EXPERIMENTS ON THE CONSUMPTION OF SUGAR IN THE NORMAL AND THE DIABETIC HEART.

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In a paper recently published in this journal(1) we described a method for maintaining the heart and lungs of a mammal for many hours in a state approximating normal. In this method the heart, fed with blood, is performing its normal amount of work, which can however be altered at the will of the experimenter. The arterial resistance, the venous volume of the heart, and the temperature may be changed at will, and may be maintained constant at any required height within physiological limits. We have kept a heart beating under these conditions for as long as eleven hours; but we are hardly justified in regarding its activity as normal beyond three or four hours. If the arterial pressure be maintained at the normal height—80 to 100 mm. Hg in the dog—the heart may begin to fail at the end of the third or fourth hour, and the failure may be shown not only by diminished output but by a gradual disappearance of blood from the venous reservoir coincident with the development of pulmonary œdema. To avoid this contingency it is important to keep the venous pressure as low as is consistent with a good output, since the failure of the heart is more easily induced by over-filling than by increased arterial resistance. In many experiments we have noted that after three or four hours the oxygenation of the blood is apparently insufficiently carried out; the blood becomes darker, and it is not possible to improve its colour by increasing the pulmonary ventilation. In other cases the heart begins to fail even though the blood remains of a good colour. In either case a fresh lease of life can be given to such a heart by withdrawing all the blood from the reservoir and replacing it with fresh blood from a newly killed animal. In one or two cases we have also noticed prolonged improvement of the heart towards the end of the experiment on adding a small trace of adrenalin to the circulating blood. The failure that supervenes at the close of an

experiment may be due to the accumulation of waste products other than gaseous in the circulating blood, or to the using up of substances, of which adrenalin may be taken as a type, normally present in the circulating blood. Experiments are at present in progress on some of these points.

For three or four hours however the heart may be regarded as normal. If the dog's heart is used, it puts out from 150 to 350 c.c. per minute, and the coronary arteries are being fed with arterialised blood under normal conditions of pressure and work. Since the whole volume of the circulating blood in our different experiments varied from 150 to 400 c.c., the renewal of the blood through the heart and lungs is a very rapid one, and it seemed to us that the preparation would present many advantages both for pharmacological investigations as well as for determining the chemical changes associated with the metabolism of the heart muscle.

One of the most important questions in muscular metabolism refers to the consumption of sugar by this tissue. A consumption of sugar in contracting voluntary muscle has been affirmed by Chauveau and Kaufmann(2) as well as by other authors. These results however have not been generally accepted, since they were based on comparative analyses of the arterial and venous blood flowing through muscle, and therefore dealt with differences which must be excessively minute. The first satisfactory demonstration of the utilisation of sugar by muscle was furnished by Locke and Rosenheim(3) in their work on the isolated heart fed with Ringer's fluid to which glucose had been added. This work has been repeated with improved methods by Rohde(4), who has measured at the same time the consumption of glucose, the consumption of oxygen, and the production of carbon dioxide, in the heart of the cat. Gayda(5) has carried out similar experiments on the rabbit's heart. His paper contains a useful summary of previous work on the subject. All these experiments however were made on a heart fed with Ringer's fluid and working under abnormal conditions, so that some loophole was still left for those physiologists who maintained that the sugar of the circulating blood could not be directly utilised by the tissues under normal conditions until it had been built up by some organ, such as the pancreas or liver, into some more complex body. It seemed to us therefore essential to determine whether there was actual consumption of sugar under the conditions of our experiments.

Methods. Most of our experiments have been performed on small dogs; a few experiments have been carried out on cats, but the greater

tendency of these animals to pulmonary œdema renders them less adapted for prolonged experiment. Moreover it was an advantage to work on a larger scale than is possible when using a cat's heart, since it was necessary to abstract samples of blood at intervals for the purpose of analysis. The preparation of the animal was carried out as described in our previous paper, especial care being taken to tie in the arterial cannula by a ligature at the extreme base of the innominate artery, so as to exclude any possible branches other than the two main ones into which this trunk divides. Even after ligaturing all the vessels other than that into which the arterial cannula is inserted, there is almost constantly a slight loss of blood from the circulation, amounting to about 20 c.c. in the hour. This loss is greater if the arterial pressure be kept high and is not to be confounded with the loss which may come on towards the end of the experiment and rapidly progresses with the development of lung œdema. The arterial pressure was as a rule maintained at about 70 mm. Hg, and the temperature between 35° C. and 37° C. The venous reservoir was graduated so that we could tell whether blood was being lost from the circulation. At the end of the experiment the amount of blood in the tubes, heart and lungs was measured by washing out with normal salt solution, so that in this way we knew the total amount of fluid in the circulation during any period of the experiment. The amount circulating during any given hour was taken to be the mean of the amounts circulating at the beginning and at the end of the hour. In most cases a certain amount of glucose in 10 p.c. solution was added to the circulating fluid at the beginning of the experiment. After the circulation had proceeded for ten minutes, a sample of blood was taken for analysis. The experiment was then allowed to proceed for an hour, when a second sample was taken, and so on, through the experiment.

