XVII. A STUDY OF THE METABOLISM IN EXPERIMENTAL DIABETES.

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I. Introduction.

The diabetes produced in dogs by the removal of the pancreas has been the theme of many investigations which have shown how similar the condition is to a severe type of human diabetes and have given hope of an explanation upon similar grounds. The questions which investigation has left debatable concern the relative importance of two factors which might be primary causes. These factors are:

1. An over-production of sugar by the organism.
2. A loss of the power to use sugar by the tissues.

According to the over-production theory the removal of the pancreas does away with a normal control exerted upon protein and fat metabolism and results in a flooding of the organism with sugar formed from these substances so that the limit of sugar usage is exceeded and a portion of the sugar wasted. The non-utilisation theory presupposes that the internal secretion of the pancreas is indispensable to the tissues in the combustion of carbohydrate. The over-production theory might well be accepted as a working hypothesis but for certain objections. These are the low level of the respiratory quotient, which points to non-utilisation of carbohydrate although the blood is hyperglycaemic, and the steadily increasing D : N ratio in the course of the diabetes when no carbohydrate is given. It is also very plain that the over-production theory would be difficult to prove until the question of the utilisation power had been settled.

The problem of utilisation has been attacked in three ways. The first method was to administer sugar and see how much of the dose could be recovered from the urine. Minkowski based his conclusion as to the non-utilisation of glucose upon results from this method, and his observations have been confirmed many times. The second method consists in observations upon the blood-sugar content. Starling and co-workers estimated the amount of sugar used by the heart by the fall in blood sugar level during an experiment with the heart-lung preparation. In the first series of experiments of this type Knowlton and Starling [1912] found evidence of a loss of the power of sugar utilisation by diabetic hearts. Later work has shown that sugar disappears from the blood perfusing diabetic hearts and has pointed out the possible sources of error in experiments of this type [Patterson and Starling, 1913; Macleod and Pearce, 1913]. Whether conclusions as to sugar usage can be safely made from the disappearance of reducing substance from the blood in such experiments is still a matter for consideration. The third method of investigating sugar utilisation in diabetes is by observing the respiratory exchanges of the intact animal or isolated tissue. The determination of the respiratory quotient gives a definite measure of the extent to which carbohydrate is being made use of by the organism. Evans and Starling [1914] have studied the respiratory exchanges of the heart-lung preparation in a series of normal and diabetic dogs. They find that the respiratory quotient of the diabetic preparation is lower than the normal, taking averages of the large series of observations. They conclude that the diabetic heart shows little evidence of sugar utilisation because of the low level of the respiratory quotient in spite of the fact that the blood is hyperglycaemic. Porges and Salomon [1910] maintain that the carbohydrate utilisation is unimpaired, since they observed high respiratory quotients in diabetic dogs whose abdominal organs were excluded from the circulation. Murlin, Edelmann and Kramer [1913] point out, however, that a high respiratory quotient in such a condition is of no significance as regards sugar usage. It is evident, as these authors state, that the abrupt change in circulatory conditions, coupled with respiratory and blood reaction variations incident upon the clamping of the abdominal vessels, must be followed by an abnormal respiratory exchange. In the extensive observations of Benedict and Joslin [1910, 1912] upon human diabetes the severity of the disease has been found to increase with the lowering of the respiratory quotient. The respiratory exchange in diabetes indicates a loss or an impairment of the power of sugar utilisation, at least in the light of our present knowledge.

In view of the unsettled state in which the question of sugar usage in diabetes still remains it seemed desirable to undertake an investigation of the metabolism in dogs before and after depancreatisation. Observations were made upon the respiratory exchange of these animals and detailed analyses of the urine and faeces carried out. The main objective has been the question of sugar utilisation. The results are reported in the following paper.

In this report we wish to acknowledge gratefully the considerable assistance we have received from the members of the staff and other workers at the Institute of Physiology. Dr Plimmer and Mr Reeve have done the urine analyses throughout the research. Dr Cruickshank carried out the tissue and faeces analyses. We take pleasure in thanking these and all the workers at the Institute for help in every stage of the research. We are greatly indebted to Professor Starling, at whose suggestion the work was undertaken, for performing the necessary operations and for his help and advice throughout the research[1].

II. Methods.

Animals. Bitches were used in all the experiments. Care was taken in the selection of young and normal animals and in training them to lie quiet in the apparatus. While under observation the dogs were kept in metabolism cages in a well-lighted, heated and ventilated room.

Feeding. The animals were usually fed once a day at about 5 p.m., no food being left in the metabolism cage over night. The food given was carefully weighed and precautions were taken to prevent wasting of the weighed amount.

Diets and Food Analyses. The diet given to normal dogs varied in character for purposes of experiment. Full notes of the diet will be found in the protocols. The standard diet given to diabetic animals was chopped horse meat and ox pancreas, in the proportion of four to one. Samples of the food were analysed from time to time and estimations of total nitrogen, carbohydrate, phosphate and fat were made where necessary. The following table gives these analyses and the corresponding abbreviations will be used in the protocols when referring to these foods.

[1] Dr Plimmer wishes to thank the Government Grant Committee of the Royal Society for the grant defraying the expenses of the chemical work in connection with this research.

The expenses in connection with the analyses of fats were partially covered by a grant from the Royal Society to Dr Cruickshank.

TABLE I.

	Abbreviation	N %	Carbohydrate %	P_2O_5 %
Horse meat	M.	4·0 to 4·4	—	0·5 to 0·66
Ox pancreas	P.	2·89	—	1·16 to 1·30
Caseinogen	C.	11·0	—	—
Gelatin	G.	14·0	—	—
Puppy biscuit	P.b.	3·2	60·0	—
Palmine	Pal.	—	—	—
Cod liver oil	C.L.O.	—	—	—

Sugar administration. The sugars administered were glucose and fructose. The dose given was 20 g. by the mouth, as the dogs were found to take this amount dissolved in water readily, and during succeeding respiratory observations would remain quiet in the apparatus. Rectal enemata of sugar solutions were also given, but owing to difficulties in controlling the amount given, liability of the enemata being expelled and varying rate of absorption, the results cannot be regarded as regular enough for comparison. With intravenous injections difficulties arose from the subsequent uneasiness of the animals in the apparatus. The effects of intravenous sugar on the respiratory exchange of diabetic animals will be studied in a future research. The standard dose of glucose or fructose in these experiments was 20 g. given per os.

Operations. The operations were all performed by Professor Starling. The dogs were given a small dose of morphine about two hours before the operation and full anaesthesia was produced by ether-chloroform mixture. Due aseptic precautions were taken and no serious wound infections were observed. All the animals recovered quickly from the operation and showed no uneasiness on the following day.

The method of Hédon was employed in the total extirpations of the pancreas and six dogs were operated upon in this manner. They were dogs I, II, III, IV, V, VI.

Two dogs, VII and VIII, were partially depancreatised and as the procedure was different the operations will be described separately.

Dog VII. All pancreatic tissue was removed from the abdominal cavity, leaving a small portion of the tail, about one square centimetre, free with its blood supply intact in a pedicle of mesentery. This portion was implanted under the skin of the abdominal wall. The graft was functional, because there was a discharge of clear juice for a short period after the operation. This juice digested fibrin slowly. The appearance of sections of the graft is

noted later. In histological structure the tissue showed little evidence of degeneration a month after the operation.

Dog VIII. This animal had two operations performed on it.

Operation 1. All the pancreatic tissue was removed with the exception of a mass about the size of a pea, which was left close to the main pancreatic duct. The duct was ligated on the peripheral side of the mass. This small portion was evidently functional as the dog, after having a slight glycosuria for a week, became apparently normal and only showed a slightly reduced sugar tolerance for glucose and fructose.

Operation 2. The remainder of the pancreas was removed and the abdominal wound again closed. The dog immediately developed a marked glycosuria with a D : N ratio of 3·2, but at the end of two weeks seemed in far better condition than is usually the case with totally depancreatised animals.

Collection of urine and faeces. The dogs were kept in metabolism cages while under observation. They were catheterised at the end of twenty-four hour periods at 10 a.m. After catheterisation the animals were allowed to run round a room for a short period and almost always faeces were passed at this time. Rarely the faeces were passed in the metabolism cages. The cages were thoroughly cleaned every day. Before the animals could be catheterised easily it was necessary to perform an operation under anaesthesia in which the perineum was split and the vulvar opening thus widened so that a glass catheter could be readily passed. In some instances the animal was catheterised at intervals of about four hours during the day, so as to ascertain the nitrogen excretion during respiratory observations. On these occasions the figures from the analyses are added up to obtain the whole 24 hour results. While the animals were in the metabolism cages the urine was received into bottles through a layer of filter paper, and some crystals of thymol were placed in the bottles to inhibit bacterial growth.

Weight of animals. The animals were weighed carefully before observations in the respiration apparatus. The weighing was generally carried out in the morning after catheterisation and when faeces had been passed and before feeding.

Urine analyses.

Total nitrogen; Kjeldahl's method.

Ammonia; Folin's method.

Urea; Soya bean urease method, as described by Plimmer and Skelton [1914, 1].

Allantoin was estimated by difference between the figures obtained for urea by Folin's method and by the urease method [Plimmer and Skelton, 1914, 1]. For the estimation of allantoin in diabetic urine the method has been modified by Plimmer and Skelton [1914, 2].

Creatinine; Folin's colorimetric method.

Uric acid; Folin's colorimetric method.

Amino nitrogen; estimation by the difference between the formaldehyde titration and the figure for ammonia by Folin's method.

P_2O_5; Neumann's method as modified by Bayliss and Plimmer.

Acetone and acetoacetic acid; two distillations, the first from acid and the second from alkaline solution, were carried out. The amount of total acetone was estimated by Messinger's iodometric titration. Due precautions were taken to have the tube under the surface of the fluid in the receivers.

Hydroxybutyric acid; estimation by the extraction method of Hurtley. The estimation was discontinued because of the very small quantity present in the dog's urine.

Sugar; the figures obtained by Benedict's method are those given in the tables. The estimations were controlled by the use of Bertrand's method, polarimetric readings, and fermentation in some cases.

Faeces.

Fat; estimation of fat in the dried sample by the Kumagawa-Suto method.

Tissues.

Glycogen; estimation in heart and liver tissue was carried out as described by Cruickshank [1913].

Fat; the method of Kumagawa using Mottram's modification for the removal of unsaponifiable and resinous substances.

Respiration apparatus.

The small respiratory calorimeter devised by Benedict [1912] for animal experimentation was used. The reader is referred to Benedict's articles for a complete description of the apparatus. The construction of the apparatus is essentially the same as that described by Benedict, the only modification being an arrangement for regulating the temperature of the chamber in which the animal is placed. Two incandescent lamps were fixed inside the chamber and the amount of heat given off by them was regulated by a lamp and coil resistance interposed in the circuit outside the box. A thermometer

reading to tenths of a degree centigrade was placed inside the chamber and a temperature of about 24° C. was maintained during the observations.

Testing the apparatus. The apparatus was tested in the various ways mentioned by Benedict. The parts were first connected to a water manometer and subjected to a pressure of about 100 cm. of water. When the parts were made air-tight separately the whole apparatus was set up and blank determinations made. The weight of the carbon dioxide absorption sets was checked during these blank periods, so as to ensure full absorption of water vapour in the large sulphuric acid bottles. Also the temperature of the box was maintained constant and the level of the spirometer observed for long periods to make sure that the apparatus was air-tight. Finally, an alcohol lamp was burnt inside the chamber and carbon dioxide and oxygen measurements carried out. This test was frequently done in the course of the research and is a very valuable indication of the reliability of results.

Carbon dioxide measurements. The carbon dioxide was estimated by the gain in weight of the soda lime bottle and Williams flask together. The weighings were carried out to 0·01 g. on a large balance capable of weighing ten kilograms. The absorption set was carefully cleaned especially at the coupling joints before every weighing. The blank of determinations showed an error of ± 0·02 to 0·05 g. The absorption set weighed four to five kilos.

The soda lime was made according to the directions given by Benedict and the absorption set was frequently replenished with fresh material. The limit of absorption of the set is about 75 g., but the bottles were replenished before they had increased in weight 50 g.

Oxygen measurements. The oxygen used was that prepared by the electrolytic process and was 99 % pure. It was sent into the apparatus through a gasometer reading to 10 cc., which had been tested for its mechanical error at the ordinary room temperature and for various rates of flow of the gas. This error was:

at rates of 50 to 60 cc. per min.—reading × 0·992.

at rates of 70 to 80 cc. per min.—reading × 0·993.

In many experiments the oxygen was supplied from a small cylinder and the readings of the gasometer were checked by the loss of weight of the cylinder. When the results were satisfactorily checked the readings of the gasometer, corrected for mechanical error, temperature and pressure, were taken, and a large oxygen cylinder could be used.

The spirometer pointer was levelled at the beginning of periods with a fixed pointer and at the end of periods brought to lie along this level again.

One millimetre rise or fall of the spirometer represents 22 cc. It was found that one-tenth of a degree rise or fall of temperature in the chamber caused a movement of the spirometer corresponding to 30 cc. Care was therefore taken to maintain a constant temperature during periods of observation.

