The fuel of life : experimental studies in normal and diabetic animals / by John James Rickard Macleod.

THE FUEL OF LIFE

JOHN JAMES RICKARD MACLEOD

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THE FUEL OF LIFE

Experimental Studies in Normal and Diabetic Animals

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THE FUEL OF LIFE

Experimental Studies in Normal and Diabetic Animals

BY

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LOUIS CLARK VANUXEM FOUNDATION

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PREFACE

THE subject matter of this volume formed the basis of four lectures delivered in March 1928 under the Louis Clark Vanuxem Foundation of Princeton University. It reviews what appears to me to be of importance in our present knowledge concerning the preparation of the food materials for combustion in the animal organism and deals more especially with the question as to whether fats, as well as proteins, form carbohydrate before being used as fuel. It has not been attempted to include all the literature bearing on the subject, but only that of recent date, and if too much weight may appear to have been given to investigations conducted during the last few years under my own direction I can only offer in excuse that it was primarily with the object in view of reviewing this work as a whole that I undertook the preparation of the volume.

I wish to thank my colleagues and pupils for their enthusiastic cooperation in the researches which are here brought under review, and Professor Laurence Irving and Miss Maynard Grange for preparing the index.

J. J. R. MACLEOD.

Toronto, Canada
1928

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CHAPTER I

THE problem in the metabolism of animals, which is discussed on these pages, may seem to be rather narrow in its interest. At the outset, therefore, let me give some of my reasons for choosing it. One of these is that the research activities of my laboratory, during the past few years, have been directed very largely towards attempts at the solution of this problem, and I should like to give a general account of what we have found. My purpose is to present, in as nontechnical language as possible, evidence for a viewpoint of the physiology of animal nutrition which differs somewhat from that held by many workers in this field, and although most of the details have already been published in various scientific journals the main results have not as yet been summed up.

Another reason for my choice is that the problem of the preparation of fuel for combustion comprehends, perhaps as well as any other, a large part of the recent work in animal metabolism in many other laboratories than my own, and therefore offers a suitable one under which not a little of modern biochemistry and physiology may be reviewed.

General statement of hypothesis. What then is the hypothesis which we propose to support? It centers on the fact, established through the researches very largely of A. V. Hill and Meyerhof, that the energy of muscular contraction is derived in the long run mainly, if not entirely, from the combustion of carbohydrate. The particular form which carbohydrate takes in the muscle is the substance known as animal starch, or glycogen, and this is built up out of

sugar carried to the muscle by the blood and derived from the liver, where it has its source in further stores of glycogen which have been formed, either out of the carbohydrates absorbed from the alimentary canal or from proteins and fats. It is particularly in asserting that fat as well as protein is converted into glycogen before it can be used as fuel for combustion in the muscles, that our hypothesis differs from that of many physiologists. These, led in this country by Graham Lusk, admit that protein forms glycogen but they deny that fat can do so, believing that the preparation of this foodstuff for the production of energy involves nothing more than a splitting of its large molecule into small fragments which are then more or less directly oxidized. According to our view neither protein nor fat is burned by the muscles until it has been converted into carbohydrate or some related substance, mainly by the liver.

It will be remarked that the muscles are not the only tissues in which energy is set free, the glands, the brain and even the nerves being also energy machines. But the proportion of the total energy production of the animal which is contributed by these other sources is comparatively small, and there is not wanting some evidence to show that it may also depend on processes which are akin to that observed in muscle as, for example, in such glands as the salivary, where the utilization of O_2 and the heat production behave, after momentary activity, much in the same way as they do in muscle.

The new production of glycogen, or gluconeogenesis as it is called, is chiefly carried on in the liver but we do not affirm that it is restricted to this organ. Under certain circumstances it may also take place, although to a much less marked degree, in the muscles.

Having thus briefly stated our hypothesis we may now proceed to examine the evidence which supports it, and

to present the reasons why we consider the older one as unsound and untenable.

Relationship between fats and carbohydrates in plant life. When we consider the relationship between fat and carbohydrate, not in animals only but also in plants, there is no doubt that the transmutation between the two substances is a reversible one. It proceeds in plants according to the general equation: carbohydrate ⇋ fat. The fat is built up out of the carbohydrate manufactured in the leaf and is broken down to this form again before being used in the production of energy. Thus, when oil-rich seeds, such as linseed, hempseed or poppyseed are allowed to germinate the fat decreases and the carbohydrate increases, as the following figures from the work of Ivanoff will serve to show.

		IN SEED (PER CENT)	IN 4 DAYS SEEDLING (PER CENT)	IN 8 DAYS SEEDLING (PER CENT)
Linseed	Fat	33.6	26.4	16.0
	Carbohydrate	4.5	6.7	17.6
Hempseed	Fat	31.3	17.8	11.3
	Carbohydrate	2.8	7.9	10.2
Poppyseed	Fat	47.0	38.5	36.3
	Carbohydrate	1.2	6.8	17.4

(*From Leathes and Raper*)

Nothing definite is known as to the nature of the chemical reactions by which the long chain of methyl groups of the fatty acid molecule is divided and modified, or as to how oxygen becomes added to these groups, to form carbohydrate, but there can be no doubt that the latter transformation is a preliminary step in the oxidative process by which the energy necessary for growth is set free. In a series of researches in which the energy value of seeds was determined before and after germination on distilled water in the dark, Terroine and his several collaborators have shown that more energy is lost in the case of seeds which are relatively rich in fats and proteins than in those with high propor-

tions of carbohydrate. Since no processes involving an increase in energy value can proceed under the conditions of germination in this experiment it is clear that the energy which is lost by the seeds which are rich in fat and protein must be expended in converting these food materials into carbohydrate. These workers have also found that when fat is thus transformed the loss of energy in the seedling, as compared with the seed, is 23 per cent, a fact of great significance, since this value agrees exactly with that derived from the formula by which Zuntz proposed to indicate the conversion of fat into carbohydrate in the animal body. Thus, on the assumption that 2 molecules of a fat, having the formula $C_{57}H_{110}O_6$, are converted into 19 molecules of glucose ($C_6H_{12}O_6$), the loss of energy is exactly 23 per cent, when the calorie value of fat is 9.4 and that of glucose 3.76 (Zuntz). Not only, therefore, is there no doubt that the transformation of carbohydrate into fat is a readily reversible process in plants, but there is also evidence to show that when the germinating plant must use fat for building up its new tissues the energy which is expended corresponds to that required to convert fat into carbohydrate.

There being therefore no doubt that fatty acid and carbohydrate are readily changeable the one into the other in plants is it not inherently probable that the same processes will also occur in animals? In them it is well known that carbohydrate is readily changed into fat—the fattening of stock being a sufficient example—why, then, it may be asked, should it be considered improbable that they can carry out the reverse process?

As the first piece of evidence indicating that they must do so we will consider the fact that the only fuel used by isolated frog muscle is carbohydrate in nature, and it may not be out of place if I briefly outline the main experiments which have led to this conclusion, especially since there is

probably no other division of physiology which is more interesting in its recent development.

The chemistry of contraction in isolated frog muscle. The initial step in this development was taken when Fletcher and Hopkins clearly worked out the conditions under which lactic acid accumulates in frog muscle. They showed that when contraction is performed in the absence of oxygen, lactic acid accumulates until it reaches a concentration of about 1.8 mg. for each gram of muscle, when complete fatigue ensues. On the other hand, when contraction occurs in the presence of oxygen lactic acid fails to accumulate and, most significant of all, when a muscle which has been fatigued, by stimulation in the absence of oxygen, is placed in this gas the lactic acid which has accumulated in it disappears and the muscle recovers from fatigue. Quite clearly, therefore, lactic acid formation is an important incident in the contraction of muscle and its disappearance is necessary for recovery of contractile power after fatigue.

But it remained to determine the exact relationship of these changes to the production of energy by the muscle. This was done by A. V. Hill who measured the heat output at various phases of contraction and relaxation, by means of very sensitive thermo-couples on which the excised muscle was laid. During contraction a sudden generation of heat occurs immediately the muscle first contracts, this becomes less as contraction is sustained and then increases for an instant as the muscle relaxes. During the subsequent resting condition a further outburst of heat occurs, often equal to that of all the preceding phases of contraction. This led to the significant conclusion, that energy expenditure is necessary for restoring to the fatigued muscle its power to contract again. The curve of heat production was further found to correspond to that of the oxygen consumption, so that there could be no doubt that

the disappearance of lactic acid and the utilization of oxygen are related processes, in which much of the energy set free by oxidation is used not, as in a heat engine, to drive the muscular machine but rather, as in winding up a spring, to put the muscle in a condition so that work may be performed when its stored-up energy is released.

These discoveries, based on physical methods of investigation, now raised the question, as to what material was being used to produce the lactic acid, and Meyerhof found this to be glycogen. When muscle contracts in the absence of oxygen glycogen disappears in proportion to the lactic acid which accumulates. When recovery in oxygen occurs, on the other hand, much less oxygen is used than corresponds to the lactic acid which disappears. According to Meyerhof, one in four molecules of lactic acid becomes oxidized, the other three being converted into glycogen, and it has been possible to demonstrate this formation of glycogen in isolated muscle by chemical means. One-fourth of the lactic acid produced by contraction is evidently burned in the resting state to yield energy by means of which the remaining three-fourths may be resynthesized into a molecule with a higher potential energy. The volumes of oxygen used and of carbon dioxide produced during this recovery process were found by Meyerhof to be equal, as theory would require for the oxidation of lactic acid; $C_3H_6O_3 + 3O_2 = 3CO_2 + 3H_2O$. In other words, the ratio $\frac{CO_2}{O_2}$ or the respiratory quotient, as it is called, of such a process is unity, which it must also be when any carbohydrate is oxidized, according to the general formula, $C_x(H_2O)_y$. In isolated frog muscle, therefore, there is no doubt that the energy used for contraction is dependent entirely on a series of processes which involve the combustion of carbohydrate, or of substances, such as lactic

acid, having the same relative number of oxygen, carbon and hydrogen atoms in the molecule.