The estimation of sugar was carried out sometimes in the whole blood, sometimes, and invariably in the later experiments, in the blood serum. Since the sugar is mainly present in the blood serum, it is essential, if the estimation be made in the blood, to determine at the same time the hæmoglobin value of the blood so as to guard against possible errors produced by the exudation of the plasma of the blood into the lung tissue. A relative diminution of blood plasma might thus be misinterpreted as a using up of sugar by the heart or lungs. The estimation of sugar was carried out in the following way. 10 c.c. of blood were treated with 20 c.c. of copper sulphate solution (of the same strength as that used for the preparation of Fehling's solution) previously

diluted with 50 c.c. of water, making altogether 80 c.c. of fluid. The mixture was filtered to free it from the precipitated proteins. Of the filtrate 60 c.c. were neutralised with sodium hydrate to precipitate the excess of copper, filtered, and 20 c.c. of the filtrate taken and the sugar determined in it by Bertrand's method. In the later experiments 25 c.c. of the blood were taken at each sample and centrifuged. 10 c.c. of the serum thus obtained were treated with 10 c.c. of copper sulphate solution and 20 c.c. of water. The precipitate of proteins was filtered off. 30 c.c. of the filtrate was then neutralised with 1½ c.c. sodium hydrate and filtered, and the sugar in 20 c.c. of the filtrate determined by Bertrand's method.

Control experiments. Before we can draw any conclusions from our results as to the utilisation of sugar by the contracting heart muscle, we must know the limits of accuracy of our actual sugar determinations and also how far any disappearance of sugar we may obtain is to be ascribed to glycolytic processes occurring in the blood or to destruction of sugar in the lungs. In about a dozen cases double determinations were made of the same samples of blood or serum. In most cases the results were identical to the second place of decimals. In a few cases the results differed by ·01 p.c. and in one case by ·02 p.c. We may take the error of analysis therefore as probably not exceeding ·01 p.c., as is shown from the following results:

Control determinations of sugar in serum.

·54	·16	·175	·44	·27	·46	·35	·32	·40	·62	·61
·54	·14	·185	·44	·27	·46	·35	·32	·395	·63	·62

Most physiologists agree that the blood has some power of destroying and oxidising glucose, though there can be little doubt that many of the results obtained which have been ascribed to glycolysis are really due to bacterial contamination. In our experiments the latter source of fallacy has not been definitely excluded, and there seems little doubt that in some of our earlier experiments, when we were not sufficiently alive to the importance of this factor, a considerable proportion of the disappearance of the sugar must be ascribed to insufficient cleansing of the tubes of our apparatus. These experiments we have distinguished by an asterisk. In all our later experiments care was taken to cleanse thoroughly all the vessels and tubes of the apparatus with potassium permanganate solution at the close of each day. They were then, just before the experiment, washed through with tap water and then with normal salt solution. About once a week the tubes were

taken apart and boiled. Any glycolysis due to bacterial action would tend to increase with the prolongation of the observation and it seems probable that infection would be more marked in diabetic blood with its lowered powers of resistance than in normal blood. In a number of cases we have determined the actual disappearance of sugar in the blood taken at the end of the experiment and maintained at body temperature for two or three hours while a current of air was led slowly through it. All conditions were thus present for increased bacterial contamination, so that the results obtained in this way can be regarded as maximal. We are of opinion that the glycolysis under the conditions of our experiments would not amount to more than 0·01 p.c. glucose per hour, though, as will be seen from the subjoined table, the blood later on might show a loss of ·02 or even ·03 p.c. glucose per hour.