General technique of observations. The periods were usually from 40 to 60 minutes in duration. If the animal became restless towards the end of a period the observation was lengthened in order to avoid as much as possible errors from increases of lung ventilation and from changes of temperature in the chamber. The normal animals show much more restlessness than the diabetics, so that many normal observations were necessary to accustom the animals to the apparatus and thus obtain observations when the animals were quite quiet. The arrangement for recording movement described by Benedict was used and is useful as a rough means of comparing observations.

III. Results.

1. *Respiratory Quotient of Fasting Animals.*

Since the respiratory quotient is a varying ratio depending upon the extent to which protein, carbohydrate and fat are being oxidised in the organism, or being laid down as stores in the depôts of the body, it is very necessary to determine this ratio when the conditions of absorption and utilisation are as uniform as possible. In the fasting state when these conditions are most regular the respiratory quotient can be used as a basis of comparison if certain factors are controlled. The factors which can be controlled are the character of the diet and the time of the observation after a meal. Other factors which must be taken into account are the amounts of carbohydrate and fat stored in the body and available for usage. The 'fasting' respiratory quotients here referred to are observations made at least 18 hours after a meal and under conditions as nearly comparable as possible. In normal animals when meat is fed, the level of the 'fasting' quotient is found to be 0·72 to 0·77 with the usual figure of 0·75. When increasing amounts of meat are fed the quotient is somewhat higher. When large amounts of fat are given the quotient observed is 0·72 to 0·74 in the fasting state, so that whether protein or fat form the main part of the diet of dogs the character of the fasting metabolism appears to be much the same. When starchy foods are fed, the level of the 'fasting' quotient rises to 0·76 to 0·80. In the latter case evidently more carbohydrate material is available for usage or has been stored as glycogen and is taking a uniform part in the

metabolism, or perhaps some formation of fat from available carbohydrate is taking place. With the 'fasting' respiratory quotients of diabetic animals two points will be observed in the curves and protocols:

(i) the average value is about that observed in the normal combustion of fat, namely, 0·705;

(ii) the respiratory quotient varies within smaller limits than that of normal animals, viz. 0·68 to 0·74.

The lowest fasting quotient of dogs upon a diet rich in fats was 0·690, while the lowest diabetic figure was 0·670. Since the diet of the diabetic

Dog I.

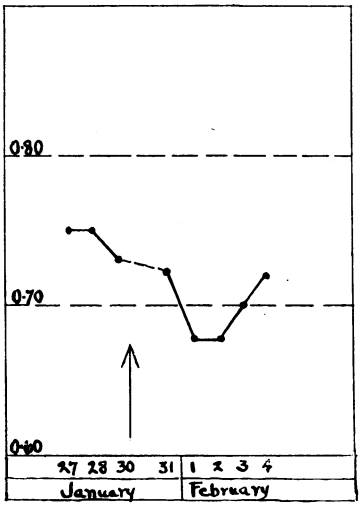


Fig. 1 *a*.

dogs was protein we must compare the 'fasting' quotients with the normal upon protein diet, which shows the diabetic quotient to be at a slightly lower level.

In Fig. 1 (*a* and *b*) the lower level of the 'fasting' quotient of totally depancreatised animals is clearly illustrated, and in most cases this level was lowest soon after the operation and tended to rise later. This rise may be only an apparent one, however, and not due to an increase in the ability to utilise carbohydrate but to a relative decrease in the amount of oxygen taken up. Later on in the course of the severe diabetes produced in totally depancreatised animals, the circulatory weakness and changes in the reaction of the blood evidently combine in causing a diminution in the oxygen intake and thus an apparent rise in the respiratory quotient.

With the partially depancreatised dogs the course of the fasting quotient varies. Fig. 2 from Dog VII shows that the quotient fell gradually to a low

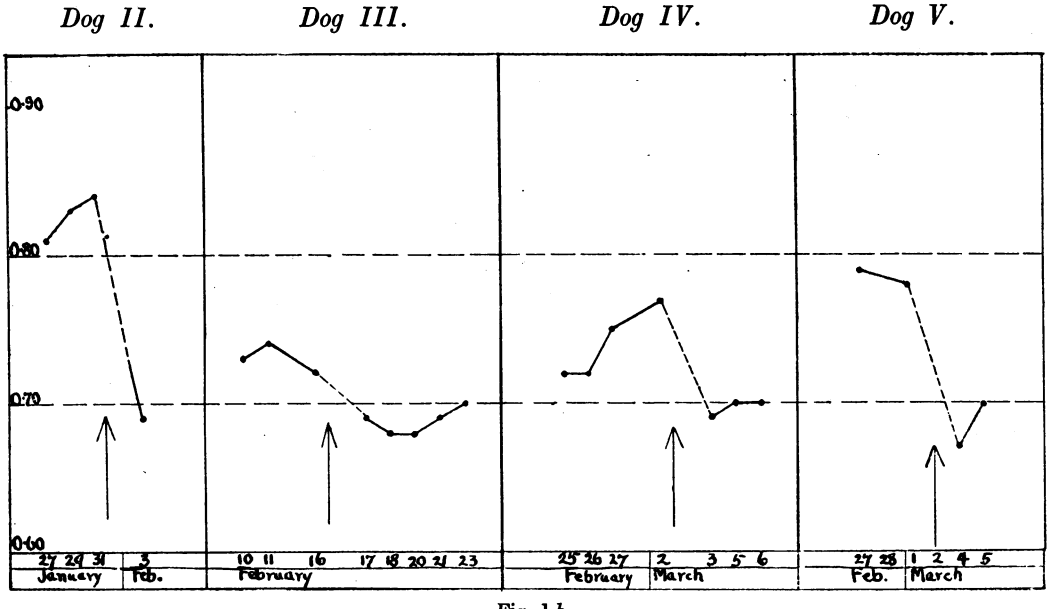


Fig. 1 *b*.

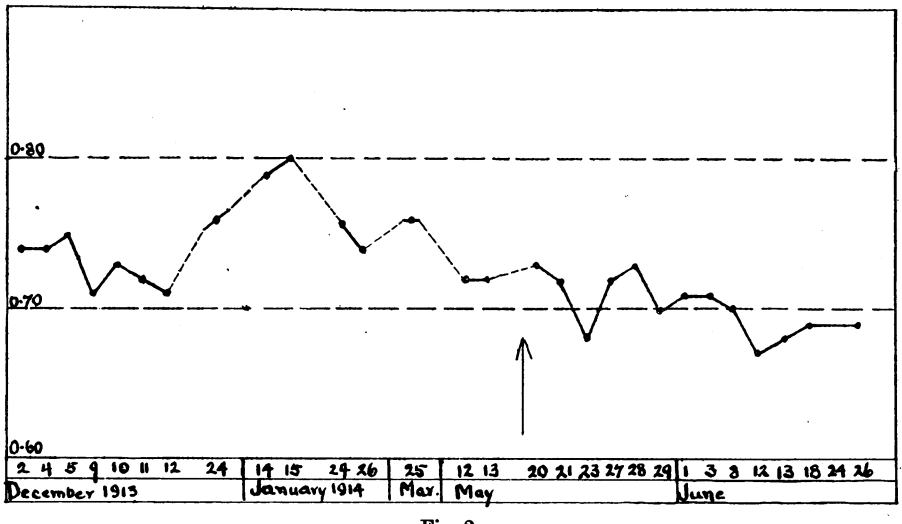


Fig. 2.

level as time went on. The course of the diabetes in this dog was very like that produced by total depancreatisation, except that the animal survived

longer. With Dog VIII partial depancreatisation produced a very transient and slight glycosuria. Fig. 3 shows that the respiratory quotient reached very low levels soon after the operation and then rose to normal and high levels. After total depancreatisation the course of the quotient was generally higher in Dog VIII than that observed in any of the other diabetic animals, and the general condition of the animal was much better than that of any of the others.

In Figs. 1, 2 and 3, the arrows show when the operations of depancreatisation were performed.

Dog VIII.

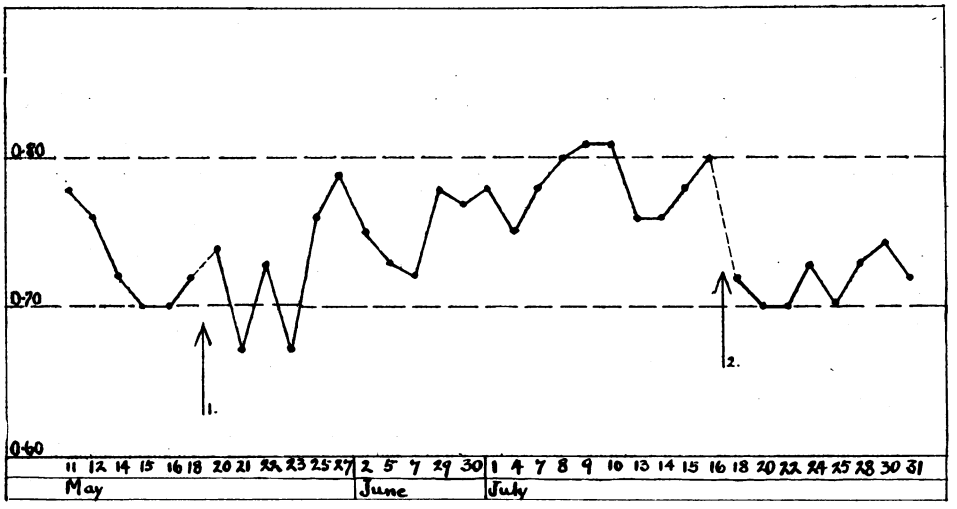


Fig. 3.

2. *Total Metabolism as judged by Oxygen Consumption.*

The figures for the oxygen consumption in the following table (II) are given in cc. per kilo. per hour. They are in every case from observations upon the animals at least 18 hours after feeding and necessarily when the animals showed the greatest degree of rest, and when the figures showed a uniformity in successive periods. Thus the first observation after the animal has been placed in the respiratory chamber has not been used on account of the usual initial restlessness, and the figures for oxygen consumption are an average of successive quiet and uniform periods. The results are therefore comparable with a fair degree of accuracy. Before remarking upon these results it is necessary to mention that there are certain objections to a comparison between

the total metabolism of normal and diabetic animals based upon the oxygen consumption in cc. per kilo. of animal per hour.

(1) The surface extent of the animal remains approximately constant while the weight of the animal decreases very rapidly when diabetes ensues. There is therefore a relatively greater area where heat loss may occur and one might expect a compensation for this in an increase of metabolism per unit of weight.

(2) The loss in weight in diabetes chiefly concerns water and fat which in the normal condition are in themselves not active consumers of oxygen, while organs and tissues which are constantly and actively engaged in metabolism preserve their normal rate of oxygen consumption. If this occurred, an apparent increase of total metabolism per unit of weight would be the result.

Another consideration in the comparison of total oxygen figures is the fact that the animals become more and more accustomed to and therefore quiet in the apparatus as the observations are continued. This consideration does not apply in the case of the partially depancreatised dogs which were under observation for some time as normal animals. It would apply in the case of the totally depancreatised dogs. Also the depressed state of these diabetic animals must be taken into account. As far as observation and the kymograph records indicate the muscular activity, the diabetic animals were invariably quieter than the normal.

It will be seen from Table II that there is an increase in the oxygen per kilo. per hour amounting to 10–15 % in the diabetic state, taking averages of all normal and diabetic observations. It will be noted that the figures show a steady increase from the time of operation up to the termination. In the last stages it has been remarked that the oxygen intake may decrease markedly owing to circulatory disturbances. The increase in the total metabolism becomes more striking when we remember that the diabetic animals are always quieter than the normal. This increase in the total metabolism we think ought to be regarded as a real increase, not an apparent one, depending upon the two considerations mentioned above. A comparison of the weights and oxygen consumption of Dog VIII in Table II shows that a decrease of weight does not necessarily cause an apparent increase of the total metabolism. This table is divided into six sections so that the figures obtained after both operations and under varying conditions may be compared. It will be noted that a steady decrease in weight occurs in sections II and III without a parallel increase in the oxygen consumption.

This indicates that the part played by a decrease in the more inert elements of the body or a decrease in weight with the same surface area, in causing an apparent increase in oxygen figures per unit of weight, is at any rate a minor factor. The complications which septic infection of the animals would

TABLE II.

Weight (kilos.) and O_2 consumption (cc. per kilo. per hour).

Dog IV.

Normal				Diabetic			
Date	Weight	O_2		Date	Weight	O_2	
1913							
Feb. 27	8·000	541		Mar. 5	7·600	615	
Mar. 2	8·000	517		„ 6	7·300	591	
		529	Average			603	Average

Excess diabetic over normal O_2 consumption = 14 %.

Dog VI.

July 21	7·100	563		July 30	5·900	615	
„ 23	7·000	512					
		537	Average			615	Average

Excess diabetic over normal O_2 consumption = 14 %.

Dog VIII.