Analogies between gaseous exchange of muscular activity in isolated frog muscle and in man. But we cannot assume that the chemical processes responsible for contraction in isolated frog muscle are of the same nature as those occurring in intact animals. It might be, for example, that isolated muscle could only use carbohydrate because, in the absence of a blood supply, it was not furnished with other fuel materials. To ascertain whether the two processes are akin it was necessary to devise methods by which it could be determined whether some at least of the changes occurring in frog muscle also occur in intact animals. But for some time it was impossible to devise a method which could be used for this purpose, for obviously most of those employed in the case of isolated muscle, such as determination of glycogen and lactic acid, or thermometric measurements are inappropriate. The only hope lay in comparing the respiratory exchange of an animal at rest with that resulting from activity, and it is again to the work of A. V. Hill and his collaborators, Long and Lupton, that we owe the development of a satisfactory method for making this comparison.

It has, of course, been known for long that the intake of O_2 and the output of CO_2 increase greatly during muscular exercise and that the ratio between these two, the respiratory quotient, may undergo certain changes, but these facts only tell us that the general metabolism has increased and become altered in type. The observations on frog muscle indicate that what we must rather know is the extent to which O_2 consumption and CO_2 production have increased over the resting state, not only during the exercise but also for some time after it. Will the respiratory quotient of the increased combustion be unity, as in frog muscle? The observations necessary to test this question are

most suitably made on man, since the degree and duration of exercise can be adequately controlled and the respiratory exchange accurately measured at short intervals of time. When the exercise, such as "marking time" rapidly, is moderate and of brief duration, the oxygen consumption may return to the resting level within 5 or 6 minutes after the exercise, but it does not do so for an hour or more when this is more strenuous and prolonged. This indicates that the exercise creates a continued demand for more oxygen, and we may conclude, in the light of the frog muscle experiments, that the excess is largely used in the recovery process.

But the fact of greatest interest for us at present is that the ratio between the extra O_2 used and the extra CO_2 excreted during both the exercise and the recovery phase (the total excess metabolism) often works out at unity. At least this is so for short bouts of moderate exercise, which indicates that the material undergoing combustion must be carbohydrate in nature. Even when the preceding diet has been excessively rich in fats and poor in carbohydrates, so that the resting R.Q. is 0.71, short periods of exercise still raise the excess quotient to unity, but it begins to fall when the exercise is prolonged more rapidly than in normally fed persons. But for other types of exercise quotients that are well below, or even well above, unity may be obtained for the extra metabolism (Hill, Furusawa, L. J. Henderson). Thus, Furusawa has found that the R.Q. for the extra metabolism is below unity when the exercise is continued for a long time and may be greater than unity after very strenuous exercise. If we assume that the muscular activity itself is exclusively due to carbohydrate combustion the only interpretation which seems possible for these lower quotients is that the metabolic processes which are going on, apart from those of contraction, must have changed in type during the exercise. Since it is

necessary, in determining the extra metabolism, to allow the O_2 consumption to come back to the level at which it stood before the exercise was started—the resting level—it is obvious that if some of the retained oxygen was being used to convert fat or protein into carbohydrate during the exercise the R.Q. of the extra metabolism would become lower. These results indicate that a complex series of changes occur in the intact animal when exercise is prolonged and it seems to me impossible, in the light of present knowledge, to determine their exact nature from studies on the respiratory exchange. The outstanding fact is that extra quotients of unity are often obtained under certain conditions; for this is, at least, circumstantial evidence for the view that carbohydrate, and carbohydrate only, is being oxidized by the muscles.[1]

Another fact of interest for us is that lactic acid accumulates in the blood and appears in the urine during strenuous exercise in proportion as the increased intake of oxygen fails to succeed in keeping pace with the increased demands of the muscles for oxygen. Even the greatest increase in breathing in man cannot cause more than 4, or at most 5 liters, of oxygen to be absorbed in a minute, which is far from sufficient to resynthesize the lactic acid which may be formed in this time. As a consequence lactic acid overflows, as it were, from the muscles into the blood, and although some of this excess may be converted into glycogen in the liver, a large proportion remains over in the blood and muscles. It is in order that this may be synthesized into glycogen (in the muscles?) that O_2 continues to be absorbed in excess for some time after the exercise. Being unable to absorb oxygen rapidly enough for synthesis of glycogen to occur during the exercise "an oxygen debt"

[1] While these pages were in press Rapport and Balli have published a paper (*Am. Journ. Physiol.*, LXXXIII, p. 450) in which it is shown that the R.Q. of exercise and recovery in dogs working on a treadmill corresponds closely to the resting R.Q.

has to be incurred. This debt is at first rapidly repaid after the exercise has ceased and then more and more slowly, the rapid repayment occurring while lactic acid still remains in the muscles and the slow repayment, while the lactic acid which has accumulated in the blood and other tissues gets gradually reabsorbed into the muscles, where it is then dealt with. During gentle exercise lactic acid is not produced more quickly than the oxygen required for oxidative synthesis can be supplied, by the increased breathing, and a "steady state" becomes established. There comes to be a balance between the formation and removal of lactic acid in the muscles, and when the exercise ceases only a small debt of oxygen remains to be repaid.

It may be of interest to note the extent to which such oxygen debts can be incurred. After very strenuous exercise for 13 seconds in a man weighing 140 lbs. a debt of 7.1 liters was incurred, whereas in another who ran for 33 minutes at a moderate speed it was only 7.9 liters. In the former case lactic acid was produced so rapidly that it was out of all proportion to the greatest possible intake of oxygen by the lungs, whereas in the latter, its more gradual production did not greatly exceed the oxygen intake. These observations indicate that the process of metabolism concerned in muscular activity must be of the same nature in man as in the isolated amphibian muscle, and this view is further supported when we compare the oxygen intake with the rate of disappearance of lactic acid from the blood and tissues of the body. Of course we can only determine the latter value approximately, by measuring the percentage of lactic acid in the blood and then multiplying by a factor to allow for the total volume of the blood and tissues, but the results are of sufficient accuracy for our purpose. If we compare the rate of disappearance of lactic acid with the intake of oxygen for a period say of ten minutes during recovery from exhausting exercise the significant result

is obtained that only about one-fourth as much oxygen is found to have been retained as would be required to oxidize the acid which has meanwhile disappeared.

Although the brilliant series of researches which I have just reviewed offers us results fitting closely to the hypothesis, that muscular contraction in man, like contraction in isolated amphibian muscle, is dependent on the breakdown of glycogen into lactic acid, yet the fact cannot be disregarded that we have had to assume that some other chemical process besides oxidation of carbohydrate, with a quotient much less than unity, also becomes established. Later on we shall discuss further evidence to support this view, but meanwhile let us turn our attention to some other experimental facts which have a bearing on our subject.

The respiratory metabolism of finely cut-up tissues. In the first place it is of significance that respiratory quotients approaching unity are commonly obtained when the O_2 consumption and CO_2 production are measured in finely cut-up tissue kept alive by being suspended in isotonic saline solutions with abundant free oxygen.[1] Not only is this true for tissues containing muscle but also for others, such as the kidney (Barcroft and Brodie). As an example may be given results recently obtained by Ahlgren. Frog muscle cut in fine pieces and suspended in a solution of phosphates was placed in a microrespirometer. After two hours the following results, calculated in cubic millimeters per gram of muscle and per hour, were obtained:

	CO_2	O_2	R.Q.
1	145	142	1.02
2	134	138	0.97
3	117	126	0.93
4	104	107	0.97
5	103	110	0.94
6	111	114	0.97

[1] The small extent of the respiratory exchange in many of these experiments demands, of course, the use of highly refined methods for its measurement, the principle usually adopted being to observe, with a sensitive manometer (micro-

The respiratory metabolism of isolated mammalian muscle. The experiments in this case are much more difficult to carry out than those on cold-blooded muscle since it is usually impossible, on account of the much more rapid metabolism of mammalian muscle, to provide adequate nutriment and oxygen for each fiber by merely suspending in oxygenated blood serum or saline solution. With very thin muscles this method is possible, and Takane, working with strips of the diaphragm of the rat suspended in serum, observed, as an average in four experiments, an R.Q. of 0.79.