TABLE I. *Table showing glycolysis in blood.*

Time	1st analysis p.c.	2nd analysis p.c.	Difference p.c.	Difference per hour p.c.	Temperature
3 hours	·33	·24	·09	·03	38—45
2 ,,	·91	·89	·02	·01	36—40
2 ,,	·35	·31	·04	·02	36—40
1½ ,,	·22	·175	·045	·03	35—40
1½ ,,	·22	·18	·04	·0265	35—40
1½ ,,	·62 ·63	·61 ·62	·01	·007	35—40
1½ ,,	·61 ·62	·59	·025	·016	35—40

On the consumption of sugar in the lungs only one experiment was made and in this case the sugar content of the blood was found identical at the beginning and at the end of an experiment in which blood was transfused through the lungs of a dog during one and a half hours. It is possible that there may be a slight consumption of sugar in the lungs, since later experiments by Evans in this laboratory point to a gaseous metabolism in lungs and blood together amounting to about one-fifth of that taking place in the heart-lung preparation.

In all these experiments we are judging of the metabolism of a tissue by determining the amount of glucose present in a small sample of circulating blood and then multiplying the results obtained by a factor which may vary between 10 and 40, in order to determine the total sugar consumption. All our errors will be increased in the same proportion. Small differences in our results could therefore hardly be

regarded as evidence for or against the consumption of sugar by the contracting heart. In view of the difficulties and fallacies connected with the estimation of sugar in blood we have thought it advisable to give full details of our experimental results, in order that other workers may form their opinion as to the value of our experiments.

THE CONSUMPTION OF SUGAR IN THE NORMAL HEART-LUNG PREPARATION.

The results of these experiments can be seen in the following tabular representation (Table II). In the first five of these experiments the estimations were carried out on the blood. In experiments 6, 7 and 8 the sugar estimations were made on blood serum. The blood serum was taken as equivalent to 66 p.c. of the total blood. The utilisation of glucose varied from 2·8 to 6·29 milligrams per gram of heart per hour. We may take the average consumption as 4 mgms. per gram heart muscle per hour. Certain results in this table are worth noting with a view to subsequent investigations. Thus in one experiment in which the anæsthetic had been left on during the first 30 minutes by mistake, there was apparently no consumption of sugar. In the same way, a considerable diminution of sugar consumption was observed in one or two experiments, *e.g.*, in Exp. 2 and Exp. 7 during the last hour when the process of oxygenation of the blood seemed to fail, so that the blood was extremely venous. It is evident that the sugar consumption observed in this case could not possibly be ascribed to glycolysis in the blood, even if it were associated with some degree of bacterial change.

THE FATE OF SUGAR IN THE DIABETIC HEART.

Two theories have been put forward to account for the fatal diabetes which ensues in mammals on total extirpation of the pancreas. According to one theory, the prime cause of the disorder is an increased formation and mobilisation of sugar in the liver, this organ having been freed from some restricting influence previously exerted on it by the pancreas or by some product of internal secretion of the pancreas. According to the other theory, the essential factor in pancreatic diabetes is the inability of the tissues of the body to utilise glucose. The liver partakes of this inability and therefore suffers from glucose starvation in the midst of plenty. The increased production of sugar, *e.g.* from

TABLE II. *Consumption of sugar in the normal heart.*

No.	Time	Sugar in blood p.c. Before	After	Difference p.c.	Weight of heart gms.	Volume of blood c.c.	Mg. per gr. heart muscle	Temp.	Pressure mm. Hg.	Remarks
1. Dog	1 hour	·79	·665	·125	37·6	175	5·80	31·5°—32°	60—100	
4·5 kilos	1½ hours	·665	·59	·075	37·6	140	5·58			
2. Dog	1 hour	·70	·61	·09	42·5	230	4·89	34·5°—35·5°	*	
4·6 kilos	1 ,,	·61	·54	·07	42·5	215	4·01			
	50 mins.	·70	·68	·02	42·5	200	1·13			Blood very venous
3. Dog	1 hour	·20	·11	·09	81·7	360	3·96	35·5°—38°	70—90	
8·7 kilos	40 mins.	·32	·25	·07	81·7	275	3·52			
4. Dog	30 ,,	·33	·33	—	83·0	430	0·00	34°—35°		Anæsthetic left on by mistake
7·25 kilos	1 hour	·33	·20	·13	83·0	407	6·37			
	1 ,,	·20	·08	·12	83·0	352	5·08			
5. Dog	1 ,,	·54 ·54	·49	·05	58·4	332	2·84	34°—35°		
5·9 kilos	1 ,,	·49	·33	·16	58·4	230	6·29			
6. Dog		serum				serum				
5·2 kilos	1 hour	·89	·71	·18	50·8	155	4·64	36°—39°		
7. Dog	1 ,,	·81	·69	·12	63·5	190	3·5	36°—37°		Blood venous and output poor during second hour
	1 ,,	·69	·57	·12	63·5	150	2·8			
8. Dog	1 hr. 35 m.	·63	·50	·13	52·5	225	4·1	36·5°—37·4°		
6·75 kilos	1 ,, 30 ,,	·50	·33	·17	52·5	165	4·1			
		blood				blood				
9. Cat	1 hour	·58	·51	·07	19·1	115	4·2	35·5°—37·5°		Exp. ended by pulmonary œdema
4·6 kilos	45 mins.	·51	·39	·12	19·1	140	?			
10. Cat	1 hour	·72	·65	·07	19·0	140	5·1	36·5°—37·5°		