May 15	8·250	512	I. Normal	May 20	7·750	525	II. Partially depanc.,
„ 18	8·000	516		„ 21	7·550	518	slight glycosuria
				„ 23	7·400	532	
				„ 25	7·200	515	
				„ 27	7·000	438	
		514	Average			505	Average
June 2	6·750	490	III. No glycosuria	July 7	6·650	503	IV. On thyroid, slight
„ 5	6·750	431		„ 9	6·500	524	glycosuria
„ 9	6·750	458		„ 10	6·500	575	
„ 29	6·500	467		„ 13	6·350	535	
„ 30	6·500	466					
July 1	6·500	467					
„ 4	6·600	466					
		463	Average			534	Average
July 14	6·400	473	V. No glycosuria	July 18	6·250	493	VI. Totally depanc.
„ 15	6·450	450		„ 20	6·000	524	D : N = 3·1
„ 16	6·500	431		„ 22	5·800	548	
				„ 24	5·650	553	
				„ 25	5·600	560	Excess period VI over
				„ 28	5·350	552	period I = 8 %
				„ 30	5·200	590	
				„ 31	5·200	623	Period VI over V = 23 %
		451	Average			555	Average

TABLE II—*Continued*

Dog VII.

1913								
Dec.	5	10·050	543	May	20	8·650	535	Partially depanc. May 18th
„	24	9·600	451	„	21	8·500	532	
1914								
Jan.	14	10·000	432	„	23	8·100	531	
„	24	9·500	545	„	27	7·100	526	
„	26	9·500	520	„	28	7·100	495	
Mar.	25	8·700	451	„	29	7·050	524	
May	12	9·150	488	June	1	6·900	524	
„	13	9·150	479	„	3	6·750	501	
				„	8	6·500	525	
				„	12	6·200	576	
				„	13	6·000	569	
				„	24	5·200	528	
				„	26	5·000	495	
			488 Average				532	Average

Excess diabetic over normal O_2 consumption = 9 %.

Dog I.

1913								
Jan.	27	7·000	563	Jan.	31	6·400	628	
„	28	7·000	579	Feb.	1	6·350	670	
				„	2	6·100	688	
				,	3	5·800	637	
				,	4	5·600	664	
			571 Average				657	Average

Excess diabetic over normal O_2 consumption = 15 %.

Dog III.

Feb.	10	7·600	518	Feb.	17	7·200	575	
„	16	7·500	486	„	21	6·750	595	
				„	23	6·500	594	
			502 Average				588	Average

Excess diabetic over normal O_2 consumption = 16 %.

introduce in comparison of oxygen figures are absent, as the post-mortem examinations revealed no evidence of a generalised septicaemia.

3. *Percentage energy distribution.*

(*a*) *Protein.* From the data obtained in the measurement of the respiratory exchange and estimation of the respiratory quotient it becomes possible to calculate the percentage part of the total metabolism taken by protein, carbohydrate and fat. This calculation is made by subtracting from the total figures the amounts of carbon dioxide and oxygen corresponding to the protein metabolised and estimating the respiratory ratio of the remainder,

which indicates quantitatively the extent to which carbohydrate and fat are being used. An example may be given:

Dog VII. Dec. 3, 1913.

	CO_2	O_2	R.Q.
Fasting 23 hrs. Starch diet	4160	5418 ⟶	0·760
Nitrogen, g. per hour 0·110	537	664	
	3623	4754 ⟶	0·762

In this example we find that the protein portion amounts to 12 % of the total and the quotient of the remainder shows that the carbohydrate percentage is 17·0 and the fat 71·0 %. The figures for one hour are used. The nitrogen excretion is calculated in this example from the 24 hours analysis and the figures for CO_2 and O_2 corresponding to protein are taken from Schumburg and Zuntz's table quoted by von Noorden [1907]. It is evident that, in order to obtain a correct value for the protein percentage, the nitrogen excretion should be estimated during the respiratory observation. An estimate based upon the 24 hours nitrogen in the above example is not so liable to error as it would be if a protein rich diet were being fed. In the latter case such a nitrogen figure would give too high a percentage for protein during a fasting period and too low in observations after feeding. In the normal dogs 15 to 20 % was found to be the usual protein percentage of the fasting metabolism when meat was fed, and where direct determinations of

TABLE III. *Percentage energy distribution. Fasting observations. Normal.*

No. of animal	Date	Hours after feeding	Diet	N	Carbohydrate	Fat	% energy distribution: Protein	Carbohydrate	Fat	
	1914									
III	Feb. 10	18	P.b.	—	—	—	15·0	6·0	79·0	Protein p.c. assumed
	„ 16	17	Meat	4·0	—	—	22·0	0·0	78·0	
VI	July 23	23	Meat	8·0	—	—	15·0	12·0	73·0	„
	1913									
VII	Dec. 3	23	P.b.	4·5	120·0	—	12·0	17·0	71·0	
	„ 4	18	P.b.	1·6	60·0	—	12·0	8·0	80·0	
	„ 5	19	P.b.	3·3	135·0	—	13·0	13·0	74·0	
	„ 24	18	Mixed	11·0	70·0	—	15·0	14·0	70·0	„
	1914									
	Jan. 15	18	Mixed	4·7	60·0	—	15·0	30·0	55·0	„
	Mar. 25	24	C. and Pal.	6·6	—	40·0	15·0	15·0	70·0	„
	May 9	17	Meat	10·0	—	—	15·0	10·0	75·0	„
	„ 14	22	Meat	10·0	—	—	15·0	0·0	85·0	„
VIII	„ 18	24	Meat	8·0	—	—	15·0	3·0	82·0	„

nitrogen excretion have not been made, this figure (15 %) has been assumed. In the tables it is indicated where the protein percentage has been directly calculated and where it has been assumed.

Table III contains a summary of these calculations from the respiratory and nitrogen figures of normal fasting animals. In Table IV will be found the observations made after feeding protein and fat. The results obtained after administration of sugars will be found in the section dealing with carbohydrate.

TABLE IV. *Percentage energy distribution in digesting animals. Normal.*

No. of animal	Date	Hours after feeding	Diet	N	Carbohydrate	Fat	R. Q.	% energy dist. to: Protein	Carbohydrate	Fat
	1914									
III	Feb. 12	4 to 6	Meat	4·0	—	—	0·806	44·0	19·0	37·0
	„ 13	5 to 7	Meat	4·0	—	—	0·798	41·0	18·0	41·0
	1913									
VII	Dec. 13	2–3	Meat	8·0	—	—	0·818	79·0	10·0	11·0
	„ 15	2–3	Meat	10·0	—	—	0·802	90·0	1·3	8·7
	„ 15	6	Meat	10·0	—	—	0·786	77·0	3·0	20·0
	„ 22	3–5	Meat	4·0	—	—	0·820	68·0	17·0	15·0
	1914									
	Feb. 2	3	Butter	—	—	50·0	0·733	9·5	7·0	84·0
	„ 9	3	P.b.	3·0	60·0	—	0·900	17·0	75·0	8·0
	„ 13	3	P.b.	3·0	60·0	—	0·855	20·0	60·0	20·0
	„ 24	5–7	Meat	8·0	—	—	0·802	62·0	11·0	27·0
	Mar. 5	7	Meat Erept.	9·0	—	—	0·803	74·0	7·5	18·5
	„ 11	3	C. and Pal.	—	—	—	0·767	17·0	13·0	70·0
	„ 18	4	C. and Pal.	—	—	—	0·744	29·0	0·0	71·0
	„ 19	1	C. and C.L.O.	—	—	—	0·750	33·0	0·0	67·0
	„ 20	3	C. and C.L.O.	—	—	—	0·707	10·0	0·0	90·0
	„ 23	4	C. and Pal.	—	—	—	0·730	20·0	0·0	80·0
VIII	June 22	2	Bread and Meat	—	—	—	0·921	34·0	66·0	0·0
	July 2	3	P.b.	—	—	—	0·861	30·0	43·0	27·0

When the animals are diabetic calculations of the percentage energy distribution become more complicated, owing to the fact that part of the protein which normally makes up the protein quota of the total metabolism is lost to the organism as excreted sugar. Beside this sugar formation from protein which we attempt to allow for in the calculations of percentage energy distribution, other processes may occur, such for example as the formation of sugar from fat or of the acetone bodies, which may still further complicate such energy estimations. The calculations, therefore, even after allowing for sugar formation from protein, are more a rough indication than a quantitative index, because of complexities for which one cannot allow. We have, however, thought that some indication would be afforded of the

character and extent of the carbohydrate and fat portions. In order, therefore, to get some idea of the energy distribution, a side calculation has been made allowing for sugar formation from protein based upon the observed D : N ratio. If one allows for the sugar formation from protein, as in the following example, the respiratory quotient of the remaining protein carbon dioxide and oxygen can be easily calculated. As the dextrose-nitrogen ratio increases from 2·6 to 4·0 the respiratory quotient decreases from 0·725 to 0·620. It can be readily seen, therefore, how much a perversion of protein metabolism with, say, a D : N ratio of 3·8 would contribute to the lowering of the respiratory quotient of diabetics. Such an example is:

Dog IV. Mar. 3, 1914.

Fasting 48 hours. Total N 5·10. D : N 2·9	CO_2	O_2	
Nitrogen per hour 0·210	1026	1268	Protein to sugar correction
Glucose „ 0·60	444	444	
	582	824	

	CO_2	O_2	R.Q.
Total figures observed	3643	5146	→ 0·700
Protein minus glucose	582	824	
	3061	4322	→ 0·707

In this example the protein percentage is 16 % and the remaining 84 % has a quotient indicating fat combustion. A number of such calculations have been made and are given in the tables. The percentage part in the total metabolism played by protein is increased in diabetes according to our observations. The protein percentage ranges from 16 to 32 % on the first and second days after depancreatisation, with an average of 25 %.

Table VII (p. 200) contains a summary of the figures for percentage energy distribution in the diabetic dogs in the fasting and digesting state. This table contains a number of observations which may be compared with those in Tables III and IV. In the digestion of a protein meal the level of the respiratory quotient is much lower in the diabetic animals than in the normal, and the allowance for sugar formation from protein in most cases indicates that the energy is distributed to protein and fat and that the oxidation of the fat portion is complete.

In Table V are given the results of calculations of the percentage energy distribution in diabetic dogs on the early days after operation. The protein portion is reckoned on the total 24 hours nitrogen. It will be noted that this protein percentage varies greatly, but the usual figure is distinctly higher

than the normal percentage when no protein has been given for periods of 48 to 60 hours. The excessively high figure in the case of Dog VII after partial depancreatisation is surprising. In this dog there was an extremely slight glycosuria and in the calculation no allowance is made for sugar form-

TABLE V.

No. of dog	Depanc.	Days after operation	Prot.	Carb.	Fat
I	Total	1st	32·0	4·0	64·0
		2nd	30·0	0·0	70·0*
III	Total	1st	24·0	0·0	76·0*
IV	Total	1st	16·0	0·0	84·0
V	Total	2nd	18·0	0·0	82·0*
VI	Total	2nd	21·0	10·0	69·0
VII	Partial	2nd	30·0	10·0	60·0
		3rd	24·0	7·0	69·0
VIII	Partial	2nd	62·0	0·0	38·0*
	Total	1st	52·0	2·0	46·0
	Total	2nd	19·0	2·0	79·0

ation from protein, since practically no sugar was excreted, but the results indicated the probability of such formation and also that the sugar, if formed, was unutilised since the quotient of the remainder after deducting the protein portion is far below that of the combustion of fat. The full figures of this observation will be given:

Dog VII. May 20. 72 *hours since food was given.*

	CO_2 1 hour	O_2 1 hour	R.Q.
Total N 9·5 g. Total sugar excretion 2·25	2889	3894	→ 0·742
Protein 1 hour N 0·40	1954	2415	
Carbohydrate and fat ..	935	1479	→ 0·632
Protein 62 %.	Fat 38 % (incomplete?).		

With this dog it will be noted that two months later, on the first day after the total depancreatisation, the protein figure is again very high, but when one allows for the sugar formation from protein on the basis of the observed D : N ratio, one sees that the combustion of the fat portion is complete.

Dog VII. July 18. 44 *hours since any food was given.*

	CO_2	O_2	CO_2	O_2	R.Q.
Total N 9·8 g. N 0·408	1994	2463	2198	3084	→ 0·712
D : N 2·8, Gl. 1·14	847	847	1147	1616	
	1147	1616	1051	1468	→ 0·715
Protein 52·0.	Carbohydrate 2·0.		Fat 46·0.		

It therefore appears very probable that in the first instance sugar was being formed from protein, that this sugar was not being utilised to any great extent and also that it was not being excreted. We obtained no striking evidence of incomplete combustion of fat in an increase of acetone bodies excreted, which indicates that the conclusions are justified.

(*b*) *Fat.* The percentage energy distribution to fat in the normal animals in a fasting condition can be seen from the results in Table VIII to be uniformly high. Feeding the animals upon fat alone was not carried out with success, but with large amounts of fat fed with protein the fat takes a predominant part in the total metabolism (Table IV, p. 186) with a resultant lowering of the respiratory quotient. We obtained no evidence of incomplete combustion of fat in the normal animals. Great interest centres around the question of the combustion of fat in diabetes. In Table V the figures for fat marked (*) are cases in which the combustion of fat was apparently incomplete as the remainder quotient after deducting for protein was lower than the normal fat quotient. We find no parallelism between this apparent incomplete combustion of fat and the excretion of acetone bodies. The latter increase steadily throughout the course of the diabetes and frequently show fluctuations which cannot be correlated with any marked change in the respiratory quotient. The course of excretion of acetone bodies seems to bear a closer relation, though not very close, to the protein breakdown. Also in the cases in which fat was fed to the animals the excretion of fat in the faeces never indicated a deficient absorption of the fat fed. The fats, therefore, as far as these observations upon dogs indicate, are apparently well dealt with by the diabetic organism. In some cases evidence of incomplete combustion of fat appears. In the greater number of cases the low respiratory quotient of diabetic dogs appears to be caused, not so much by a perversion of the fat metabolism as by the disturbance of the normal combustion of protein.