To keep most mammalian muscles alive, however, it is necessary to maintain the circulation of blood through them. The respiratory exchange is then measured by comparing the volumes of O_2 and CO_2 in the blood entering and leaving the muscle, which is by no means an easy task, since not only must the percentage of the gases in each of these bloods be determined but also the volume of blood flow in a unit of time. In this way, Doisy and Beckman found that the average R.Q. of 14 observations made on the hind limbs of dogs was 0.80, and that one of unity was obtained only in two cases. The results of the different experiments were, however, very irregular. More recently Himwich and Castle have reported similar observations in which the blood was taken from the vessels supplying the gastrocnemius muscle of the dog. Besides the R.Q. of the muscle itself these authors simultaneously determined that of the animal as a whole and found, in general, that the two quotients ran parallel. Although the experiments were carried out with extreme care the quotients obtained varied considerably, but nevertheless the authors felt justified in concluding

respirometer), the degree of change in pressure occurring in the atmosphere of a small vessel which contains the tissue. The CO_2 can be absorbed and measured by having some caustic alkali solution in a side pocket of the vessel and when this is done the pressure reduction from this absorption is a measure of the absorption of O_2.

that "resting muscle under normal conditions oxidizes besides carbohydrate either fat or protein or both, probably in the same proportions as does the rest of the body." The extreme difficulties in carrying out these experiments have led various workers to use what is known as an eviscerated preparation, that is, one in which the muscles are left undisturbed, but the abdominal viscera are removed. In such a preparation the remaining active tissues are mainly the muscles and the great advantage is gained that the respiratory exchange can be measured by collecting and analyzing the expired air, instead of analyzing the blood.

The respiratory metabolism in mammals after evisceration. The earliest experiments of this nature were those performed by Porges, in which the circulation to the abdomen and lower extremities was cut off by ligating the abdominal aorta and the inferior vena cava at the level of the diaphragm in anaesthetized rabbits. After waiting for about ten minutes, so that the breathing, which had been temporarily disturbed by the operation, might return to normal, the expired air was collected through a cannula placed in the trachea. In eight animals which survived the operation for a sufficient length of time, analysis of the respired air, collected in each case for a period of one hour, gave respiratory quotients which ranged between 0.878 and 0.928, except in two animals in which they were 0.985 and 0.997. In three rabbits similarly anaesthetized, but in which the liver circulation was not disturbed, the quotients were 0.726, 0.717, and 0.698 respectively. The removal of the liver and other abdominal viscera from the circulation, therefore, caused a marked increase in the quotient, but this was not sufficient to bring it to unity, except in two cases. This makes the result inconclusive and the research has failed to find its place in physiological literature for another reason, namely because, a few years after its publication, Murlin, Edelmann, and Kramer challenged the view

that the rise in the quotient depended on a change in the type of metabolism. These workers found, in experiments of a similar nature to those of Porges, that a decrease in the CO_2-content of the arterial blood always accompanied a rise in R.Q. and they considered that increased ventilation of the blood in the lungs, through exaggerated breathing, or a more rapid pulmonary blood-flow, or the appearance of organic acids in the blood, caused excess of CO_2 to be discharged, hence the higher quotient. Moreover, they found that the clamping of the vessels sometimes had no influence on the quotient or might even cause it to be lowered, the arterial CO_2 remaining constant in the former case and increasing in the latter.

Porges and Salomon also determined the quotient in dogs from which the pancreas had been removed two days previous to the tying of the vessels. Quotients close to unity were obtained in three of the animals (1.13, 0.920, and 1.19), but in a fourth one it was only 0.859. It is pointed out in this paper that excessive breathing cannot be held accountable for the high quotients, since one of them (1.19) was observed in an animal that survived the ligation of the vessels for $2\frac{1}{2}$ hours. The chief interest of these results is that they show that similar changes in R.Q. occur in completely diabetic animals as in normal ones, when the influence of the abdominal viscera is removed, thus indicating, contrary to the belief of many, that the muscles in diabetes have not lost the power to oxidize carbohydrate.

There is no further record of experiments of a similar type until comparatively recently, when either eviscerated, decapitated animals, or dehepatized animals, have been used. Let us first of all consider the results obtained in the former. Following decapitation the animal (cat) is maintained alive by artificial respiration, and the stomach, the intestines, the spleen and the pancreas, are removed, after ligation of the coeliac and mesenteric arteries. Although

the liver still remains *in situ* it is practically excluded from the circulation,[1] since its entering blood vessels (portal vein and hepatic arteries) are tied. Such an eviscerated preparation is one in which the muscles, the heart, and the lungs are the only remaining active tissues. One of the most striking effects which follows evisceration is that the percentage of sugar falls rapidly in the blood, so that injection of sugar is necessary in order to maintain the eviscerated animal in good condition. Burn and Dale found the respiratory quotient under these conditions to stand close to unity and they provisionally suggested that this "may indicate the use by muscle of dexterose alone as the source of its energy requirements." In view of the importance of this result, it seemed worth while to repeat these experiments, and this has recently been done in my laboratory by Kilborn.

After decapitation the trachea of the animal was connected with a respiratory pump constructed in such a way that with each stroke a definite volume of air (about 40 cc.) was sent into the lungs, after which a previously closed side tube in the pump was automatically opened so that the expired air could be collected in a rubber bag (of 13 liters capacity). The dead space between pump and trachea was as small as possible and there was no resistance to expiration, which depended on the elastic recoil of the thorax. The minute volume of the pump was constant and the period of collection of expired air for analysis (in Haldane's apparatus) was 15 minutes. I mention these details because, as we shall see later on, the rate of ventilation in such an experiment has a prepotent influence on the results. Several samples of expired air having been collected at intervals all the abdominal viscera were removed with the exception of the liver, after which samples of air were again

[1] It is not entirely excluded, however, because some blood still gets to it through diaphragmatic vessels, and the hepatic veins are still unobstructed.

collected from hour to hour.[1] Typical results are shown in Table I:

TABLE I

CAT NO.	R.Q. BEFORE EVISCERATION	R.Q. AFTER EVISCERATION				
		1ST HR.	2ND HR.	3RD HR.	4TH HR.	5TH HR.
1	0.79, 0.80	0.90, 0.86	0.99, 0.86			
2	0.74, 0.72, 0.74	1.04, 0.94	1.13, 1.20	1.29, 1.62		
3	0.71, 0.73	0.98, 0.92	0.84, 1.05	1.38		
4	0.72, 0.80	0.93, 0.97		1.28	1.07	1.07
5	0.78, 0.80	0.94, 0.94	1.01, 0.98	1.25		
6	0.72	1.03	1.04	1.05	0.87	

(*Kilborn*)

In all cases the quotient rose sharply immediately following evisceration, and in subsequent hours it either remained fairly steady at about unity, or it rose, sometimes considerably, above this level. Burn and Dale's results were thus in general confirmed, but their suggestion that the rise in the quotient might depend on the exclusive oxidation of carbohydrate seemed doubtful, because of the gradual rise above unity, which suggests rather that a relatively excessive discharge of CO_2 from the blood must have been responsible, as had previously been pointed out by Murlin, Edelman, and Kramer. This might be due either to excessive ventilation of the lungs (a blowing-off of CO_2) or to a lessened CO_2 carrying-power of the blood and tissues, dependent on the production of some fixed acid in the preparation. With regard to the former possibility, it must be remembered that although the rate and volume of the pump remained unchanged after evisceration in Kilborn's experiments, the active tissues of the animal had been much reduced by this operation (by about 40 per cent according to Hunt and Bright), so that a relative over-ventilation

[1] Although thus deprived of its main circulation it must nevertheless be remembered that some blood still finds its way through the liver by way of diaphragmatic veins and also that the blood in the patulous hepatic veins may still carry into the vena cava substances derived from the liver cells, a certain ebb and flow movement being conferred on this blood on account of the respirations.

became established. That this might account for the rise in R.Q. will be evident from the following rough calculations.

As an average of 15 experiments the volume of air respired per hour was 45 liters and it contained 2 per cent CO_2, or 900 cc. per hour before evisceration. During the hour following evisceration the percentage of CO_2 fell to 1.4, or a total of 630 cc. so that the hourly output was decreased by 270 cc. Since the rate of metabolism usually fell by about forty per cent after evisceration the CO_2 output should have been 540 cc., so that about 90 cc. extra CO_2 was added to the expired air from the animal's body. Assuming the blood volume of a cat of 3 Kg. body weight to be 200 cc. then a reduction of 10 per cent in blood CO_2 would contribute 20 cc. The remaining 70 cc. must therefore have come from the tissues and this can all be accounted for if these be taken as weighing 1400 gm. and as having suffered a reduction of 5 per cent in their CO_2 content. Krogh also calls attention to the very large amount of CO_2 which may be pumped out of a rabbit by artificial ventilation.

In view of these considerations the CO_2 content of the arterial blood was analyzed by van Slyke's barometric method, with the result that a marked falling off was found to occur following evisceration, corresponding in general to the rise in R.Q., as shown in the curves of Fig. 1.