* Where not expressly mentioned, the pressure was kept at about 70 mm. Hg.

proteins, is merely a secondary result of this glucose starvation[1]. The loss of power on the part of the tissues to utilise glucose may on this theory be ascribed either to the absence of some co-ferment or similar body, normally produced in the pancreas and forming the necessary link in the process of sugar assimilation, or to the accumulation in the body of some substance normally destroyed by the pancreas, which acts as a poison and has a special inhibitory influence on the sugar-assimilating properties of the tissues. Although the lack of power to assimilate and oxidise sugar is the most prominent feature of the disorder, it must be remembered that the disability applies in a large measure also to the other foodstuffs; amino acids, for instance, occur in increased amounts in the urine, and the final death of the animal is largely due to the accumulation of products arising from defective fat oxidation, *e.g.*, oxybutyric and aceto-acetic acids.

It seemed to us that a distinct step in advance would be gained if we could determine whether the heart of a diabetic animal, isolated by our method, was able to consume sugar, as we had proved to be the case with the normal heart. In a number of dogs we therefore produced diabetes by the removal of the pancreas. This operation becomes relatively simple if carried out in the manner indicated by Hédon(7). Hitherto the operation has been a lengthy one and has necessitated the application of many ligatures to the vessels running between the pancreas and duodenum, and a great danger has always been the sloughing of the duodenum during the first few days after the operation. In Hédon's method the only ligature which is applied is to the main pancreatic duct, which is tied just as it enters the duodenum. The rest of the pancreas is then torn out bit by bit, using only the fingers, the glandular substance being stripped off the vessels which run into and through the pancreas. The main part of the gland having been removed, small portions adherent to the duodenum are then picked off with the fingers so that not a trace of this gland is left. The omentum is then wrapped round the duodenum, which is replaced in the abdomen, and the abdominal wall is closed. Half an hour suffices for the whole operation, including the anæsthetisation and preparation of the animal. The operation being shortened in this way, the animal very soon recovers from the anæsthetic and seems little the worse for the operation. The great advantage of this quick method probably depends on the fact that the diabetic state does not come on for two or

[1] A useful account of the enormous mass of literature dealing with this subject will be found in the papers by de Meyer(6).

three hours, during which time the phagocytes and fluids of the body have time to destroy any bacteria which have accidentally infected the wound. If rigid asepsis be observed, it thus becomes possible to remove the pancreas in one operation and yet to attain a perfect healing of the abdominal wound. The animals which had been operated on in this way were fed during the day after the operation on milk, and after that on lean meat (horse flesh). The day before they were going to be killed in order to test the sugar consuming powers of their heart, they were placed in a metabolism cage, and the 24 hours' urine collected in order that the D/N ratio might be determined. Even the day after the operation the urine will be found to contain 7 p.c. of sugar. The animals have been killed at varying intervals, from the second to the seventh day after extirpation of the pancreas. They have presented the usual phenomena which have already been described by many observers in pancreatic diabetes. Thus the blood is often found loaded with fat globules, so that a cream-like layer rises to the top of the plasma or serum. With this lipæmia is associated an advanced condition of fat infiltration of the liver. The lipæmia and fat infiltration seemed to us more marked in the animals which had a considerable store of fat in the body at the time of the operation, suggesting that the main factor is the mobilisation of the fat and a carrying of this fat to the liver in order that it can be broken down and utilised to take the place of the failing carbohydrate metabolism.