(*c*) *Carbohydrate.* The extent to which carbohydrate is oxidised in diabetic as compared with normal dogs is, of course, the question which is the most important to us at present. It is necessary, therefore, to find out what percentage part of the total metabolism is taken by carbohydrate in the normal animal under various conditions.

In the normal animal it is obvious that the amount of sugar used in the oxidations will depend firstly upon the supply available. Thus in the fasting animal the percentage distribution to carbohydrate may vary from 0 to 30 % depending upon the diet, whether rich or poor in carbohydrate, and the carbohydrate storage in the tissues. Table III (p. 185) contains the figures

calculated as described above from a series of normal fasting observations.

Dog III.

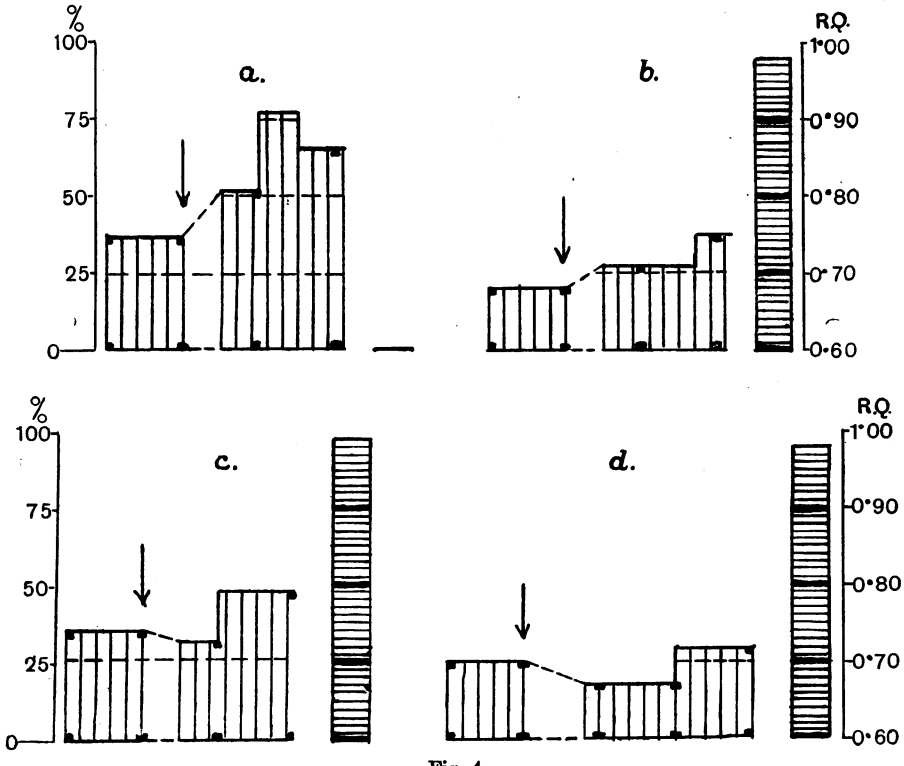


Fig. 4.

Dog IV.

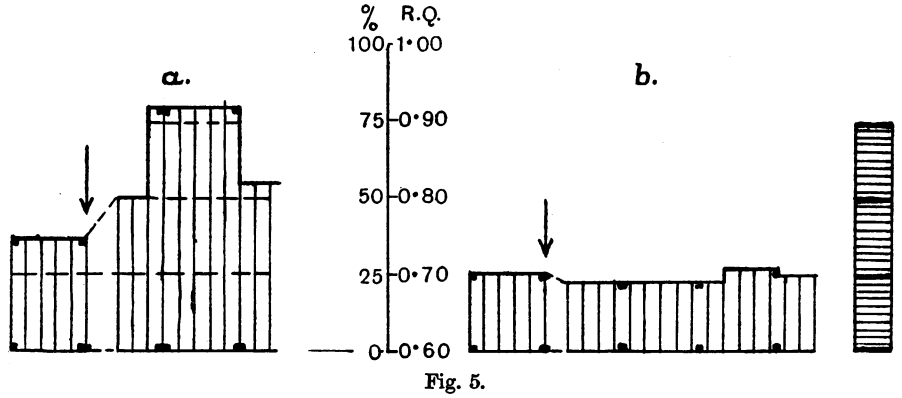


Fig. 5.

When glucose or fructose is given to normal animals the utilisation is shown by a marked rise of the respiratory quotient. Within an hour after

Dog VII.

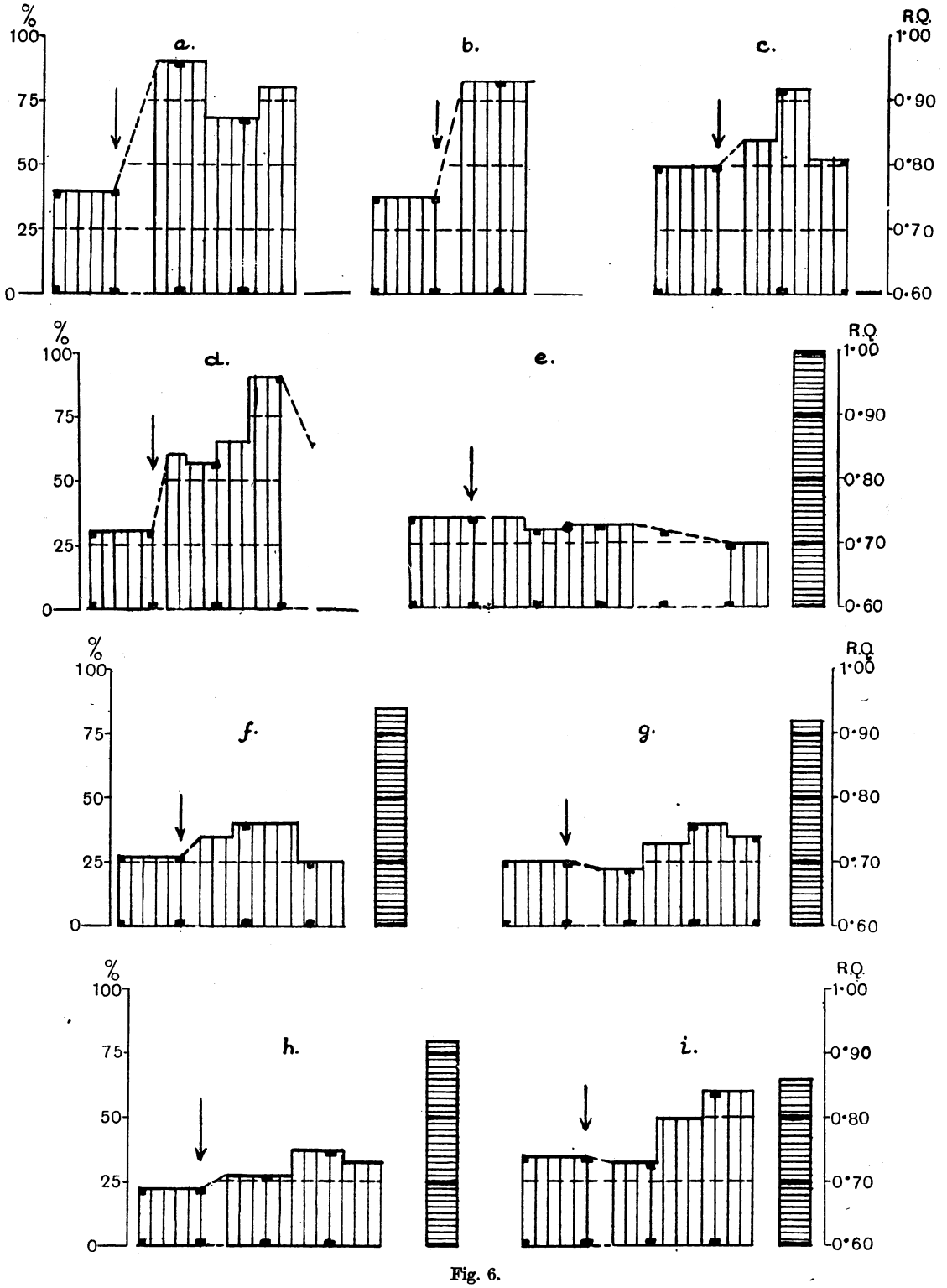
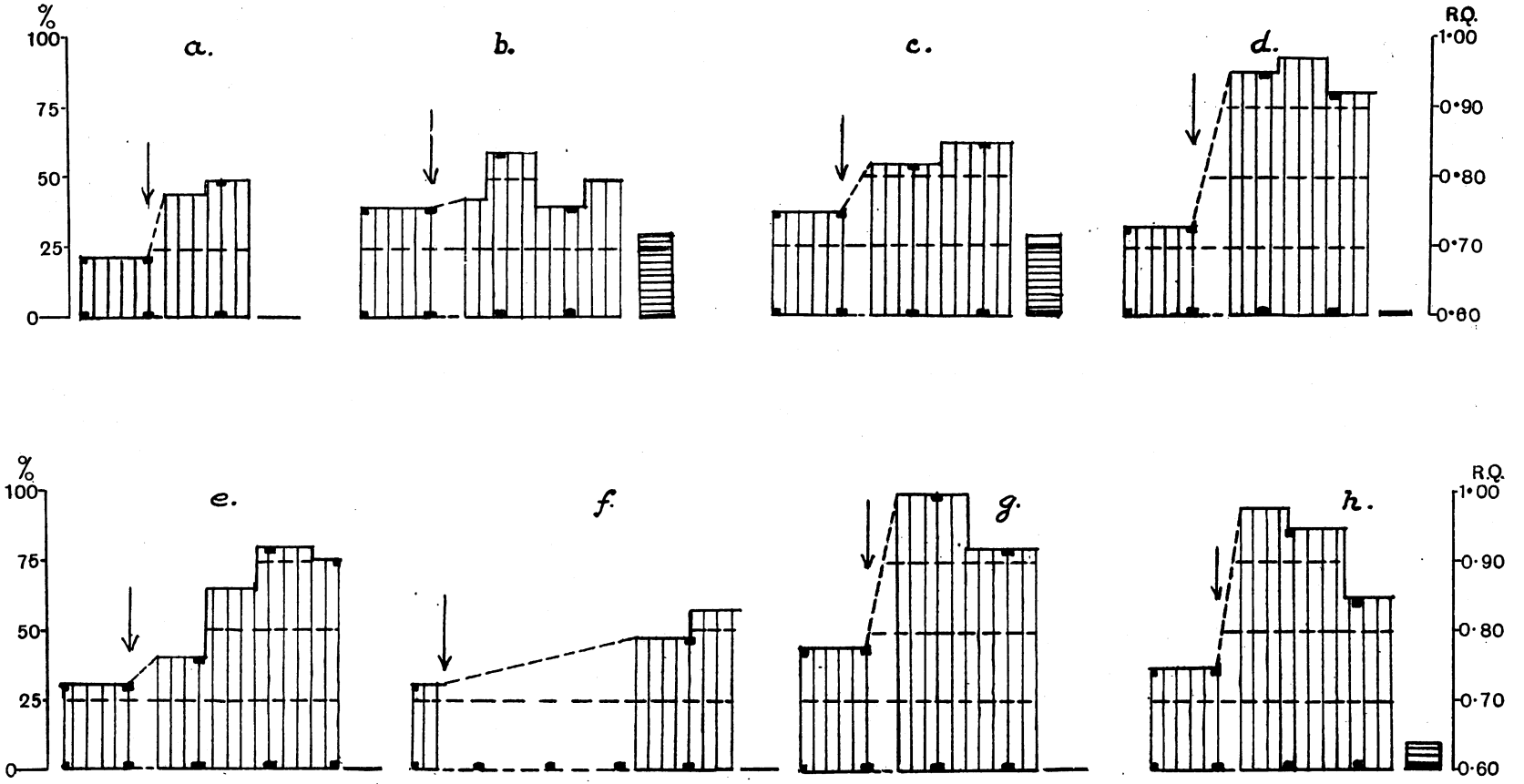


Fig. 6.

Dog VIII.