It is entirely possible, therefore, that the mere blowing off of CO_2 from the blood and tissues may account for the rise in R.Q. and if this be so then it should be possible to make it return to its level before evisceration, either by reducing the rate of ventilation or by adding CO_2 to the inspired air. The first of these methods was adopted in cat 25 and it will be seen from the curve that the R.Q. immediately fell parallel with an increase in the CO_2 of the blood. But a certain degree of anoxaemia also resulted from the curtailment of the ventilation, and much more satisfac-

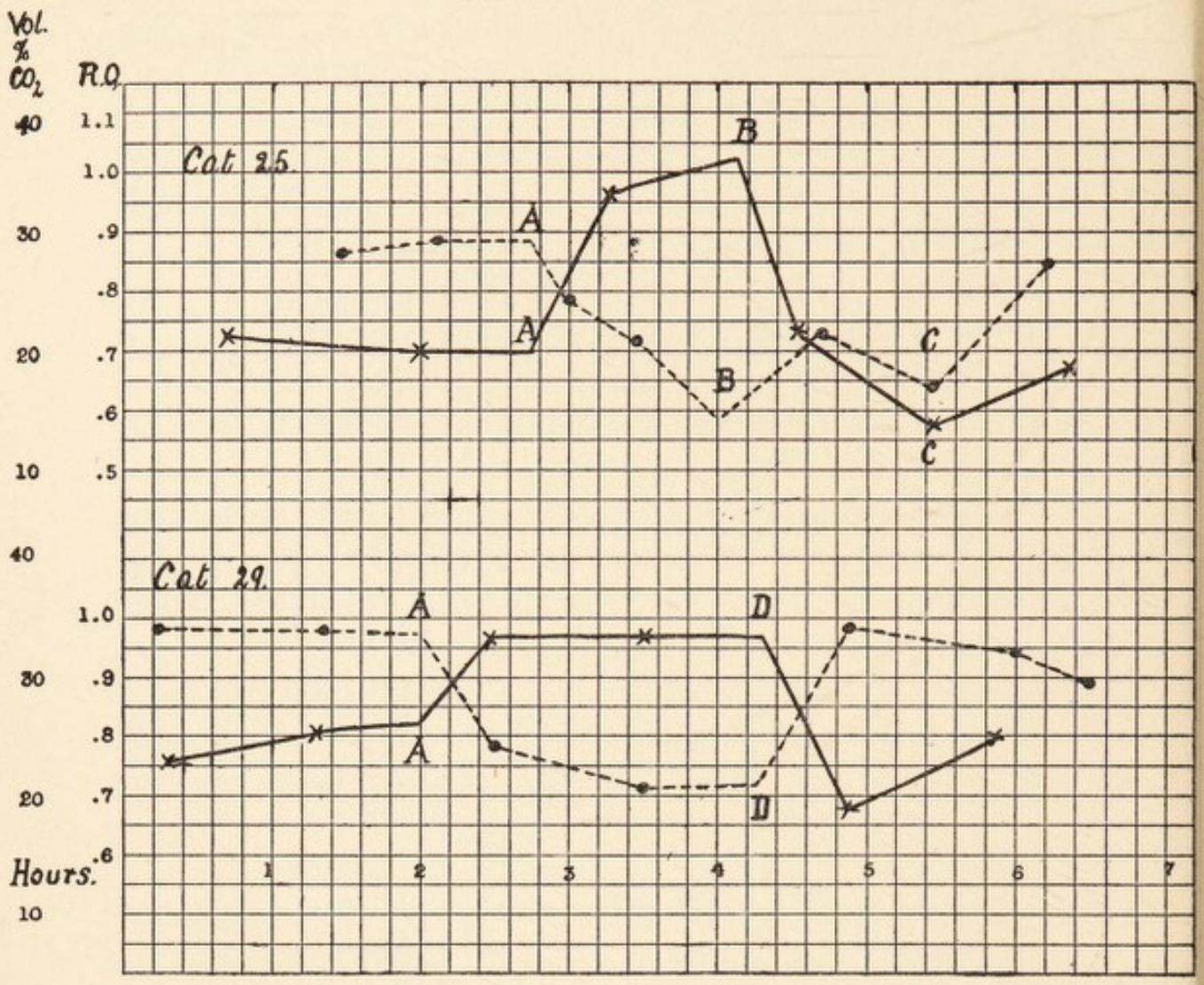


FIGURE 1. Curves showing behavior of R.Q. and CO_2 content of arterial blood following evisceration and alterations in breathing. R.Q.—continuous lines; CO_2 of blood—broken lines. *Cat 25.* Evisceration at A; minute volume of pump reduced from 850 cc. to 612 cc. at B; and to 230 cc. at C. *Cat 29.* Evisceration at A; began breathing air containing 3.4 per cent CO_2 at D. Ordinates—volume per cent CO_2 and R.Q. Abscissa—time in hours.

tory results were obtained by the second method, as shown in the curve of cat 29. It seems fairly clear, therefore, that variations in R.Q. after evisceration are related to variations in the CO_2 content of the arterial blood.

But another factor besides over-ventilation may come into play to assist in the expulsion of CO_2, namely, the appearance of unoxidized organic acids in the organism.

The steady decline in the O_2-intake from hour to hour following evisceration shows that the tissues were gradually dying and this suggests the possibility that lactic acid may have been accumulating in the blood and tissues, owing to a failure of its oxidative synthesis into glycogen (p. 9). In two experiments, therefore, lactic acid was determined in the blood, with the result that it rose from an average of 0.015 per cent before evisceration to 0.060 per cent and 0.067 per cent in 2 and 4 hours, respectively, after it. Further evidence that fixed acids had appeared was obtained in one of the experiments in which the blood, prior to analysis for CO_2, was equilibrated in an atmosphere containing a partial pressure of 40 mm. CO_2, for it was found that 25 per cent less CO_2 was taken up after evisceration than before it. These results show that we may not depend on observations of the R.Q. in eviscerated, decapitated cats as an index of the type of metabolism going on in the preparation, and this leads us to the question as to whether similar observations made on other animals after elimination of the liver may be of assistance.

The respiratory metabolism after elimination of the liver in dogs. Partly by the method devised by F. C. Mann and his collaborators, and partly by one worked out in collaboration with Soskin, Markowitz removed the liver in dogs, without disturbing the circulation through the remaining viscera, and then observed the gaseous exchange of the animals during several consecutive hours in a respiratory cabinet of the Benedict type, the accuracy of which was repeatedly tested by burning alcohol or ether in it. Unfortunately, in view of Kilborn's experiments, the CO_2 content of the blood was not also determined but, for reasons which will be apparent later, it is improbable that the rise in R.Q. which was observed was dependent on a blowing off of CO_2 or on acidosis. Before considering these researches, however, let me briefly say something about the

general behavior of the hepatectomized animals and of some earlier observations of their respiratory exchange.

For some hours after the removal of the liver the animals do not show any noteworthy symptoms. But signs of collapse ultimately appear, followed by convulsive seizures which may be quickly fatal. Mann and Magath made the important observation that these symptoms supervene when the percentage of sugar in the blood has fallen to a certain level and, more significant still, that they are almost instantly removed, and the animal restored to a practically normal state, by the intravenous injection of glucose solutions. Under these conditions the animal may live for sixteen or twenty hours. By using dogs trained to breathe into a mask connected through valves so that the expired air was collected in a spirometer, Mann found, by analysis of this air, that the respiratory quotient rose and the O_2 consumption fell following hepatectomy.

In Markowitz's experiments the respiratory exchange was observed from hour to hour after hepatectomy, both before and after the injection of sugar. Normal, as well as depancreatized, dogs were used, since it was considered important to ascertain whether removal of the liver would cause the quotient to rise to an equal extent in both, it being still held by some physiologists that diabetes is essentially due to a loss of power on the part of the tissues (muscles) to oxidize sugar.

Typical results (from ten successful experiments) in a normal and a depancreatized dog hepatectomized by Mann's 3-stage operation are given in Table II, the former being also shown in curve form in Fig. 2.

In two hours after hepatectomy in the non-diabetic animal the quotient stood at 0.87 this being very much above the level of about 0.7 which is usual in normal animals sometime after taking food. This result, which is typical of several others, was in many ways a disappointment, for we

TABLE II

TIME	CONDITION	CALORIES PER KILO PER HR.	R.Q.	REMARKS
Non-diabetic				
9:40 a.m.	Hepatectomy completed			Wt. 7.8 Kg.
10:15–10:30	Recovered from anaesthetic and put in cabinet			Blood sugar 0.074 per cent
11:00–12:00		2.00	0.869	Animal quiet
12:10 p.m.	Removed			Hypoglycaemic symptoms Blood sugar 0.052 per cent Glucose injected with prompt recovery. Animal replaced in cabinet.
12:50–1:50		2.14	0.990	Animal quiet
1:50–2:50		2.25	0.987	Animal quiet
2:50–3:50		2.43	0.936	Animal quiet
3:50–4:50		2.32	0.902	Animal quiet
6:00				Blood sugar 0.100 per cent Animal in good condition
Diabetic				Wt. 12 Kg. Depancreatized 2 days previously.
9:00–9:40	Hepatectomy completed and put in cabinet			
10:00–11:00		2.65	0.857	Very quiet
11:00–12:00		2.63	0.886	Very quiet
12:00–1:00		2.28	0.887	Very quiet
1:00–2:00		2.05	0.855	Convulsions at end of period
2:05	Removed from cabinet			Blood sugar 0.054 per cent Glucose injected—recovery
2:35–3:35		1.70	0.831	Quiet
3:35–4:35		1.63	0.851	Unconscious part of time
5:30				Blood sugar 0.042 per cent

had hoped that the quotient would rise to unity, as had been found by Burn and Dale after elimination of the liver in decapitated cats. It was only after glucose was administered to antidote the symptoms of hypoglycaemia that quotients approaching unity were obtained. Thus, for the four hourly periods following the administration of glucose, quotients of 0.99, 0.987, 0.936, and 0.902 were observed. This attainment of unity following the injection of