The final experiments on the heart were carried out in exactly the same way as had been previously done with the normal hearts. The animal was generally bled at the beginning of the experiment, and the defibrinated blood was later added to that pumped out by the heart on connecting it to the apparatus, so that the diabetic heart was fed with diabetic blood. In one or two cases it was necessary to sacrifice a second diabetic animal in order to obtain sufficient blood. The following table (Table III) shows the results obtained in seven such experiments (Exps. 12 to 18).

In three of the experiments, namely, 14, 15 and 16, the sugar consumption in the heart, though less than observed in the normal animal, was still considerable. In this experiment however the apparatus had been in use for some time and had not been taken to pieces or sterilised. On the supposition that the small effect might be due to bacterial change, the apparatus was taken to pieces, and it was found that a slimy growth had occurred on the inner surface of the rubber tubing. The whole apparatus was thoroughly cleaned and boiled, and in all

TABLE III. *Fate of sugar in heart of diabetic dog.*

No.		Time	Sugar in blood p.c. Before	After	Difference p.c.	Weight of heart gms.	Volume of blood c.c.	Mg. per gr. heart muscle	Temp.	Remarks
12.	Bitch	1 hour	·59	·60	—	50·0	230	0·00	35°—36°	Day after excision of pancreas. Urine contains 6·9 p.c. sugar. This is the second hour, the first blood sample having been lost.
13.	Bitch		Serum				Serum			
	6·4 kilos	55 mins.	·74	·74	—	55·4	240	0·00	36°—38·5°	3rd day after excision of pancreas.
*14.		1 hr. 5 m.	·35	·29	·06	69·0	245	1·80	35°—36·5°	2 days after excision of pancreas. Urine contains 8·4 p.c. sugar. No glucose added to blood. Between 2nd and 3rd periods diabetic blood replaced by normal blood *plus* glucose. Blood very venous during 4th period.
		1 hr. 20 m.	·29	·16, ·14	·14	69·0	160	2·43		
		1 hour	·49	·40	·09	69·0	214	2·79		
		45 mins.	·40	·29	·11	69·0	140	2·95		
*15.	Bitch	1 hour	·83	·67	·16	62·5	193	4·90	35·8°—36·2°	3 days after excision. D/N ratio (on milk diet) = 7. Between 2nd and 3rd periods some normal blood and more glucose added. Blood venous. Output bad.
		1 hr. 15 m.	·67	·53	·14	62·5	140	2·63		
		50 mins.	·86	·74	·12	62·5	137	3·10		
*16.		2 hours	·28	·175, ·185	·10	29·5	173	2·90	35·7°—36·5°	3rd day after excision. D/N ratio (on meat) = 2·2. Between 1st and 2nd periods normal blood and glucose added.
		2 ,,	·65	·440, ·440	·21	29·5	187	6·60		
17.		1 hour	·58	·600	—	36·2	190	0·00	36·3°—37·5°	6th day after excision. Meat diet. D/N = 3·2.
18.		1 hr. 30 m.	·55	·55	0	56·5	—	0·00	36·5°—37·2°	3rd day after excision. D/N (of *urine in bladder*) = 2·6. Pulse rate = 100 per min.

* Between Exps. 16 and 17, the apparatus was taken apart and thoroughly cleaned and sterilised.

subsequent experiments potassium permanganate was run through the apparatus at the close of the day's work. In the other experiments, namely, 12, 13, 17 and 18, there was practically no consumption of sugar in the heart.

In each case the analysis of the blood at the beginning and end of the hour either gave identical results or results which differed only within the limits of experimental error. We consider ourselves justified therefore in concluding from these experiments that after pancreatic diabetes has been produced by total removal of the pancreas the heart muscle is unable to utilise the blood sugar for its metabolic requirements.

On the nature of the influence exercised by the pancreas on the utilisation of sugar.

The experiments by Minkowski(8) on the effects of transplantation of a small portion of the pancreas with the removal of the rest, show conclusively that the action of the pancreas must be chemical, *i.e.* must be effected through the intermediation of the circulation and not of the central nervous system. It has been suggested that the action of the pancreas on the sugar consumption or utilisation is not a direct one but involves the interaction of other organs, such as the suprarenal and the thyroid glands. It seemed to us possible that we might be able to resolve not only this question but also the further question as to whether the action of the pancreas is the production of some substance or hormone necessary for the assimilation of sugar by the tissues, or of some substance which would act as an antitoxin to another substance which would otherwise accumulate in the blood and poison the sugar-assimilating or oxidising functions of the cells. We may say at once that our experiments appear to point to the simpler of these possible explanations as the correct one, and indicate that the pancreas normally produces some substance which is discharged into the blood stream and the presence of which is necessary for the utilisation of sugar by the tissues or, at any rate, by the contracting heart muscle; further that this substance is continually being removed from the blood and used up by the tissues, so that it must be constantly renewed if the animal is to continue to be able to utilise the glucose of the blood. If the antidiabetic hormone is being continually produced by the pancreas, it should be present in diabetic blood. On feeding a diabetic heart therefore with normal blood we should be able to restore to the heart