%
100
75
50
25
0
a.
b.
c.
d.
R.Q.
1·00
0·90
0·80
0·70
0·60
%
100
75
50
25
0
e.
f.
g.
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R.Q.
1·00
0·90
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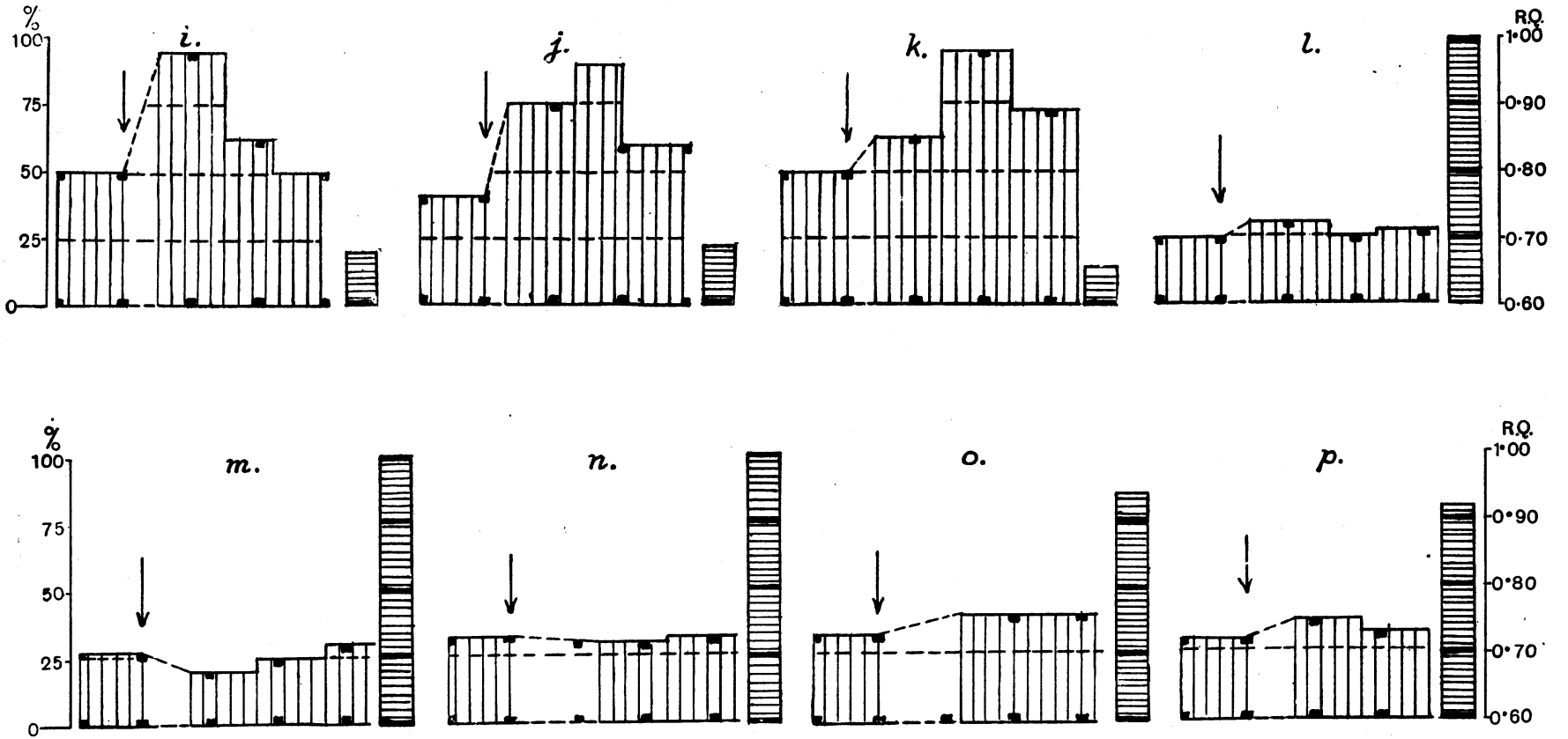
0·70
0·60


%
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o.
p.
RQ.
1·00
0·90
0·80
0·70
0·60

Fig. 7.

the sugar has been taken the quotient has risen to 0·90 to 1·00 and for three or more hours remains between 0·80 and 0·90, gradually returning to its original level. If calculations are made of the percentage energy distribution, the carbohydrate portion increases up to 80 to 90 % (see Table VI). The following is a protocol of an observation before and after the giving of glucose:

Dog IV. Feb. 27, 1914.

Period No.	Duration mins.	CO_2 cc. per min.	O_2 cc. per min.	R.Q.	Protein	Carbo-hydrate	Fat
I	50	54·1	72·2	0·749	15·0[1]	10·0	75·0
		30 g. glucose given 20 mins. before II period.					
II	20	85·2	106·0	0·803	15·0	27·0	68·0
III	40	84·6	92·2	0·917	15·0	67·0	18·0
IV	30	77·7	84·5	0·919	15·0	70·0	15·0
V	30	71·5	87·6	0·816	15·0	35·0	50·0

[1] Assuming protein to be 15 %.

With the doses of glucose given (20 to 40 g.) no sugar appeared in the urine of normal dogs. In the normal rise of the quotient after giving sugar the minute figures for carbon dioxide and oxygen both increase, but the amount of the carbon dioxide increases far more than the oxygen intake. The rise in the respiratory quotient is due to an increase in the output of CO_2.

In Figs. 4, 5, 6 and 7 (pp. 190–193) a series of observations before and after the giving of sugar are plotted as curves. The curves hatched vertically represent the levels of the respiratory quotient during the successive periods. Five of the vertical lines represent a duration of one hour. The arrow marks the time at which the sugar was given. The second part of the curve is hatched horizontally and shows the percentage of the sugar which was recovered from the urine, over and above the amount of sugar corresponding to the nitrogen excreted on the basis of the D : N ratio of the day before and after. The protocols corresponding to Figs. 4, 5, 6, 7 will be found in Table VI. In the normal animal it is seen from these results that the rise of the respiratory quotient after sugar is a well-marked one. If we compare these results with the effect of giving sugar to the diabetic animals, two important points are brought out:

(i) The rise of the respiratory quotient in the diabetic animals is diminished in all cases, and frequently fails to appear.

(ii) The level of the quotient after giving sugar corresponds very closely to the percentage excretion of the sugar given. If all the sugar is excreted the quotient shows little or no rise, and if only a slight excretion occurs, as in the case of Dog VIII, the increase in the respiratory quotient approaches the normal.

TABLE VI. *Respiratory quotient, minute figures for CO_2 and O_2 and percentage energy distribution before and after sugar administration.*

No. of animal	Date	Period No.	Duration min.	Sugar	CO_2 cc. p. m.	O_2 cc. p. m.	R.Q.	% energy distribution: Protein	Carbohydrate	Fat	Notes
	1914										
III	Feb. 11	I	60		66·75	89·5	0·745	15·0	9·0	76·0	Normal.
	a.			Gluc. 30 g.							
		II	30		76·0	94·0	0·806	—	—	—	
		III	30		67·26	75·9	0·908	15·0	65·0	20·0	
		IV	35		68·5	79·43	0·862	—	—	—	Depancreatised Feb. 16.
	Feb. 18	I	60		57·86	85·5	0·680	24·5	0·0	**75·5**	D : N = 3·2.
	b.			Gluc. 30							
		II	70		55·5	78·3	0·713	26·8	3·2	70·0	
		III	20		59·8	79·2	0·754	—	—	—	Sugar excreted = 95 %.
	Feb. 19	I	40		54·2	73·0	0·740	25·0	11·0	64·0	D : N = 3·4.
	c.			Gluc. 20							
		II	20		59·5	82·1	0·724	26·0	20·0	54·0	
		III	30		52·6	66·6	0·789				
		IV	30		77·5	98·1	0·790	—	—	—	Sugar excreted = 95 %. Dog very restless.
	Feb. 21	I	60		48·05	67·9	0·699	21·0	4·0	75·0	D : N = 3·4.
	d.			Gluc. 20							
		II	40		49·3	74·4	0·672	20·0	0·0	**80·0**	
		III	30		48·5	72·3	0·671				
		IV	60		58·15	81·3	0·723	18·0	6·5	75·5	Sugar excreted = 95 %.
IV	Feb. 27	I	50		66·85	89·1	0·750	—	—	—	
	a.			Gluc. 30							
		II	20		85·2	106·0	0·803	—	—	—	
		III	40		84·6	92·2	0·917	—	—	—	
		IV	30		77·7	84·5	0·920	—	—	—	
		V	30		71·5	87·6	0·820	—	—	—	Depancreatised Mar. 2.
	Mar. 6	I	60		51·05	72·4	0·705	15·0	2·0	83·0	D : N = 3·8.
	b.			Gluc. 30							
		II	43		58·4	84·0	0·696	—	—	—	
		III	41		57·3	83·7	0·685	—	—	—	
		IV	40		56·0	81·3	0·688	13·0	0·0	87·0	
		V	40		55·0	76·7	0·716	—	—	—	
		VI	32		55·97	79·03	0·708	15·0	2·0	83·0	Sugar recovered 75 %.
	1913										
VII	Dec. 3	I	45		69·34	90·3	0·760	12·0	17·0	71·0	Normal.
	a.			Gluc. 30							
		II	45		83·55	86·95	0·96	12·7	82·0	5·3	
		III	45		67·98	77·98	0·87	—	—	—	
		IV	45		73·24	79·95	0·92	—	—	—	
	Dec. 5	I	45		68·50	91·0	0·750	13·0	13·0	74·0	
	b.			Fruct. 30							
		II	60		95·55	102·98	0·930	11·0	73·0	16·0	

TABLE VI—*Continued*

No. of animal	Date	Period No.	Duration min.	Sugar	CO_2 cc. p. m.	O_2 cc. p. m.	R.Q.	% energy distribution: Protein	Carbohydrate	Fat	Notes
	1914										
VII	Jan. 15	I	30		64·3	79·77	0·807	15·0	30·0	55·0	
	c.			Gluc. 10							
		II	30		64·8	77·3	0·840	—	—	—	
		III	30		73·8	80·4	0·920	15·0	70·0	15·0	
		IV	30		68·0	84·5	0·805	—	—	—	
	May 14	I	40		52·8	73·0	0·720	15·0	0·0	85·0	
	d.			Gluc. 20							
		II	20		74·8	89·35	0·837	—	—	—	
		III	30		68·7	83·5	0·822	—	—	—	
		IV	30		65·5	75·86	0·860	—	—	—	
		V	30		70·94	73·4	0·966	15·0	85·0	0·0	Partially depancreatised May 18.
	May 28	I	60		46·1	61·9	0·743	32·0	13·0	55·0	D : N = 2·9.
	e.			Gluc. 20							
		II	33		49·0	65·8	0·744	—	—	—	
		III	40		51·77	71·7	0·720	—	—	—	
		IV	60		55·5	75·5	0·730	28·0	10·0	62·0	Sugar excreted 100 %.
		V	32		46·0	66·4	0·691	30·0	0·0	70·0	4 hrs. after the sugar.
	June 3	I	60		40·55	56·4	0·718	14·0	0·0	86·0	D : N = 3·1.
	f.			Gluc. 20							
		II	30		39·0	53·0	0·736	—	—	—	
		III	60		47·9	63·17	0·758	17·0	12·0	71·0	
		IV	40		44·52	63·55	0·700	15·0	0·0	85·0	85 % of sugar excreted.
	June 8	I	60		39·9	56·94	0·700	14·0	4·0	82·0	D : N = 3·7.
	g.			Fruct. 20							
		II	40		52·3	76·2	0·686	20·0	5·0	75·0	
		III	43		46·16	62·8	0·734				
		IV	37		44·13	58·1	0·759	23·0	20·0	57·0	
		V	30		47·3	64·1	0·738				Sugar excreted 80 %.
	June 13	I	50		38·6	56·90	0·678	20·0	0·0	80·0	D : N = 3·4.
	h.			Gluc. 20							
		II	60		37·33	52·16	0·715	2·0	5·0	93·0	
		III	45		43·06	57·4	0·749	2·0	15·0	83·0	
		IV	35		42·0	57·0	0·736	—	—	—	Sugar excreted 80 %.
	June 24	I	60		34·35	46·32	0·741	20·0	17·0	63·0	D : N = 3·8.
	i.			Gluc. 20							
		II	40		53·8	73·9	0·727	—	—	—	
		III	40		46·17	58·0	0·796	—	—	—	
		IV	40		39·05	46·6	0·837	23·0	40·0	37·0	Sugar excreted 65 %.
	June 18	I	80		54·81	77·4	0·707	40·0	7·0	53·0	D : N = 3·5.
				Gluc. 20							Intravenous injection.
		II	35		54·0	77·4	0·698	—	—	—	
		III	35		53·8	76·1	0·706	—	—	—	
		IV	30		55·9	79·0	0·708	39·0	5·0	56·0	

