.		
20	100	180	216	162	·75		
40	120—133	207	234	172	·73		4 c.c. extract
40	144—150	180	226	204	·90	Mean	2 c.c. extract
40	114—109	125	244	208	·85	= ·82	
40	120—134	115	251	200	·80		5 c.c. extract
40	137—128	100	238	198	·83		4 c.c. extract

Exp. 17. Dog operated three days before experiment, and fed on glucose after operation until 24 hours before experiment, when the last dose of 50 grams was given. Heart 66 grams. Arterial pressure 100 mm. Hg. Temp. 36° C. Both lungs used.

Period min:	Pulse rate per min.	Output c.c. per min.	Gaseous exchange per hour c.c. O_2	c.c. CO_2	R.Q.		
40	96	238	209	148	·71		
40	97	234	239	148	·62		7 c.c. extract
40	100	235	216	159	·74	Mean	4 c.c. extract
40	98	216	229	178	·775	= ·72	
60	96	180	225	165	·735		5 c.c. extract

Exp. 18. Dog operated five days previously. 0·8 gram glucose added to circulating blood of preparation before experiment. Weight of heart 65 grams. Temp. 35·3° C. Arterial pressure 100.

Period min.	Pulse rate per min.	Output c.c. per min.	Gaseous exchange per hour c.c. O_2	c.c. CO_2	R.Q.		
60	108	183	162	116	·715		
60	169	133	195	138	·71	Mean	2 c.c. extract
60	108	96	163	128	·785	= ·747	

In Exps. 15 and 16 there was a distinct rise in the respiratory quotient, of ·13 and ·07 respectively, and this increased quotient represented the mean of experiments lasting in the first case 2½ hours and in the second case 3⅓ hours after the addition of the pancreatic extract, so that it was in both cases a well-sustained rise. But in the other two experiments there was practically no effect, and negative results were also obtained in a number of other experiments which were of short duration, and which are not given here. In Exp. 17 there was practically no cardiac acceleration, and this may mean that the extract was to be considered as inactive; this was not the case with Exp. 18 however, yet the change in the respiratory quotient was negligible.

These experiments on the addition of pancreatic extracts cannot be considered to lend much support to the view that they contain a specific hormone, though two of them might be interpreted in this way. But when taken in conjunction with the fact that by direct chemical estimation of sugar usage, the glucose consumption of the heart is increased,

they indicate that some further experiments in this direction are desirable, with possibly a different method of preparing the pancreatic extracts.

Conclusions.

1. The average respiratory quotient for the normal dog's heart is ·85, and the heart uses on the average 3·2 c.c. of oxygen per gram per hour when doing moderate work.

2. From the respiratory exchanges it is calculated that the heart uses about 1·6 mg. of carbohydrate per gram per hour, when the animal is fed on a mixed diet. This amount is in agreement with that found by direct chemical methods.

3. The lung tissue per gram of heart in similar manner uses not more than ·5 mg. of sugar per hour, as calculated from the gaseous exchanges.

4. The respiratory quotient of the diabetic heart in pancreatic diabetes has an average value of ·71. The quotient is not raised by the addition of glucose, nor by the acceleration brought about by the addition of adrenalin. These results indicate a depression or abolition of the power of the diabetic tissue to utilise carbohydrate, but there is also some indication that there are other profound modifications of tissue metabolism which may mask a moderate carbohydrate consumption.

5. The diabetic state is fully developed the day after the operation for removal of the pancreas, and the condition is thereafter constant for several days, or probably until just before death.

6. The average oxygen usage of the diabetic heart is 3·1 c.c. per gram per hour, from which it is inferred that the energy requirement of the diabetic tissue is the same as that of the normal one.

7. The normal heart does not alter its metabolism when fed with diabetic blood, apart from the effect of the added sugar. The diabetic heart is also unchanged when fed with normal blood.

8. Extracts of pancreas prepared by boiling with dilute acid have an effect on the normal heart which resembles that of adrenalin. There is acceleration, with the same type of disturbance of the quotient, but the mean quotient is unaltered. On the diabetic heart the effects are on the whole similar, though in two cases definite and prolonged rises of quotient have been obtained.

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