muscle the power of utilising glucose, in virtue of the hormone present in the normal blood. The following two experiments (Table IV) show the effect of feeding a diabetic heart with normal blood. In the second experiment the results are somewhat irregular, but the average consumption for the three hours is practically normal, the consumption per gram of heart muscle in the three successive hours being 5·13, 1·87 and 3·05 mgms. In the first experiment there was a continuous increase in the utilisation of sugar during the course of the experiment, the exact figures being 2·9, 5·28, and 8·1 mgms.

The third experiment of this series (Exp. 21) is the reverse of the first two. A normal heart containing presumably a certain amount of the antidiabetic hormone was fed with blood derived from a diabetic dog. In this case there was a continuous diminution in the consumption of sugar during the first three hours of the experiment, the figures obtained being 3·5, 2·45 and 1·68 mgms. per gram of heart muscle per hour. In this case the rate of the heart beat was slower than normal, a phenomenon which we had observed constantly in our series of experiments on the diabetic heart fed with diabetic blood. It seemed probable that the diabetic blood contained some constituent, perhaps of an acid character, which had the action of slowing the normal cardiac rhythm. It seemed possible that this substance might also be responsible for the diminishing consumption of sugar in this experiment and for the inability of the diabetic heart to oxidise sugar. In the third hour of this experiment we therefore added 2 c.c. of 10 p.c. sodium bicarbonate to the blood in the reservoir. The effect of this addition was to increase the rate of the heart beat from 120 to 135 per minute. No effect however was produced on the sugar consumption, which continued to fall, so that the consumption during the third hour was the lowest obtained throughout the experiment.

Although not definitely excluding an antitoxic action of the pancreas, these three experiments are most readily explained by the assumption that the tissues and blood normally contain some substance, the presence of which is essential for the direct utilisation of sugar by the tissues. This substance is gradually used up in the tissues and therefore has to be continually replaced from the blood if the utilisation of sugar is to continue. Since this substance is absent from the blood and tissues when the pancreas has been entirely removed, it seems probable that it is normally produced by this gland and turned into the blood. The question at once arose, whether it might not be possible to replace this substance in diabetic blood by the addition of pancreatic extracts.

TABLE IV. *Effects of feeding a diabetic heart with normal blood, and a normal heart with diabetic blood.*

No.		Hour of Exp.	Sugar in serum p.c. Before	After	Difference p.c.	Weight of heart gms.	Volume of serum c.c. (estimated)	Mg. per gr. heart muscle	Temp.	Remarks
19.	Dog	1st	·64	·61	0·03	47	300	2·90	35°—37°	4th day after excision of pancreas. Pronounced lipæmia. Heart fed with fresh blood plus glucose from a normal dog.
	5·25 kilos	2nd	·61	·52	0·09	47	276	5·28		
		3rd	·52	·36	0·16	47	240	8·10		
20.	Bitch	1st	·74	·67	0·07	34·5	253	5·13	36°—38°	4th day after excision of pancreas. D/N ratio (meat diet) = 3·4. Heart fed with blood plus glucose from a normal dog.
	3·75 kilos	2nd	·67	·64	0·03	34·5	213	1·87		
		3rd	·64	·58	0·06	34·5	175	3·05		
21.	Dog	1 hr. 30 m.	·56	·44	0·12	53·5	235	3·50	36°—37°	Normal heart fed with blood plus glucose from a dog from which pancreas had been excised 2 days previously. Pulse rate 120 per min. Before 3rd period added to blood 2 c.c. 10 p.c. Na_2CO_3. Pulse rate quickened slightly (to 134 per min.).
		1 hr. 25 m.	·44	·34	0·10	53·5	195	2·45		
		1 hour	·34	·27 ·27	0·07	53·5	160	1·68		