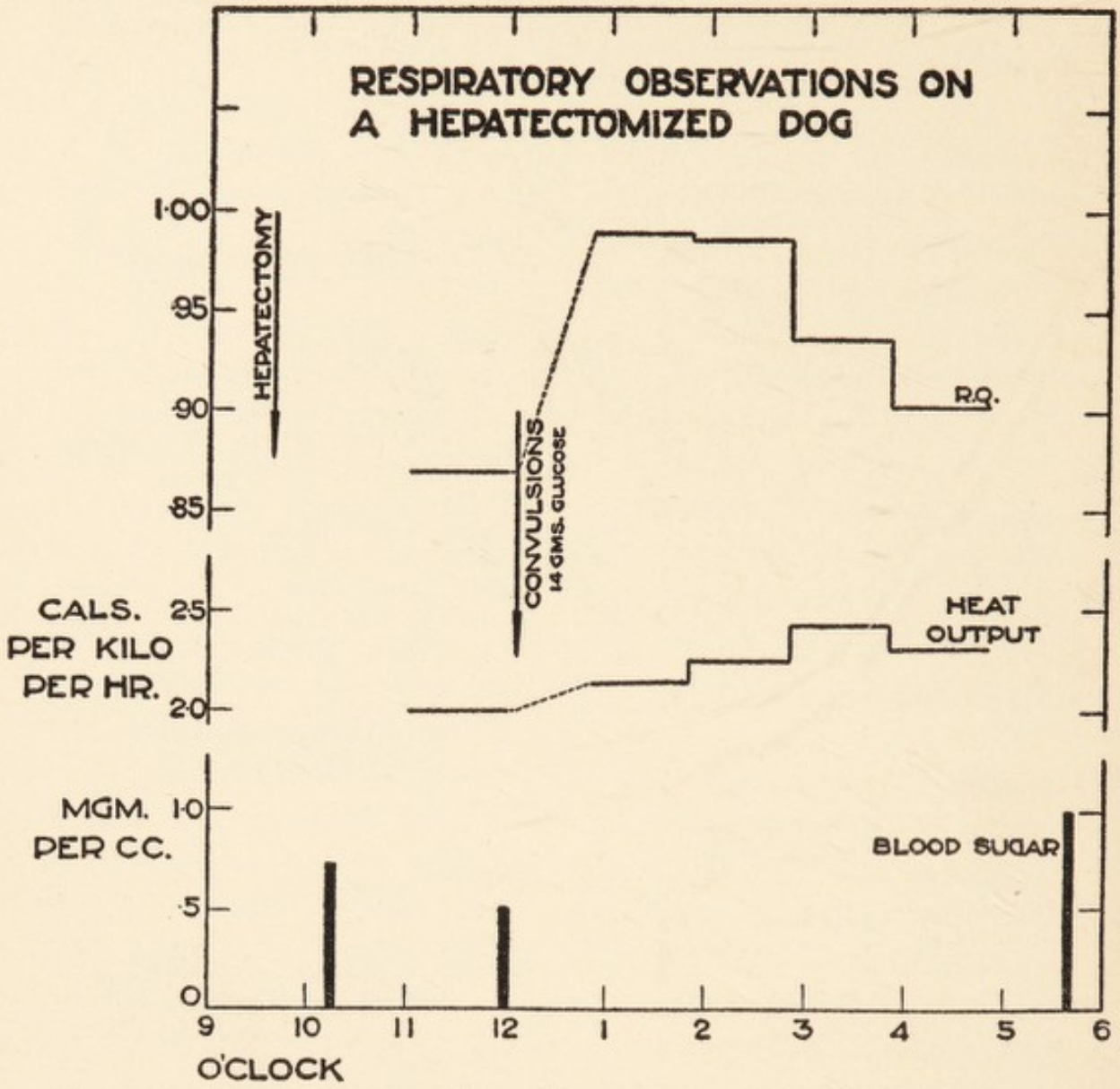


FIGURE 2. Curves showing effect of hepatectomy, followed by injection of glucose, on R.Q. and heat output in a dog. The percentage of blood sugar is also shown by the vertical columns. The figures on the abscissa give the actual times during the day at which the various observations were made; those on the ordinates give the value of R.Q. (uppermost figures), cals. per Kg. and hour (middle figures) and the blood sugar (lowermost figures). At noon 14 gm. of glucose was injected. (Markowitz)

glucose indicates that the hepatectomized animal *can* use carbohydrate as its exclusive fuel, but the lower quotient observed prior to glucose injection shows that it *does not actually do so*. That administration of sugar raises the quotient makes it improbable that a blowing off of CO_2

from the blood can have been responsible for the rise in the quotient which followed the hepatectomy. This result further suggests that the percentage of blood sugar has to be at or above the level observed in normal animals in order that preformed carbohydrate shall be burned exclusively, and that the muscles assume gluconeogenic functions of their own when the supply of sugar to them becomes inadequate and so cause the quotient to stand about midway between that of the fasting animal and one consuming preformed carbohydrate exclusively. Of course, a direct combustion of non-carbohydrates would also explain the lower quotient.

It is important to note in these experiments that the energy expenditure after hepatectomy stood at 2 calories per kilo body weight and hour. This is not much below the normal value which for a normal dog of about 6 Kg. and after 18 hours fasting is given as between 2.4 and 2.9 (L. Hédon). This relatively high caloric expenditure, hour after hour, shows that only a small proportion of the fuel burned can have come from the glycogen originally present in the muscles. Even if all this glycogen were used up it could not account for the calories for more than one hour in any of the ten experiments. After the injection of sugar the energy expenditure rose gradually during the next three hours to 2.4 calories, a similar result being obtained in four out of eight experiments. This illustrates the influence of the carbohydrate plethora spoken of by Lusk, and it shows that hepatectomy has not caused any serious weakening of the energy transforming powers of the muscles.

But perhaps the most important results are those obtained on depancreatized dogs, in that they show as marked a rise in R.Q. following hepatectomy as occurs in normal animals. This would seem to shatter the belief, so fondly held by many, that the essential cause of diabetes is the loss by the tissues of their power to oxidize sugar.

Thus, in the observation of our Table the average quotient during four hours after hepatectomy in a previously depancreatized animal was found to be 0.87, which is the same as that of a normal animal after this operation, and is much above the usual diabetic quotient of 0.66 to 0.68. Hypoglycaemic symptoms do not develop in diabetic animals as quickly as in normal ones, because of the high blood sugar to start with, but when they do develop they are removed with the usual promptness by injecting glucose, although the quotient rises only slightly, or not at all. Soskin has also observed this peculiar effect of glucose and although we cannot suggest any explanation, we nevertheless consider it as very significant.

We shall return to these experiments again in other connections. Meanwhile the important fact to bear in mind, insofar as the behavior of the R.Q. is concerned, is that it affords no evidence that the diabetic animal is any less capable than the normal one to oxidize carbohydrate after removal of the liver, although after this operation, in both of these states, some process other than the combustion of carbohydrate comes into play in the metabolism of the muscles. This may be a process of gluconeogenesis, thus accounting for a quotient less than unity.

The respiratory metabolism of the isolated mammalian heart. It is one of the achievements of experimental physiology that suitable conditions have been discovered under which the mammalian heart can be kept beating powerfully and efficiently for hours, either after its removal from the body, or with the circulation through the aorta short circuited, through suitable resistances, so that the lungs alone are supplied with blood. The advantage of this heart-lung preparation, as it is called, is that the respiratory exchange of the heart can be measured, by collecting and analyzing the air which is made to enter and leave the lungs by means of a pump. What interests us at present is the respiratory

quotient of this air, since it will indicate the nature of the respiratory process in the heart. If the heart muscle, like skeletal muscle, performs its work by oxidizing preformed carbohydrate exclusively then the quotient should be unity. As a matter of fact, E. H. Starling and C. Lovatt Evans have found that the average quotient of the normal dog's heart is 0.85, but considerable variations are shown in the 51 results upon which this average is based. Quotients as low as 0.70 and as high as 1.09 are recorded, and it is doubtful whether the metabolism of the heart-lung preparation can be considered as really proceeding in a normal way. An observation which would seem to justify this criticism is that the addition of glucose to the perfusion fluid, although it raises the quotient, does not succeed in carrying it above 0.9, whereas, as we have seen, a quotient of 1.0, or near it, is usually reached under similar conditions after hepatectomy.

Incidentally it may be pointed out that quotients exceeding 0.9 were obtained in the heart-lung preparation when, previous to the experiment, the animal had been fed on a diet composed mainly of carbohydrates. On the other hand, much lower quotients, averaging in 17 experiments 0.71, were observed when the heart-lung experiment was performed on animals from which the pancreas had been removed some days previously, and the quotient in these cases did not become raised by adding glucose to the perfusion fluid. This result would seem to supply direct evidence that the heart had lost the power to oxidize carbohydrate, although as Starling and Evans themselves point out, profound modifications in tissue metabolism may have masked a moderate carbohydrate consumption. It seems to me that the heart-lung preparation is not a suitable test object for investigation of the nature of tissue metabolism in the body as a whole. The remarkable power of the heart to continue beating for many hours after isola-

tion from the body with a perfusion fluid containing no organic constituents, and the peculiar increase in glycogen which is known to occur in it, both in acute starvation and in diabetes, indicates that important metabolic differences exist between it and skeletal muscle. The heart muscle may possess the power of gluconeogenesis within itself to a much greater degree than skeletal muscle.