TABLE VI—*Continued*

No. of animal	Date 1914	Period No.	Duration min.	Sugar	CO_2 cc. p. m.	O_2 cc. p. m.	R.Q.	% energy distribution: Protein	Carbohydrate	Fat	Notes
VIII	May 16	I	30		53·26	77·1	0·690	10·0	0·0	90·0	Normal.
	a.			Gluc. 10							
		II	35		62·1	79·3	0·782	—	—	—	
		III	35		55·7	70·0	0·796	10·0	30·0	60·0	
	May 25	I	60		46·9	61·8	0·758	15·0	14·0	71·0	Partial depancreatisation on May 18.
	b.			Gluc. 20							Slight glycosuria.
		II	20		52·4	67·8	0·773	—	—	—	
		III	40		48·25	57·25	0·842				
		IV	40		50·9	66·5	0·765	15·0	28·0	57·0	Total sugar exc. = 11·0 g.
		V	30		54·66	68·4	0·798				
	May 29	I	60		43·75	58·4	0·748	15·0	10·0	75·0	
	c.			Gluc. 20							
		II	60		48·66	59·36	0·820	—	—	—	
		III	60		52·08	61·5	0·846	15·0	43·0	42·0	Total sugar exc. = 5·9 g.
	June 5	I	35		35·6	48·57	0·733	15·0	6·0	79·0	No glycosuria.
	d.			Fruct. 20							
		II	42		54·05	56·9	0·949	—	—	—	
		III	40		50·4	51·7	0·974	15·0	85·0	0·0	
		IV	40		51·9	56·3	0·921	—	—	—	No sugar excreted.
	June 9	I	40		37·3	51·6	0·723	15·0	0·0	85·0	
	e.			Gluc. 20							
		II	40		42·75	56·0	0·763	—	—	—	
		III	40		50·4	58·2	0·864	—	—	—	
		IV	45		49·66	53·8	0·922	15·0	70·0	15·0	
		V	20		49·6	54·8	0·904	—	—	—	No sugar excreted.
	July 1	I	60		39·3	50·66	0·777	15·0	20·0	65·0	
	g.			Gluc. 20							
		II	60		54·9	52·85	1·03	15·0	85·0	0·0	No sugar excreted.
		III	60		49·95	54·3	0·920				
	July 4	I	45		38·44	51·33	0·749	15·0	9·0	76·0	Thyroid administered.
	h.			Gluc. 20							
		II	40		52·4	53·25	0·984	10·0	90·0	0·0	
		III	50		62·6	66·2	0·946	—	—	—	
		IV	40		46·3	54·37	0·851	—	—	—	Total sugar exc. = 2·1 g.
	July 9	I	60		47·57	58·95	0·806	15·0	26·0	59·0	
	i.			Gluc. 20							
		II	60		65·66	67·08	0·978	15·0	85·0	0·0	
		III	40		52·8	62·17	0·849	—	—	—	
		IV	40		49·75	62·17	0·800	15·0	25·0	60·0	Total sugar exc. = 4·3 g.
	July 14	I	60		38·55	50·47	0·764	15·0	16·0	69·0	
	j.			Gluc. 20							
		II	60		44·66	49·65	0·900	—	—	—	
		III	40		49·0	51·1	0·958	15·0	80·0	5·0	
		IV	50		49·36	58·68	0·841	—	—	—	Total sugar exc. = 4·8 g.

TABLE VI—*Continued*

No. of animal	Date	Period No.	Duration min.	Sugar	CO_2 cc. p. m.	O_2 cc. p. m.	R.Q.	% energy distribution: Protein	Carbohydrate	Fat	Notes
	1914										
VIII	July 16	I	60		37·65	46·7	0·806	15·0	29·0	56·0	Thyroid stopped.
	k.			Gluc. 20							
		II	60		39·6	46·5	0·852	—	—	—	
		III	60		44·77	45·73	0·979	10·0	90·0	0·0	
		IV	60		41·9	47·2	0·887	—	—	—	Total sugar exc. 3·3 g. Totally depancreatised, July 17.
	July 20	I	60		37·39	53·4	0·700	36·0	0·0	64·0	D : N = 3·0.
	l.			Gluc. 20							
		II	71		39·3	54·8	0·717				
		III	40		40·35	57·45	0·702	35·0	1·0	64·0	Sugar excreted 100 %.
		IV	50		38·36	53·92	0·711				
	July 22	I	60		37·63	53·01	0·709	20·0	4·0	76·0	D : N = 3·1.
	m.			Gluc. 20							
		II	60		38·25	55·93	0·684	—	—	—	
		III	60		40·2	57·03	0·705	20·0	0·0	80·0	
		IV	40		38·8	53·4	0·720	—	—	—	Sugar excreted 100 %.
	July 24	I	60		38·06	52·13	0·730	20·0	10·0	70·0	D : N = 3·1.
	n.			Gluc. 20							
		II	62		38·0	52·64	0·721	—	—	—	
		III	60		39·16	53·66	0·730	20·0	7·0	73·0	Sugar excreted 100 %.
	July 28	I	60		36·13	49·26	0·733	20·0	12·0	68·0	D : N = 3·1.
	o.			Fruct. 20							
		II	60		38·76	50·8	0·763				
		III	60		39·1	51·53	0·758	20·0	20·0	60·0	Sugar excreted 85 %.
	July 31	I	50		38·88	54·0	0·720	15·0	7·0	78·0	D : N = 3·1.
	p.			Gluc. 20							
		II	60		44·02	58·73	0·749				
		III	60		42·33	57·95	0·730	14·0	13·0	73·0	Sugar recovered 80 %.

When the respiratory quotient shows no rise, as for instance with Dog IV *b*, Fig. 5, the minute figures for carbon dioxide and oxygen will be found to show a parallel increase; see protocol IV *b* in Table VI.

In some of the totally depancreatised dogs in the later stages, it was observed several times that the respiratory quotient might rise noticeably, simulating the elevation after giving sugar. The cause of this rise was found to be a diminution in the oxygen intake with a very constant carbon dioxide output. The following is an instance:

Dog I.

Feb. 4th, 1913.	CO_2	O_2	R.Q.
Period I. 40 mins.	45·67	62·72	0·728
Given 15 g. glucose			
Period II. 30 mins.	45·60	60·2	0·750
Period III. 42 mins.	46·70	54·0	0·863
Feb. 5th. No sugar given			
Period I. 30 mins.	39·6	63·6	0·624
Period II. 45 mins.	39·4	46·7	0·844
Period III. 40 mins.	39·7	53·5	0·742

This animal was in a very bad condition when these observations were made. This condition may lead to confusing results and the quotient is of no value unless the minute figures are analysed. The variations in the taking up of oxygen may be due to a periodic type of breathing, but we regard the duration of the observation as long enough to equalise any such effect, and think that the blood changes must be responsible for the continual decrease in the taking up of oxygen observed. The respiration rate has been observed to vary from 15 to 43 per minute without any irregularity in the oxygen intake—no measurement of the depth of respiration was made. Several instances such as the above have been observed when the animals showed great circulatory and muscular feebleness, and were so quiet in the apparatus that the possibility of errors in the oxygen measurement is excluded.

As is seen in Figs. 5, 6 and 7 the rise of the respiratory quotient caused by sugar is absent in observations shortly after depancreatisation and later may occur in a very diminished form. In the totally depancreatised Dog III some evidence of sugar utilisation is present shortly after the operation, but the last observation (Fig. 4) indicates practically no utilisation of sugar. Dog IV when diabetic showed no indication that sugar was being used (Fig. 5 *b*). The series of observations shown in Fig. 7 on Dog VIII gives a very good comparison of the partially and totally depancreatised state. In this figure *b* and *c* are made shortly after the partial depancreatisation and show less marked increase of the R. Q. than the succeeding observations when practically none of the sugar was recoverable from the urine. The dog apparently recovered its normal power of utilising glucose. The observations *l*, *m*, *n*, *o* and *p*, after the remnant of pancreas was removed, show very little evidence of sugar utilisation. Dog VII shortly after partial depancreatisation gave no indication of sugar utilisation, as is seen in Fig. 6 *e*. Later the graft apparently established its disturbed circulatory conditions and a return of the power of oxidising sugar is evident from the rise of the respiratory quotient (Fig. 6 *h*, *i*).

The percentage energy distribution to carbohydrate has been seen to vary in the normal fasting condition and after the giving of sugar to constitute a large part, 80 to 90 %, of the total metabolism. In such calculations in the fasting state of diabetic animals the figures, after allowance for sugar formation from protein, indicate that a small part is taken by carbohydrate in some cases. The results will be found in Table VII. Whether this is an apparent or real utilisation of carbohydrate can be seen to depend upon the accuracy with which the sugar formation from protein can be estimated from the D : N ratio observed. Thus, if too great an amount of sugar is taken to correspond with

TABLE VII.

Respiratory quotients and percentage energy distribution in diabetic animals.

No. of animal	Date	Hours after	Diet	N	Carbo-hydrate	Fat	R.Q.	% energy dist. Protein	Carbo-hydrate	Fat	D/N	Notes
I	Jan. 31	40	Meat	12·0	—	—	0·717	32·2(1)	4·0	64·0	3·1	
	Feb. 1	64	Meat	12·0	—	—	0·691	30·0	0·0	**70·0**(2)	3·0	
	,, 2	24	Meat	8·0	—	—	0·688	29·0	0·0	**71·0**	2·7	
	,, 3	20	Meat	4·0*	—	—	0·708	24·0	3·0	73·0	3·2	* Intake less—dog vomiting.
	,, 4	18	Meat	4·0*	—	—	0·720	9·0	9·0	82·0	3·2	
III	Feb. 17	41	Meat	4·0	—	—	0·692	24·0	0·0	**76·0**	2·7	
	,, 18	3	M. and P.	4·0	—	—	0·680	24·5	0·0	**75·5**	3·2	
	,, 19	3	M. and P.	4·0	—	—	0·740	25·0	11·0	64·0	3·4	
	,, 20	7	M. and P.	4·0	—	—	0·680	41·0	0·0	59·0	3·8	
	,, 21	18	M. and P.	4·0	—	—	0·690	21·0	4·0	75·0	3·4	
	,, 23	19	M. and P	4·0	—	—	0·705	22·0	3·0	75·0	3·4	
IV	Mar. 3	48	Meat	4·0	—	—	0·700	16·0	0·0	84·0	2·9	
	,, 4	5	M. and P.	5·5	—	—	0·691	21·0	0·0	79·0	3·5	
	,, 6	20	M. and P.	4·0	—	—	0·705	15·0	2·0	83·0	3·8	
V	Mar. 4	67	M. and P.b.	8·0	50·0	—	0·677	18·0	0·0	**82·0**	3·0	
	,, 5	4	M. and P.	4·0	—	—	0·700	36·0	0·0	64·0	3·0	
	,, 7	36	M. and P.	4·0†	—	—	0·670	10·0	0·0	**90·0**	4·0	† Dog vomiting.
VI	July 29	60	Meat	8·0	40·0†	—	0·731	21·0	10·0	69·0	3·1	Fructose day before.
VII	May 20	72	Meat	8·0	—	—	0·733	30·0	10·0	60·0	2·6	Milk 100 cc. 19 hrs. before.
	,, 21	24	Milk	1·0	10·0	6·0	0·725	24·0	7·0	69·0	2·6	
	,, 23	18	M. and P.	8·5	—	—	0·676	34·0	0·0	**66·0**	3·2	
	,, 27	17	M. and P.	8·5	20·0	—	0·715	42·0	3·0	55·0	2·8	Glucose day before.
	,, 28	18	M. and P.	8·5	—	—	0·743	32·0	13·0	55·0	2·9	
	,, 29	18	M. and P.	7·5	20·0	—	0·693	36·0	0·0	**64·0**	2·9	Glucose day before.

	June 1	24	M. and P.	7·5	—	—	0·706	30·0	0·0	70·0	2·8	
	„ 2	3	M. and P.	7·5	—	—	0·693	43·0	0·0	**57·0**	2·8	
	„ 3	22	M. and P.	7·5	—	—	0·710	14·0 N.O.	0·0	86·0	3·1	
	„ 8	24	M. and P.	9·5	—	—	0·700	14·0 N.O.	4·0	82·0	3·7	
	„ 13	18	M. and P.	10·0	—	—	0·693	20·0 N.O.	0·0	80·0	3·4	
	„ 18	3	Cas. and P.	6·0	—	—	0·707	40·0 N.O.	7·0	53·0	3·5	
	„ 24	18	Gel. and P.	6·0	—	—	0·741	20·0 N.O.	17·0	63·0	3·8	
	„ 26	24	Cas. and P.	6·0	—	—	0·696	19·0	0·0	81·0	3·7	
VIII	May 20	72	Meat. Milk 100 cc. day before	8·0	—	—	0·742	62·0(1)	0·0	**38·0**	—	Total sugar 2·25.
	„ 25	24	M. and P.	5·5	—	—	0·758	36·0	6·0	58·0	—	Total sugar 11·0.
	„ 29	24	M. and P.	5·5	—	—	0·748	27·0	9·5	63·5	—	Total sugar 5·9.
	June 22	2	Bread and Meat	6·5	100·0	—	0·921	34·0	61·0	5·0	—	No glycosuria.
	„ 29	18	P.b.	5·0	60·0	—	0·780	34·0	14·0	52·0	—	„ „
	„ 30	18	Meat and P.b.	7·0	60·0	—	0·770	31·0	12·0	57·0	—	„ „
	July 2	3	P.b. and Meat	7·0	80·0	—	0·861	30·0	43·0	27·0	—	
	„ 7	24	P.b. and Meat. Thyroid	7·0	60·0	—	0·785	28·0	18·0	54·0	—	
	„ 8	19	„ „ „	7·0	60·0	—	0·809	29·0	26·0	45·0	—	
	„ 9	18	„ „ „	7·0	60·0	—	0·806	35·0	22·0	43·0	—	
	„ 18	44	M. and P.	11·0	20·0	—	0·712	19·0	2·0	79·0	2·8	
	„ 25	18	„ „	11·8	20·0	—	0·710	20·0*	2·0	78·0	3·1	
	„ 27	4	„ „	5·0	—	—	0·780	33·0	28·0	39·0	3·1	
	„ 29	3	„ „	5·9	20·0	—	0·740	44·0	17·0	39·0	3·1	
	„ 30	19	„ „	5·9	20·0	—	0·745	16·0(1)	15·0	69·0	3·1	
	„ 31	18	„ „	11·8	—	—	0·720	15·0(1)	7·0	78·0	3·1	

(1) Protein metabolism has been calculated from 24 hours nitrogen excretion, except where marked * when the percentage has been assumed, and N.O. where the nitrogen excretion has been determined during the respiratory observation.