Reasoning from the behaviour of substances belonging to the class of hormones, such as secretin and adrenalin, we might guess that the pancreatic hormone would be a body diffusible, soluble in water, unstable in alkaline solution, but more stable in slightly acid solution, and not destroyed immediately at the temperature of boiling water. We therefore adopted the following means for the preparation of a pancreatic extract which should contain this hypothetic hormone. The pancreas of a dog or cat was rubbed up with sand, a few drops of N/10 HCl being added to keep the reaction slightly acid. About 35 c.c. of Ringer's solution, also rendered slightly acid, were then added and rubbed up with the gland. The mixture was carried rapidly to the boiling point, a few drops more of dilute acid being added during the ebullition, if necessary, to keep the mixture slightly acid while boiling. It was then filtered, the filtrate being obtained practically free from coagulable protein. Five experiments in all were made to test the effect of such an extract (Exps. 22–26, Table V). In the first four experiments, the heart of a diabetic animal was fed with its own blood for an hour, blood samples being taken at the beginning and end of the hour in order to test the consumption of glucose. Ten c.c. of the pancreatic extract, neutralised with a few drops of sodium carbonate solution, were then added to the blood, and after the lapse of ten minutes another sample of blood was taken and tested. The experiment was then allowed to run for a further one or two hours, a blood sample being taken at the end of each hour.

In every case it will be seen that the addition of the extract of pancreas, whether derived from the dog or cat, raised the sugar consumption considerably. In the second experiment of the series the removal of the pancreas was incomplete. The operation of excision had been carried out in two stages and in removing the graft a small piece of pancreas, about 2 cms. in diameter, was by accident left attached to the pedicle. In this experiment, although the dog was passing sugar in its urine, it had evidently not lost entirely its power of utilising sugar, the consumption of this substance in the first hour amounting to 3·68 mgms. per gram heart muscle. The effect of the addition of pancreatic extract was to raise the consumption of glucose to 5·2 mgms. per gram heart muscle. In the other three experiments the sugar consumption during the first hour was only about 0·5 mgm. per gram per hour, *i.e.*, within the limits of experimental error. During the hour after the addition of pancreatic extract the consumption rose to 3·5, 2·8 and 3 mgms. per gram per hour, *i.e.* to a figure not far removed from that found in normal hearts fed with normal blood.

TABLE V. *Effect of pancreatic extract on the utilisation of sugar by the diabetic heart.*

No.	Intervals	Sugar in serum p.c. Before	Sugar in serum p.c. After	Difference p.c.	Weight of heart gms.	Volume of serum c.c.	Mg. per gr. heart muscle	Temp.	Remarks
22. Bitch	1 hr. 15 m.	0·58	0·57	0·10	41·50	267	0·48	36°—37·5°	3rd day after excision of pancreas. Heart fed with own blood. Pancreatic extract (10 c.c.) added between 1st and 2nd periods.
	1 hr. 15 m.	0·54	{0·46 / 0·46}	0·08	41·50	234	3·55		
23. Bitch	1 hour	0·60	0·53	0·07	40·25	212	3·68	36°—38°	Incomplete removal of pancreas. 2nd day after second operation. Urine contained 1·8 p.c. glucose. Pancreatic extract added between 1st and 2nd periods.
	1 hour	0·52	0·37	0·15	40·25	140	5·21		
24. Bitch	1 hour	0·50	0·49	0·01	41·7	210	0·50	36°—37·4°	3rd day after excision of pancreas. D/N (on milk diet) = 5·4. 10 c.c. pancreatic extract added between 1st and 2nd periods.
3·17 kilos	1 hr. 10 m.	0·44	{0·35 / 0·35}	0·09	41·7	130	2·80		
25. Bitch	1 hr. 30 m.	0·53	0·52	0·01	43·6	227	0·33	36°—37·2°	6th day after pancreas excision. D/N (meat diet) = 2·8. Between 1st and 2nd periods added 10 c.c. of an extract of *cat's* pancreas.
5·5 kilos	1 hr. 30 m.	0·45	{0·32 / 0·32}	0·13	43·6	153	3·00		
26. Bitch	1 hour	0·57	0·540	0·03	45·7	230	1·50	36·8°—37·5°	6th day after pancreas excision. D/N (meat diet) = 2·7. During 1st period added 10 c.c. extract of muscle and kidneys from same dog. Pulse rate 108. Before 2nd period added 10 c.c. of pancreatic extract. Pulse rate rose to 162. Before 3rd period replaced blood by normal blood plus pancreatic extracts.
	1 hour	0·51	{0·400 / 0·395}	0·11	45·7	175	4·25		
	1 hour	0·65	0·550	0·10	45·7	207	4·60		
Glycolysis in diabetic blood of 26	1 hour	0·42	0·400	0·02	—	—	—		