Résumé of evidence afforded by examination of respiratory metabolism. To sum up, we may say that the evidence which is furnished by the R.Q. shows that the fuel used in the production of energy by the excised muscle of cold-blooded animals is exclusively carbohydrate in nature, but that it does not necessarily show this to be the case for the muscles of warm-blooded animals. When these are kept alive by perfusion with blood, as in an eviscerated preparation, the high quotient which is observed may depend on a "blowing off" of CO_2 rather than an exclusive combustion of carbohydrate; whereas when a hepatectomized animal, or the isolated beating heart is used the behavior of the quotient indicates a partial utilization of non-carbohydrate material. But a quotient that is less than unity does not prove that foodstuffs other than carbohydrate are being directly oxidized, for it is possible that, when driven to it by an inadequate supply of sugar from the liver, the muscles may themselves assume gluconeogenic functions, converting protein, or fat, or both into glycogen, which is then used as the immediate fuel for the production of energy.

The supposed absence of glycogen from the muscles in fishes. Denial that carbohydrate is the immediate fuel of muscular activity has recently been voiced by C. W. Greene, from observations on the glycogen content of the muscles and liver of the salmon. He states, "That preformed carbohydrate in the usual quantities is obviously not essential for the physiological activity of the salmon" and again, that "in the absence of evidence to the contrary

we may conclude that the salmon is able to metabolize . . . fats and proteins directly." Leaving out of regard the fallacy which is involved in the assumption that because little glycogen is found present in the muscles, carbohydrate cannot be the immediate fuel used in contraction—for it might be used as quickly as it was being formed out of the blood sugar—the absence of glycogen is probably to be accounted for by its being all used up by the excessive struggling of the fish in the act of catching. This conclusion is based on observations recently made by myself and Simpson on the glycogen content of the muscles and liver of sea fish of various species. We always found glycogen in the muscles when these were removed immediately after catching the fish by hand line. The average for the muscle of 15 haddock (Melanogrammus) was 0.12 per cent and for a similar number of sculpin (Myoxocephalus), 0.276 per cent, which is not much below the percentage often found in mammalian muscle. On the other hand, when the fish were caught by a trawl which was not hauled for about an hour after setting practically no glycogen could be found in the muscles. The struggling of the fish in the water after hooking was no doubt responsible for the rapid using up of the muscle glycogen. Reserves of this carbohydrate were at the same time usually found to be still present in the liver and evidence was obtained, in the high percentages of sugar found in the blood, that these were being used to replenish the stores of muscle glycogen, although not at a rate that was sufficient to leave a detectable balance over when the fish was killed. Fish, such as the haddock, caught by trawl are usually in a greatly exhausted state when landed and only a few of them recover when returned to the sea. In several specimens that did recover when they were placed in a crib kept at several fathoms depth we observed that the glycogen reappeared in the muscles in a few days.

It will be of interest to investigate the relationship between the rate and extent of this recovery in muscle glycogen and the glycogen reserves of the liver, for there can be no doubt that new formation of glycogen (gluconeogenesis) must always be proceeding actively in fishes, since their food supply is very restricted in preformed carbohydrate. Some glycogen is always present even in the lowest forms of plankton on which the smaller fishes feed, but this original source cannot serve to account for more than a small fraction of the glycogen which is found present in the larger fishes. Most of this must therefore be manufactured out of non-carbohydrate material, and although this will no doubt include protein, fat also must be used. A further investigation of these problems in fishes would no doubt be fruitful in results. Those presented here make it advisable that Greene's evidence, for the conclusion that fishes can use fats and proteins for muscular movement without converting them into carbohydrate, be reinvestigated.

The contractility of muscle deprived of glycogen. According to the theories of Meyerhof and Hill, glycogen occupies the central position in muscular contraction so that it becomes of interest to see how the muscles behave when all glycogen is removed from them. Such a condition can be brought about by the injection of large doses of insulin, or by the repeated administration during several weeks of thyroxin. The most obvious change in deglycogenated muscles is that they go into rigor in warm-blooded animals almost immediately after the death of the animal (Hoet and Marks). Indeed in rabbits in which convulsions and coma have been allowed to persist for some time as a result of the injection of large doses of insulin, the muscles of the extremities are practically in rigor at the time of death, and they do not respond to a stimulus by the usual brief contraction and relaxation. This rigor is pecu-

liar in that it does not last long and is removed by forcibly stretching the muscles, which is not the case with the rigor mortis of normal muscles.

The contractile properties of deglycogenated muscle have been studied in frogs by Olmsted and Harvey. In these, like in other cold-blooded animals, insulin, even in very large doses, does not bring down the blood sugar to the convulsive level until after at least 24 hours. The convulsions then recur at intervals during the next day or two, until at last the animal dies with the muscle glycogen either greatly reduced, as compared with that normally present, or entirely absent. When the contractile power of the muscles (to electrical stimulation) of injected frogs was compared with that of the muscles of normal frogs no significant difference could be demonstrated, either in the strength of the contractions or in the time of onset of fatigue, which took several hours to show itself, and in four of the experiments no glycogen was detectable in the fatigued muscles of the injected frogs. But this does not prove that contraction can occur in the absence of glycogen, for not only may traces of this substance be present which cannot be detected by chemical means but it is possible when the circulation is intact, as it was in these experiments, that glycogen was being formed out of blood sugar at the same rate as it was being used up, so that no balance remained over.

This possibility was eliminated in more recent experiments in which practically deglycogenated muscles, removed from large frogs which had been for some time in insulin convulsions, were used. When these were stimulated at regular intervals they contracted almost as well and for as long a time before fatigue set in as control muscles removed from normal frogs (Olmsted and Coulthard). These results are difficult to explain unless we suppose that some carbohydrate other than glycogen was used. But no

evidence favoring such an explanation could be obtained by measuring the free sugar, or the lactic acid, of the muscles. Thus the average of five observations gave the following results:

Muscle Removed from Bull Frogs after Insulin Convulsions

	GLYCOGEN (PER CENT)	LACTIC ACID (PER CENT)	FREE SUGAR (PER CENT)
At rest	0.05	0.05	0.10
After fatigue	0.03	0.11	0.14

That small amounts of some intermediate substance between glycogen and lactic acid may have been present in the muscle to start with, was indicated, in observations made on other muscles removed from the same frogs, by a small increase occurring in lactic acid (average 0.03 per cent), without any measurable change in glycogen, when the muscle was thrown into rigor by chloroform vapor. The free phosphorus also increased somewhat as a result of rigor, but it did not change as a result of fatigue.

These results would seem to indicate that contraction can occur at the expense of non-carbohydrate substances, although the possibility remains that the muscle when deprived of carbohydrate can assume the power of gluconeogenesis. This possibility leads us to enquire as to the behavior of the fatty ingredients of muscle during contraction.

The inability of isolated muscle to use fat directly. If fat can be used directly, or indirectly, by a contracting muscle, changes in its amount should be detectable by chemical means, but all attempts to demonstrate any such change have been strikingly negative in their results. Thus, Leathes found the amount of fat to be practically the same in corresponding muscles of the two legs of frogs, after those on one side had been tetanized for several hours, by stimulation of the sciatic nerve. Indeed when any slight difference could be detected it was in the direction of show-

ing that the stimulated muscles contained more fat than the controls (Leathes). Similar observations, conducted on excised frog muscles with great care by Winfield, yielded equally negative results, although again there was, in some of the observations, a decided surplus of fat in the stimulated muscles. Since Winfield's observations were made partly on muscles which were stimulated to exhaustion anaerobically, then allowed to recover in oxygen and this repeated many times, and partly on muscles in rigor, it is certain that fat is not used by excised amphibian muscles under conditions which cause very marked changes in the carbohydrate content.

These experiments should be repeated with deglycogenated muscles, for it may be that fat is only used when no carbohydrate is available. In any case, they do not finally prove that fat may not be used by contracting muscle, especially since Lafon has found, by comparison of the fat which could be extracted by ether from the blood of the artery and vein supplying the muscles of chewing in the horse and ass, that more fat is removed from the blood when the muscles become active. This investigator also states, although less emphatically, that more fat disappears from the blood supplying the muscles of the hind leg of the dog under chloralose anaesthesia when they are stimulated electrically than when at rest. But it must be remembered that it is extremely difficult to determine the fat of blood with any degree of accuracy and moreover, it would be necessary to take into consideration the marked changes in blood flow which accompany muscular activity before concluding, from changes in the percentage of ether-soluble material, that more or less fat had been utilized by the muscle.

The rôle of protein in muscular activity. Since muscle is composed very largely of protein and also contains large amounts of various nitrogenous substances, such as creatin,

purin bodies, etc., it might be expected that its activity would entail some changes in these materials. Direct chemical examination of excised muscle has indeed shown that the purin bases and the creatin are greater in fatigued, as compared with resting muscle (Burian, Cathcart). But such results are open to many interpretations, so that the problem has been mainly attacked by observing in the intact animal whether the excretion of nitrogen becomes altered as a result of exercise, it being assumed that any significant changes in muscle protein, caused by exercise, would be reflected in the nitrogen balance of the animal. While this is no doubt a correct enough assumption in a general way, it is nevertheless necessary to bear in mind the possibility that protein might be more extensively broken down in the muscles without any immediate increase occurring in the excretion of nitrogen. The carbon of the protein molecule might be oxidized, but the nitrogen used over again, in the sense that it was recombined with other carbon atoms, derived perhaps from fat or carbohydrate, and the resynthesized protein again used as fuel. In such a case there might be no change in the excretion of nitrogen, although energy was set free through oxidation of the carbon.