(2) Figures in black type denote that the calculation indicated an incomplete combustion of fat or other disturbance associated with abnormally low quotient.

the nitrogen excretion, deduction of the carbon dioxide and oxygen figures corresponding to protein minus sugar will indicate an apparent combustion of carbohydrate. In the majority of cases this amount of carbohydrate apparently utilised is quite small and frequently after sugar is given does not show any increase whatever, which is difficult of explanation if sugar is really being utilised.

4. *Results of Urine Analyses.*

Table VIII contains the results of the urine analyses as well as the total intake of food, balance sheets for nitrogen and phosphate, and general notes as to the condition of the animals.

(*a*) P_2O_5. It was thought that the increase of excreted phosphate indicated the tissue break-down in the diabetic animals. When a balance is estimated over a period, however, there is very little discrepancy between the intake and output. Dog VII showed a loss of 2·5 g. and Dog VIII a gain of 0·5 g. during the period.

(*b*) *Acetone bodies.* In Table VIII the figures for acetone represent the total acetone preformed and present as acetoacetic acid in the urine. The excretion of acetone bodies starts on an upward course soon after depancreatisation and shows many fluctuations from day to day with the general increase as the condition becomes more severe. We can find nothing definite to account for the considerable rise in the amount of acetone bodies in Dog VII on June 9 (Table VIII). 10 g. of sodium bicarbonate were administered on that day, but since on other occasions this does not cause a marked rise in acetone excretion, it may be due to something else. The question as to the chief source of the acetone bodies will be dealt with later in the report.

(*c*) *Sugar.* The sugar excreted in the diabetic urine was almost always dextro-rotatory and apparently glucose. When glucose was given to the diabetic dogs it was excreted in large amount. If the animals were catheterised after three to four hour periods, the rate of excretion of the sugar given could be ascertained. If we compare the sugar and nitrogen excretion after giving sugar (see Table IX) we see that the sugar is excreted in this case in large proportion during the first three hours after administration. On June 8 (Table IX) 20 g. fructose was administered and a small proportion of the sugar excreted was laevo-rotatory. On June 18 the animal received a protein meal before the first four hour period and the results illustrate the fact that the sugar is excreted before the nitrogen of the protein given.

(*d*) *Nitrogen excretion.* The total nitrogen balance shows a marked loss of nitrogen from the body in the early stages of diabetes. Later this loss becomes smaller and with a high protein intake equilibrium may be reached. Since the nitrogen loss is so marked in the early stages and the proportion of

TABLE IX. *Dog VII. Excretion of sugar and nitrogen after giving glucose and fructose.*

Date	No. of Period	Dur'n hours	Total glucose	Total nitrogen	D/N	N g. per hour	Notes
1914							
June 3	I	4	0·325	0·470	0·70	0·117	
	II	2·5	15·0	0·390	38·4	0·156	Given glucose 20 g. at beginning of period II.
	III	17·5	24·5	6·80	3·6	0·388	Meat and pancreas = 7·5 g. N at beginning of period III.
		24	39·8	7·66	5·1	—	Totals.
		24	38·5	7·14	5·3	—	Control on 24 hrs sample.
June 8	I	3	1·20	0·49	2·4	0·163	
	II	3	10·30[1]	0·80	13·0	0·266	Fructose 20 g. at beginning of period II.
	III	18	43·5	9·9	4·4	0·55	
		24	55·0	11·19	4·9	—	Totals.
		24	51·0	—	—	—	Control on 24 hrs sample. Glucose 48·0 Fructose 3·0
June 13	I	2	0·10	0·42	0·24	0·210	
	II	3	12·9	0·056	212·0	0·019	Glucose 20 g.
	III	19	35·7	9·13	3·9	0·480	Caseinogen and pancreas = 9·6 g. N at beginning of period III.
		24	48·7	9·61	5·0	—	Totals.
June 18	I	4	14·4	2·2	6·5	0·55	Caseinogen and pancreas = 6·0 g. N at beginning of period I.
	II	4	7·35	2·1	3·5	0·525	Intravenous injection 1·5 g. glucose at beginning of period II.
	III	16	21·8	7·0	3·1	0·44	Caseinogen and pancreas = 6·9 g. N at beginning of period III.
		24	43·5	11·3	3·8	—	Totals.
June 24	I	4	3·7	0·70	5·2	0·175	
	II	3	14·0	0·68	20·0	0·226	Glucose 20 g. at beginning of period II.
	III	17	41·7	11·82	3·5	0·695	Gelatin and pancreas = 12·9 g. N at beginning of period III.
		24	59·4	13·20	4·5	—	Totals.

[1] Dextrose 8·42. Fructose 1·88. Meat and panc. = 10·0 gm. N at beginning of period III.

sugar to nitrogen relatively small, and later increasing, the impression is given that the protein break-down in the earlier stages is resulting in the formation of sugar which accumulates in the blood to no purpose and overflows through the kidneys when a certain level is reached. This indication

has been mentioned above in connection with the increased protein metabolism in the partially depancreatised Dog VIII when practically no sugar was excreted. The total nitrogen excretion in the case of Dogs I and V is large directly after the total extirpation and decreases to a very small amount. This is accounted for by the fact that the animals on the fourth day after the operation vomited most of the food taken and the nitrogen excretion therefore indicates the break-down of the animal's own tissues.

(*e*) *Ammonia.* In Table VIII the ammonia is given both as g. and % of nitrogen. When expressed as percentage of total nitrogen the ammonia figure increases somewhat after total depancreatisation in the case of Dog III. In Dog VII the ammonia nitrogen percentage of the total nitrogen shows a slight increase when diabetes was produced. The increase is from 2 % to 3 % above the normal figure.

(*f*) *Urea.* As can be seen from Table VIII the percentage of urea nitrogen to total nitrogen is slightly lower in the diabetic animals than in the normal fed upon the same diet. In Dog VII, when normal and fed on meat and pancreas, the urea percentage was 85 to 90 % and when the animal was diabetic the percentage was slightly lower—80 to 87 %. Dog III before depancreatisation had a urea percentage of 90 % and after the operation 82 to 86 %.

(*g*) *Allantoin.* The average normal excretion of allantoin in the dog when meat is fed amounts to 0·2 to 0·5 g. N, or 2·4 % of the total nitrogen (see Table VIII). In the presence of sugar the allantoin estimation is upset unless special precautions are taken [Plimmer and Skelton, 1914, 2]. The allantoin figures for the diabetic dogs are of no value except in the case of Dog VIII. The allantoin nitrogen in this case increases up to 1·16 g. in the diabetic state and the percentage of the total nitrogen is about 10 %.

(*h*) *Uric acid.* As will be seen from the analyses figures for Dogs III and I, uric acid appears in the diabetic urine in estimable amounts and shows an increase as the diabetes progresses. In the normal dog the uric acid excretion is very small.

5. *Faeces.*

Analyses of the faeces for total nitrogen and fat and phosphate were carried out by Dr Cruickshank. The total nitrogen and P_2O_5 figures were used in estimating the balances to which reference has been made. The fat elimination in the faeces was generally small in normal and diabetic animals and the fats fed were evidently absorbed without difficulty from the intestine.

6. *Post-mortem Findings.*

General. The animals all showed great emaciation. The abdominal organs were examined, particularly the site of the pancreas on the duodenum. In the total depancreatisations the duodenum was removed and suspicious tissue examined histologically. No remnants of pancreatic tissue were found after the total extirpations. Portions of the heart and liver and in some cases suprarenals and thyroid were removed immediately after death.

Dog I. No signs of suppuration about wound or in the abdominal cavity. No remnants of pancreas present on the duodenum. Heart muscle flabby and pale. Liver pale. Suprarenals removed and histological appearance found to be normal.

Dog III. Some adhesions about the duodenal borders where pancreas had been removed. No pancreatic tissue found. No signs of suppuration or inflammation about wound or abdomen. Lymphatic nodes were enlarged, but this was noted at the operation. Artery walls very friable. This was noted also on many occasions by Evans, Patterson and Starling in their experiments on the heart-lung preparation of diabetic animals.

Dog VII. Some adhesions about the duodenum. Abdominal wound quite healed and closed. Graft was found to the right of the middle line under the skin of abdomen. It was hard, white and about one cm.^{2} in area. Blood supply evidently came to graft through a fold of omentum which was adherent to inner side of the abdominal wound. No remnants of pancreatic tissue in abdomen.

Histological appearance of graft. The tissue shows some, but not very marked evidence of degeneration. Acinous tissue in places looks quite normal. No islets of Langerhans could be identified with certainty.

Dog VIII. Adhesions between duodenum and colon and liver. Liver yellowish. Some small masses of tissue were removed from the duodenum and on histological examination were found to be masses of scar and lymphatic tissue—no pancreas remains found. No signs of suppuration about wound or abdomen.

Histological examination. Remnant of pancreas removed from the duodenum at operation II was found to show very little sign of degeneration. Acinous tissue was normal with some vacuolisation. Islets of Langerhans could be distinguished.

7. *Analysis of Organs for Glycogen and Fat.* (*Dr Cruickshank.*)

The glycogen estimations which have been carried out agree with the general results of Cruickshank's former investigation. The great increase of glycogen in the heart in the case of the partially depancreatised Dog VII is of interest, but more estimations are necessary before any conclusion can be

No. of animal	Organ	Glycogen %	Fat %	
VII	Heart	1·40	1·89	
	Liver	0·10	6·10	
VIII	Heart	0·924	2·6	
	Liver	0·025	10·7	
VI	Heart	0·50	2·37	
	Liver	0·20	5·84	
Normal	Heart	0·49	1·50	Average figures for
	Liver	4·0 to 7·0	2–3 %	normal animals.

drawn. The results have indicated that the heart muscle retains its power of adding to its glycogen store in diabetes and, perhaps because of the increased available amount of sugar, has actually a greater glycogen reserve than the normal. The increase in the percentage of fat in both cardiac and hepatic tissue is an evidence of the fat mobilisation in diabetes. A further analysis of the mobilisation of fat in diabetes would be of value and, it is hoped, will shortly be undertaken.

IV. Discussion and Summary.

Total Metabolism in Experimental Diabetes.

The results show a definite increase of total metabolism in pancreatic diabetes. This increase corresponds with the severity of the usual symptoms. In Dog VIII when partially depancreatised no increase was observed and the glycosuria was slight and transitory. In Dog VII partial removal of the pancreas resulted in a marked glycosuria and the average increase in the total metabolism is 9 %. In the totally depancreatised animals the increase was from 14 to 23 % and the type of diabetes very severe. Murlin and Kramer [1913] found an increase of metabolism amounting to 42 % in a depancreatised dog. Our figures represent an average throughout the course of the diabetes and the increase per cent. is found to rise during the diabetic period (Dog VIII) from 9 % to 38 %. The increase of total metabolism parallels the severity of the diabetes. Benedict and Joslin [1910, 1912] in their studies of diabetes in man calculated that the increase of

metabolism was from 15 % to 20 %. Lusk and others objected to this conclusion on the ground that the controls were unsuitable for comparison

Dog VII.

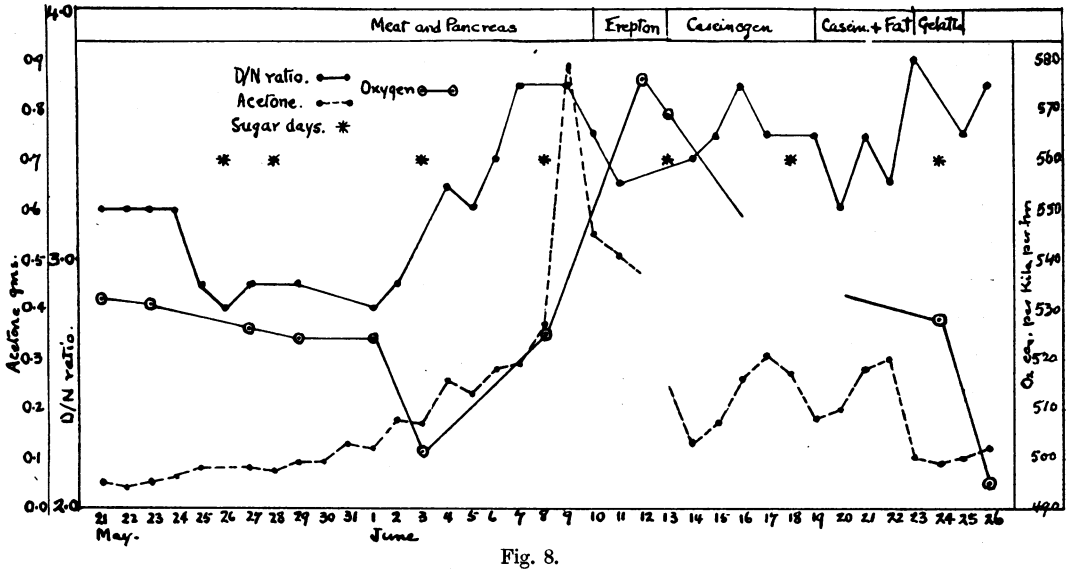

Fig. 8.

Dog VIII.