It was necessary to show that this improvement was connected specifically with the pancreas and was not due simply to the injection of any organ extract. It could not be due to any change of reaction, the alkalinity of the added fluid being so slight as not to alter appreciably the reaction of the circulating blood. In the fifth experiment we therefore during the first hour added to the blood an extract prepared from the muscle and kidney of the diabetic animal in exactly the same way as the pancreatic extract was prepared. During the first hour of this experiment the sugar consumption per gram of heart muscle was 1·5 mgms. This figure is rather higher than those obtained in the other three experiments on completely diabetic hearts and may possibly indicate some direct influence of the organ extracts on the consumption of sugar by the heart muscle. On the other hand it is not large enough to exceed the possible error due to glycolysis occurring in the blood itself, and was not comparable with the effect produced by the action of pancreatic extract. When this was added the sugar consumption rose to 4·25 mgms. in the second hour. In the third hour of the same experiment the diabetic blood was removed and replaced by defibrinated blood from a normal dog together with another 10 c.c. of the pancreatic extract. In this case the consumption of sugar during the third hour amounted to 4·6 mgms. per gram of heart muscle, indicating that a maximum result had been produced in the previous hour by the addition of pancreatic extract alone to the diabetic blood.

It was not however necessary to wait for the results of the sugar analysis in order to prove that the addition of pancreatic extract had a beneficial effect on the diabetic heart. We have already mentioned that the rate of the heart beat in the diabetic animal is abnormally slow. Within a few minutes after adding the extract of pancreas the pulse rate rose and finally attained a normal figure. Thus in the fifth experiment of the last series (Exp. 26) the rate of the heart beat during the first hour when fed with diabetic blood was 108 at a temperature of 36·8° C.; 12 mins. after adding 10 c.c. of the pancreatic extract, the heart rate was 162 per min. at a temperature of 37·2° C. Half an hour later the heart rate was 144 at a temperature of 36·8° C. After replacing the blood by normal blood together with another 10 c.c. of pancreatic extract, the heart rate quickened to 168 per min. at a temperature of 36·9° C.

So far as these experiments go, they would seem to indicate that the pancreas normally produces a substance which is continually discharged into the circulating blood, and which we may speak of as the

pancreatic hormone. This substance is soluble in dilute acid solutions, is thermo-stable, and can therefore be extracted from the fresh pancreas by grinding up this organ and boiling it with dilute, slightly acid, saline solutions. This substance is carried by the blood to all the tissues of the body, and its presence is a necessary condition for the assimilation and utilisation by the tissues of the glucose present in the circulating blood. Removal of the pancreas brings about the disappearance of this substance from the blood and therefore deprives the tissues of any possibility of utilising carbohydrates as sources of energy. It is evident however that many further experiments will be required before this conclusion can be regarded as definitely established, since, although they afford the most obvious explanation of our experimental results, we cannot claim to have absolutely excluded all other explanations. The weak point in all such experiments is the necessity of drawing conclusions from the analysis of samples of blood, so that at no time are we dealing with the *total* metabolism of the tissue, and our results have to be multiplied by a factor, which multiplies also our errors of experiment. The method we have used permits however of a direct determination of the gaseous exchanges of the heart-lung preparation, in which the total oxygen consumption and total carbon dioxide output of the tissues are measured. In thus avoiding the necessity for any multiplying factor we hope to obtain more definitive results, and especially by determination of the respiratory quotient to find out the nature of the substance which the heart muscle is using as a source of energy. Mr C. L. Evans has already commenced in this laboratory the study of the respiratory exchanges of the normal heart. An extension of these experiments to the diabetic heart may give the confirmatory evidence which we are seeking or may show the direction in which a truer explanation is to be sought.

Summary.

1. The heart muscle of the dog, fed with normal blood, consumes about 4 milligrams of glucose per gram of heart muscle per hour.

2. This power of consuming sugar is reduced to a minimum or disappears altogether in hearts taken from animals which have undergone complete excision of the pancreas.

3. The addition of a boiled extract of pancreas to the blood circulating through the heart of a diabetic animal, restores to the latter the power of utilising the glucose of the circulating blood.

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