As a matter of fact, all of the innumerable researches which have had to do with this problem, researches which date back to the famous experiments of Fick and Wislicenus, have yielded practically negative results with regard to changes in nitrogen excretion. It is only when the muscular exercise is so strenuous that a distressing degree of dyspnoea is set up, or when the diet is deficient in amount, particularly with regard to non-protein foodstuffs, that any marked increase can be detected in the excretion of nitrogen, and even under these conditions the amount of protein broken down is far from adequate to account for the work done. For some days following pro-

longed severe muscular exercise in man, as in route marching, a greater excretion of nitrogen has not infrequently been observed to occur, but factors other than the actual amount of work performed, such as the degree of comfort under which it was carried out, the physical condition of the person, the kind of day, etc., have been found to have a considerable influence. (For further details and literature see Cathcart, Zuntz, and Schumburg.)

As far as the evidence goes, therefore, all we can say is that the utilization of protein, as a whole, is not increased by muscular exercise. Protein cannot be directly burned, although it may be an ultimate source from which the direct fuel (carbohydrate) can be manufactured, and it is possible, as had already been remarked, that the gluconeogenic process, perhaps even in the muscles themselves, may be stimulated to greater activity by muscular exercise without there being any change in the excretion of nitrogen. In this connection it is worthy of note that both Embden and Meyerhof have observed that free ammonia is liberated during muscular contraction in amphibian muscle, which suggests that the same process may occur in mammalian muscle when the incoming supply of free carbohydrates is curtailed. The increase in creatinuria which follows a bout of severe exercise may depend on the breakdown of a creatin-phosphoric acid compound of muscle, such as that recently discovered by Fiske and Subarrow, and the accompanying increase in the excretion of inorganic phosphorus lends support to such a view.

CHAPTER II

THE *blood sugar as the source of muscle glycogen.* The foregoing evidence favors the view that both in normal and diabetic animals the main fuel used by active muscle is carbohydrate, and we may now turn our attention to the source of supply of this material in the muscles. One immediately assumes that it must be the blood sugar and there can be no doubt that the great bulk of the glycogen and other carbohydrates of muscle is thus derived, although a small amount may be provided in other ways. The blood sugar, therefore, occupies a position of supreme importance in metabolism. Indeed if the hypothesis which we are supporting be correct, we may consider the blood sugar to be, as it were, the connecting link between the processes of gluconeogenesis going on mainly in the liver and those of carbohydrate utilization in the muscles. When this link is broken the muscles soon use up all their stores of carbohydrate and life becomes impossible.

There is abundant evidence to justify this view of the importance of the blood sugar. In the first place, it is present in the blood, or corresponding circulatory fluids, of all animals, even of such lowly creatures as star-fish, and in the great majority of animals in which the circulating fluids have been examined the percentage of sugar is not very far removed from that present in the blood of man and the other mammals, that is, in the neighborhood of 0.10 per cent. Thus Lang and I found an average of about 0.04 per cent of reducing sugar in the clear, colorless, circulating fluid of the shore crab (Cancer productus), about the same percentage in the blood of both the cartilaginous

and the bony fishes and usually a somewhat higher percentage in the blood of amphibia.

When the concentration of sugar falls below a certain level highly characteristic symptoms supervene, indicating a serious upset in the neuro-muscular mechanism. Mann and Magath were the first to observe this important relationship. When they caused the blood sugar of dogs to become lowered to about 0.04 per cent, by removing the liver, they found that symptoms supervened, consisting in part of convulsive seizures and in part of coma. There could be no doubt that the symptoms were closely related to the hypoglycaemia, since they were entirely removed, in an amazingly short interval, after injecting glucose into the blood stream. One cannot of course say that the symptoms are actually caused by the low blood sugar; they must rather be caused by some agent the development of which is normally prevented by the presence of a certain percentage of glucose.

The blood sugar becomes reduced after hepatectomy because the muscles continue to use it in the absence of its usual source. It can be still more rapidly reduced by the *injection of insulin,* which seems to act, partly, by stopping the discharge of sugar from the liver and, partly, by increasing the rate of its removal from the blood by the tissues. In very small animals, such as mice, in which metabolism proceeds with great rapidity, the injection of insulin lowers the blood sugar so quickly that symptoms may appear well within an hour, this interval being more and more prolonged in other mammals, in proportion to their size; thus, symptoms appear in rabbits in about an hour and a half, in dogs in about three hours and in man in about four hours following the injection of large doses of insulin. There is no exception to the rule, that the symptoms are promptly removed by restoring the blood sugar to its

normal level; if this be not done they increase in severity until at last the animal dies in profound coma.

In this connection it is highly significant that during prolonged complete starvation the percentage of blood sugar does not, as a rule at least, fall to the level at which hypoglycaemic symptoms appear,[1] neither does glycogen disappear entirely from the muscles. At an early stage in starvation glycogen may practically vanish from the liver but it returns later, indicating that it is now being formed in large quantities out of non-carbohydrate materials.

There can, therefore, be no doubt that a certain percentage of blood sugar is essential to life, and the question arises as to what it is used for. Is it converted into glycogen in the muscles? The strongest evidence that such is its fate has been furnished by Best, Hoet, and Marks who found that a decided increase occurs in the percentage of glycogen in the leg muscles when insulin is injected into eviscerated, decapitated cats. This is very marked when the blood sugar is prevented from falling, by injecting glucose along with the insulin, but it also occurs in the presence of hypoglycaemia. On the other hand, no glycogen is formed in the muscles of an eviscerated, decapitated cat in the absence of insulin, however high the blood sugar may be, or, as Choi has shown, in those of one from which the pancreas alone has been removed. Although this shows that one function of insulin must be to make possible the conversion of blood sugar into muscle glycogen, it must be remembered that glycogen is always present in the muscles of depancreatized dogs.

When the blood sugar is maintained for sometime at a low level, as a result of the presence of an excess of insulin, the percentage of glycogen in the muscles rapidly falls, especially if, as is usually the case, convulsive movements

[1] Shope has found very low sugar percentages in the blood serum in fasting men. (*Jour. Biol. Chem.*, LXXV, p. 101.)

have been pronounced. When convulsions are prevented in certain groups of muscles, by section of the nerve supply, glycogen does not disappear from the denervated muscles following injection of insulin into the animal, although the blood sugar may become very low and the remaining muscles of the body show convulsions and disappearance of glycogen (Best, Hoet, and Marks). This indicates, as many other experiments to be described later also do, that the formation and breakdown of glycogen in the muscles does not primarily depend on the concentration of the blood sugar. It is not the outcome of a balanced reaction obeying the laws of mass action. It also shows that glycogen cannot be drafted, from one group of muscles containing it in abundance to others, notwithstanding that the demands for it in them may be great.

But much remains to be learned with regard to the relationship of glycogen in the muscles to the blood sugar. In the muscles of the eviscerated cat, for example, glycogen remains constant in amount during several hours, although meanwhile the animal is expending energy at the rate of about 2 calories per kilo body weight and per hour. Where does all this energy come from? The blood sugar, as we have seen, is steadily falling, but the total amount of glucose which the muscles could use as fuel from this source would be very far from sufficient to account for the energy output. Thus, as Logan and Olmsted showed, about 0.07 per cent of glucose disappears from the blood in decapitated cats each hour. If we take the blood as amounting to 70 cc. per Kg. body weight and the body fluids as of equal bulk, about 0.1 gm. of glucose disappears and this would yield 0.4 calories or one-fifth of the total. Even if enough sugar could be furnished by the blood to account for the energy expenditure, there remains to explain why, if glycogen be the immediate fuel used in muscular metabolism, no change should occur in its amount in these experiments, for it is

difficult to imagine that the utilization of this substance could exactly balance its formation. On the other hand, if we assume that the muscle contains some other fuel besides glycogen, such as some carbohydrate, which occupies a position between glycogen and lactic acid but which we do not identify by our present-day methods of analysis, then these difficulties are in part at least removed.

The source of the blood sugar. We have now to consider the source of the blood sugar and this, it will be evident, is the key problem of carbohydrate metabolism. When sugar is being absorbed from the intestine it is probably safe to conclude that the blood sugar is thus derived, and that the excess which is absorbed over what is required to maintain the physiological level is absorbed and converted into glycogen, in part by the muscles and in part by the liver. But the problem which interests us at present is with regard to the source of the sugar when no supply from outside the body is available. For example, where does the blood sugar come from during the several weeks that an animal, such as a dog or man himself, continues to live and expend energy when no food is being taken? So long as any of the glycogen which has been derived from the previously absorbed carbohydrate remains in the liver, it is natural to assume that it must furnish the necessary sugar, but this supply must soon become exhausted—a day at most in a medium-sized dog—after which the sugar can only come from protein and fat, glycogen probably being formed as an intermediate step in the process of conversion. Such was the view of Claude Bernard, as has recently been pointed out by Cramer. Bernard considered glycogen to be an internal secretion of the liver cells, a substance manufactured out of sugar when the portal blood contains it in excess, and out of other foodstuffs when this source of supply is lacking. Under the usual conditions of feeding aud muscular activity the supply of glycogen in the liver remains

fairly high—2 to 4 per cent in dogs and rabbits. In a fasting state the supply may dwindle to a very low level—0.2 per cent or less—although it never disappears entirely unless muscular activity be superadded, as for example, by prolonged severe exercise, or shivering, or convulsions, such as those induced by strychnine.[1] And even when, as under these conditions, no glycogen is found at death we cannot conclude that none was being formed, but only that an insufficient quantity was left over, as a balance between formation and transformation into glucose, to be detectable by chemical analysis. Our hypothesis, therefore, supposes that when the source of glucose from outside the body is cut off, new formation of glycogen in the liver is an essential step in furnishing the blood with sugar and hence the muscles with glycogen.