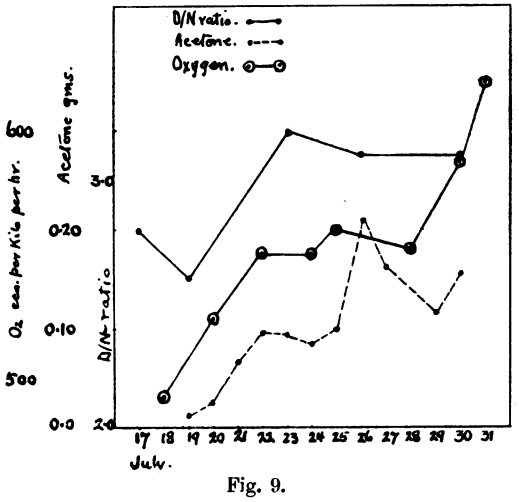

Fig. 9.

with the diabetics because of differences in size and weight. More recently, however, Benedict and Joslin in their report on cases of severe diabetes have

substantiated their former finding. Their later normal controls are strictly comparable in the matter of dimensions and the authors find the increase in diabetes more marked than previously stated. Taken as an isolated fact this increase in metabolism cannot be used as a deciding argument in favour of either the over-production or the non-utilisation theory. The fact that the total metabolism increases more and more during the course of the diabetes might be taken to indicate that this phenomenon is secondary to some other process. It is interesting, therefore, to find out what parallelism exists between the increase of total metabolism and other evidences of disturbance. In Figs. 8 and 9 the D : N ratio, the total acetone excretion, and the oxygen consumption in cc. per kilo. per hour of the diabetic Dogs VII and VIII, have been charted. The acetone excretion is subject to some fluctuation but a certain amount of parallelism exists between the course of the oxygen consumption and the acetone excretion. The three curves in Fig. 9 show a corresponding rise. In the case of Dog III when diabetic these three curves also show a parallel rise. There seems to be a relationship between the degree of disturbance of the protein metabolism and the oxygen consumption. As far as one can judge from the calculations of energy distribution an increase in fat metabolism or evidence of incomplete combustion of fat does not seem to bear a relation to the increase in total metabolism. Some interesting observations of Benedict and co-workers show that, in the normal human, an acidosis induced by a carbohydrate-free diet is accompanied by an increase in the total metabolism. It is apparently difficult to induce an acidosis in normal dogs and in some experiments with fat feeding (Dog VII, Table VIII) observations upon the acetone excretion and respiratory exchange did not show any marked variation. The part played by sugar usage in relation to the changes in total metabolism in diabetic dogs is an important consideration. The evidence as to the power to oxidise sugar, which is exhibited by animals during the course of the diabetes, consists in the level of the respiratory quotient and the degree of rise after sugar is given. From this evidence it has been seen that the degree of utilisation is sometimes less shortly after depancreatisation than later on. This is evident from the series of curves in Fig. 6 from the partially depancreatised Dog VII. In this case a limited degree of sugar usage occurred after three weeks. The course of the total metabolism does not seem to bear a close relation to the evidences of sugar usage. In the totally depancreatised animals the diminished utilisation of sugar is evident from the beginning of the diabetic period, while the oxygen consumption shows a steady increase throughout.

This comparison of the course of the two processes would seem to indicate that the disturbance of carbohydrate metabolism is the primary process and the gradual increase in total metabolism an evidence of a cumulative disturbance in protein and perhaps fat metabolism or an attempt to make up for the deficiency in the energy exchanges caused by the imperfect utilisation of sugar.

The increase in protein metabolism in diabetes has been noted by many investigators. The protein break-down is shown by the marked loss of nitrogen to the body especially in the earlier days of diabetes. In our experiments calculation of the energy distribution to protein after allowance has been made for sugar formation shows that the increase is 5 % to 10 % over the normal protein value in most cases. The actual protein break-down in the fasting condition has been shown to be enormously increased in pancreatic diabetes [Falta, Grote and Staehelin, 1907] and in phloridzin glycosuria [Reilly, Nolan and Lusk, 1898]. The protein destruction as evidenced by the nitrogen elimination shows an enormous increase, while the actual part taken by protein in the oxidations is only slightly increased. This process may be illustrated by the figures from two observations upon a dog in the normal and diabetic condition after the ingestion of meat.

(*a*) *Dog III. Normal.*

	CO_2	O_2	R.Q.
Feb. 12, 1914. Wt. 7·600	3768	4677 ⟶	0·806 (observed)
N in g. per hr. = 0·340 =	1662	2053	
	2106	2624 ⟶	0·802

in which the protein percentage is 44·0 %, carbohydrate 19 %, fat 37 %.

(*b*) *Dog III. Diabetic 4 days. D : N ratio* 3·8.

	CO_2	O_2	R.Q.
Feb. 20, 1914. Wt. 6·800	3132	4624 ⟶	0·680 (observed)

	CO_2	O_2	CO_2	O_2	R.Q.
N in g. per hr. = 0·595 =	2907	3592	1227	1912	
Sugar from protein = 2·26 g. =	1680	1680	1905	2712 ⟶	0·705
	1227	1912			

Protein 41·0 %. Carbohydrate 0·0 %. Fat 59·0 %.

Both observations are six hours after meat containing 4·0 g. nitrogen had been eaten. Hourly figures are given.

Comparison of these two observations will show that, while the protein percentage of the total oxygen distribution is practically the same in both cases, the nitrogen excretion, and therefore the protein destruction, is greatly increased.

In the protein break-down which occurs in diabetes the form in which the nitrogen is excreted might give some indication of the disturbance. Expressed in percentage of the total nitrogen the urea nitrogen tends to diminish and the ammonia to increase, and the increase in ammonia nitrogen corresponds generally with the increased excretion of acetone bodies. The balance thus maintained by the urea and ammonia nitrogen is an expression of the process by which the organism attempts to counteract the increasing acidosis, as has been generally recognised in cases of human diabetes. The destruction of tissue protein is shown also in the increase of the purine metabolism in diabetes. The allantoin excretion in one dog where the estimation in the presence of sugar is satisfactory formed up to one gram of the total nitrogen or about 10 %. The allantoin nitrogen in the normal animal under the same conditions of diet amounts to 0·5 g., or 3 % of the total nitrogen. The excretion of uric acid in the normal dog is very small and showed a definite increase in diabetes. The phosphate excretion in diabetes is slightly greater than the intake, but the phosphate balance shows such an inconsiderable loss that it cannot be taken as evidence of tissue destruction.

The protein destruction, as shown by the great loss of nitrogen to the body, starts soon after the removal of the pancreas, and the intake has to be greatly increased before a balance is reached. In this increase of the protein metabolism in the first few days of diabetes the amount of nitrogen excreted per hour is equal to that observed after a protein meal in the normal condition, but the respiratory exchanges do not show the increase in total metabolism which would occur were the protein fully oxidised. If, on the basis of the D : N ratio, we subtract the energy equivalent of the sugar formed from protein, the energy distribution to protein in the total metabolism is found to be somewhat increased. These facts indicate that the well recognised break-down of protein is closely associated with the power of the tissues to oxidise carbohydrate.

The question as to what significance can be attached to the steadily increasing excretion of acetone bodies in experimental diabetes is of interest. The possible sources of these intermediary products are the proteins or fats, and some indication has been sought for as to whether they originate mainly from alteration in the protein or fat metabolism. On a carbohydrate-free

diet normal dogs do not show any marked increase of acetone excretion. With feeding of fats to Dog VII no such increase was observed. As soon as pancreatic diabetes is established the acetone excretion increases steadily and shows a parallel course to the D : N ratio and the increase in total metabolism. If the acetone bodies had their source in incomplete fat combustion one would expect the respiratory quotient to fall in relation to the rise in acetone excretion. This, however, does not appear to be the case. The energy distribution calculations on days when the acetone excretion is highest do not show any marked indication of incomplete fat oxidation. The total acetone excretion in the diabetic dogs is not large and the production of a small amount might not influence the level of the respiratory quotient. The general course of the acetone excretion bears a closer relation to the protein metabolism. Variations in the total nitrogen are usually accompanied by parallel variations in the total acetone. In view of the fact that amino-acids, such as leucine and tyrosine, have been observed to produce acetoacetic acid, while many of the other amino-acids of protein produce sugar in diabetes, the influence of feeding different proteins and erepton was studied in Dog VII. In this dog the acetone excretion reached its highest level upon the standard diet of horse meat and pancreas. Upon erepton the acetone excretion was high and fell somewhat with caseinogen feeding. When gelatin was fed the acetone excretion fell considerably while the D : N ratio rose. We have therefore some indication that the acetone excretion depends on the character of the proteins fed. In the case of gelatin, which contains a larger proportion of sugar-producing amino-acids, the results are well marked. The most important source of the acetone bodies in the diabetic metabolism of dogs appears to be protein, and the increasing disturbance in the protein metabolism is indicated by the excretion of the acetone bodies as well as by the D : N ratio.

Utilisation of Sugar in Diabetes.

The generally low level of the respiratory quotient in diabetes is one of the chief arguments in favour of the non-utilisation theory. It might be argued, however, that in diabetes other processes which require much oxygen and yield less carbon dioxide obscure an oxidation of carbohydrate which is as effective as in the normal condition. The processes which would contribute to give very low respiratory quotients are the following:

(*a*) The formation of sugar from protein. Magnus Levy has calculated that the respiratory quotient yielded by protein after the full amount of

sugar formed is allowed for, is 0·615. In our calculations with a D : N ratio ranging from 2·7 to 3·8 the respiratory quotient of protein decreases from 0·715 to 0·640.

(*b*) The formation of acetone bodies from protein.

(*c*) The formation of acetone bodies from fat.

(*d*) The formation of sugar from fat.

We have attempted in our calculations to allow for the formation of sugar from protein. The formation of acetone bodies has not been taken into account in the calculations, because the small amounts excreted indicate that the part played by this process in the low respiratory quotient is a minor one. The respiratory quotient of the formation of sugar from fat is very low. The figures cannot be corrected for the formation of sugar from fat because there are no data to go upon. If this process occurred to any extent in diabetes the respiratory quotient should reach much lower values than those generally observed.

After making allowance for the first of the factors mentioned above, we still find very little evidence of sugar utilisation in the diabetic dogs, and the apparent utilisation when it occurs may be due to error in the use of the D : N ratio in the calculation.

The diminished rise or constant level of the respiratory quotient after giving glucose to diabetic dogs is also evidence of the inability of the tissues to oxidise glucose. The course of the respiratory quotient agrees with the percentage of sugar recovered from the urine. When a rise of the respiratory quotient does occur, after giving sugar to totally depancreatised animals it is in most cases small and within the limit of possible error. The disturbance of sugar utilisation is apparent shortly after depancreatisation and does not appear to increase with the severity of the diabetes. The giving of sugar later in the course of the diabetes resulted in a limited utilisation in the case of Dog VIII. The rise in the respiratory quotient after fructose (Table VI, protocol *o*) indicated a utilisation of 0·30 g. of sugar per hour during the observations. After glucose (protocol *p*) the utilisation was 0·25 g. per hour in the same dog three days later. In the partially depancreatised Dog VII the course of the sugar utilisation is similar, but the ability to oxidise sugar returned to a greater degree. In this latter case the return of the sugar usage can be explained by the pancreas tissue present, which evidently became more functional after a time had elapsed. An explanation of the apparent partial recovery in the power of using sugar in Dog VIII is harder to find. Remnants of pancreas tissue were carefully searched for at the

post-mortem without result. It may be that the tissues elaborate to some degree some other way of dealing with carbohydrate than that which exists when the pancreas is intact. Another possibility is that the increasing number of organisms in the intestinal tract and elsewhere account for the oxidation of sugar which occurs. The general condition of the animals has usually corresponded with the ability to use carbohydrate; thus Dog VIII after total depancreatisation was in much better condition than any of the other diabetic dogs. The results, therefore, indicate that removal of the pancreas in dogs causes a serious disturbance in the carbohydrate metabolism, in which the power of oxidising glucose is, for a time, completely lost and, while this may return to a small degree, it is never again observed to approach the normal in extent.

The recent work of Verzar [1914] on the question of sugar utilisation in diabetes has also given support to the non-utilisation theory. Verzar finds that, when sugar is injected into the circulation of diabetic animals, the normal rise of the respiratory quotient does not occur, but that this evidence of the loss of sugar utilisation is obtained only some days after the removal of the pancreas. In our experiments the effect of sugar giving was tried for the first time about the third or fourth day after depancreatisation, and only in the case of Dog III did we obtain evidence of a limited sugar utilisation two days after the operation. The results obtained from the injection of glucose and fructose therefore lead to the same conclusions as our findings in regard to the sugar utilisation in diabetes.

Summary.

1. In experimental diabetes an increase of the total metabolism occurs, amounting to 15 to 20 % on an average, and increasing with the severity of the condition.

2. The rise of the respiratory quotient which normally occurs after the giving of glucose or fructose is greatly diminished or is absent after the total removal of the pancreas, indicating that complete oxidation of sugar does not occur.

3. The increase in the excretion of acetone bodies parallels the increase in the D : N ratio and the total metabolism, suggesting that the phenomena are expressions of a similar disturbance of protein metabolism which is secondary to the impairment of sugar utilisation.

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