Differences between muscle glycogen and liver glycogen. The liver forms glycogen, the muscles use it; which implies that differences should be detectable by experimental means in the behavior of these two forms of glycogen. Is there any evidence that such is the case? It has been known, since the time of Claude Bernard, that glycogen disappears both from the muscles and from the liver very rapidly after death. This glycogenolysis, as it is called, is attributed to the action of diastatic enzymes which become very active immediately the circulation ceases, either because of the removal of some inhibiting influence of the blood, or because of the breakdown of those invisible intracellular membranes which in the living state hold enzymes and substrat apart.

These diastases are always readily demonstrable in extracts of liver, even after all traces of blood, which also contains them, have been removed, but they may fail to be

[1] Even under these conditions we have usually found *some* glycogen in the liver.

present in extracts of muscle. This absence may be due to rapid destruction of the diastase, although Meyerhof and his coworkers have recently shown that diastase, as well as a glycolytic enzyme, is always present in freshly prepared extracts of muscle. In any case, the difference between the diastatic activities of liver and muscle extracts induced Simpson and myself to compare the process of glycogenolysis in these two tissues after reducing them to a fine powder in a frozen condition, and then allowing the powder to thaw. The tissue was frozen (by liquid air) immediately after its excision from the animal, so that the living chemical processes were stopped and it is probable that the cells became considerably broken up, partly by the pulverization and partly by the freezing and thawing.

We found that practically all the glycogen disappeared from skeletal muscle within twenty minutes after thawing, and even in less time when the initial percentage was low. It also became reduced with great rapidity in the liver powder, but a considerably longer time was necessary for all the glycogen to vanish in this case, partly because of the much larger stores to start with, and partly because retardation of the glycogenolytic process sets in after the tissue has stood some time. So far as the actual rate of disappearance of glycogen was concerned, then, the processes were much alike in liver and muscles, but a striking difference was found in the nature of the breakdown products; in muscle lactic acid accumulated and only a little glucose, whereas in liver glucose alone accumulated and lactic acid, if anything, became less in amount. The lactic acid also did not accumulate in muscle at the same rate as the glycogen disappeared and this delay, which was very evident when the process was stopped in about five minutes after its start, indicates that some intermediary carbohydrate which is not glucose must be formed between glycogen and lactic acid during *post mortem* glycogenolysis (see Fig. 3). In liver

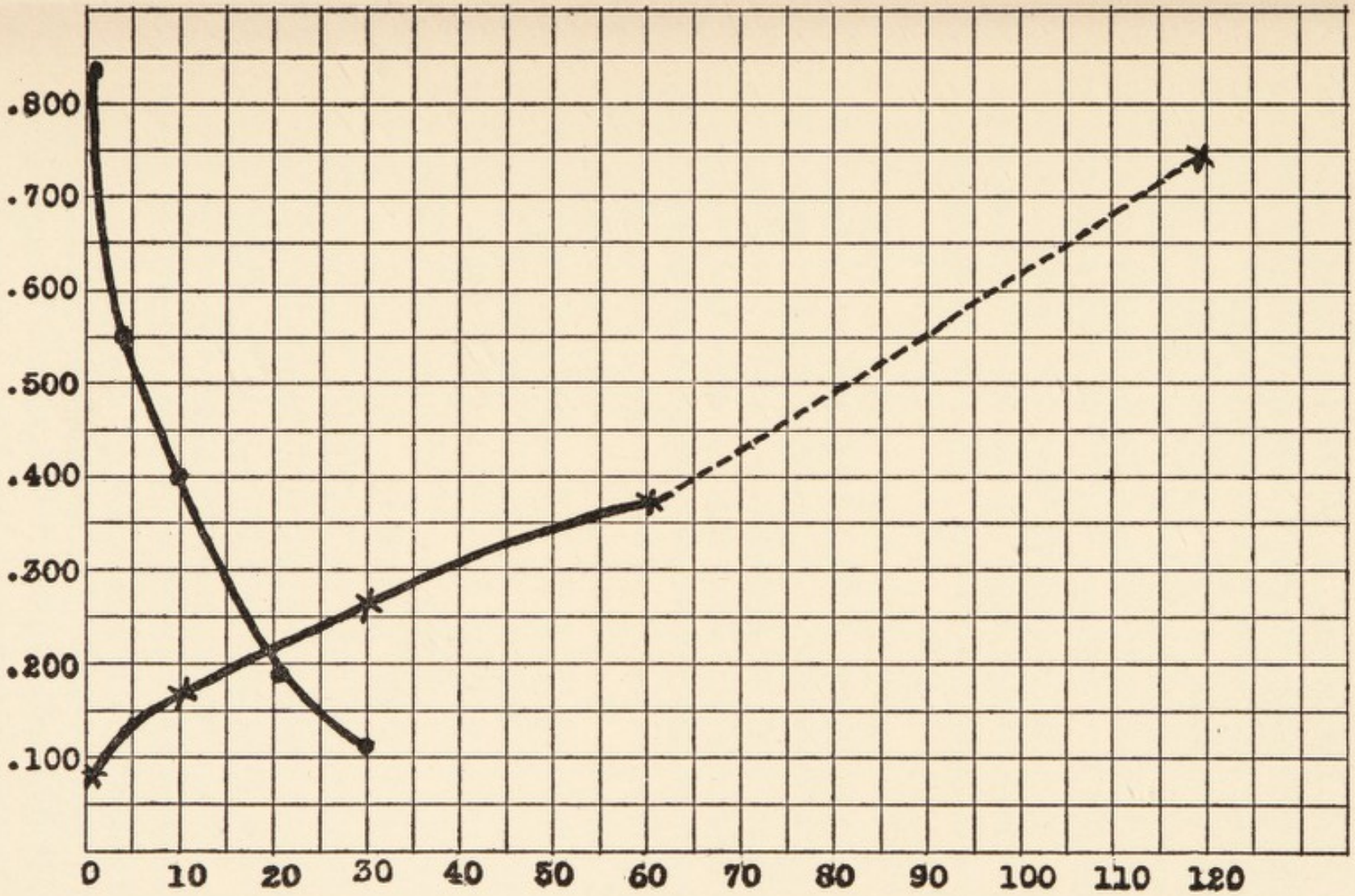


FIGURE 3. Curves showing comparative rate at which glycogen disappears (upper curve) and lactic acid accumulates (lower curve) in previously frozen mammalian muscle (cat) kept in phosphate buffer solution under nitrogen at about 3° C. The last determination of lactic acid was made on muscle that had been kept at 21° C. for 2 hours. The figures on the ordinates give the percentages of glycogen as glucose and the percentages of lactic acid. (Macleod and Thomas)

the glucose accumulated almost in proportion to the glycogen which disappeared, the differences which were actually observed being probably due to the technical difficulty of extracting all the free glucose from tissue.

If we may assume that these differences in the process of *post mortem* glycogenolysis in liver and muscle bear a relationship to what is occurring during life, we have striking evidence of a fundamental difference in the fate of glycogen in the two tissues. In the liver it is converted into glucose which is discharged into the blood, whereas in the muscle it is converted, through some unknown intermediaries, into lactic acid.

The derivation of blood sugar from glycogen already present in the liver. We may now return to the question of the source of the blood sugar, and first of all let us consider it when abundance of glycogen is present in the liver. In this case there is little doubt that the sugar is derived from the glycogen, since it becomes excessive when this is caused to break down rapidly, as in the various forms of experimental diabetes. These may be grouped in asphyxial, nervous, and hormonal, and the best known and the one of the greatest historic interest, because of the fact that its discovery in 1856 by Claude Bernard opened up new fields of research in experimental medicine, being that which follows puncture of the floor of the fourth ventricle of the brain (piqûre). Within an hour after the puncture the blood sugar rises to 3 or 4 times the normal level (hyperglycaemia) and sugar soon appears in the urine (glycosuria). These diabetic symptoms last for several hours and after they have subsided the glycogen content of the liver is found to be less than that usually present in similarly fed normal rabbits. Bernard showed that piqûre may have very little effect when it is performed on rabbits in which the stores of glycogen are reduced by starvation, etc.,

and Stewart and Rogoff have recently confirmed this finding.

The piqûre acts by setting up irritative stimulation of the nerve centers of the medulla, and the impulses are transmitted to the liver by way of the great splanchnic nerves. Hyperglycaemia may therefore be caused by electrical stimulation of these nerves, but so far as I am aware no one has shown whether its occurrence depends on the presence of glycogen in the liver.

The hyperglycaemia of asphyxia is believed to depend on the presence of glycogen in the liver (Stewart and Rogoff), but in that due to hormones, such as epinephrin (the hormone of the medulla of the adrenal gland), the relationship is more complex. That the degrees of hyperglycaemia and glycosuria which become developed following the injection of epinephrin are more or less proportional to the amount of glycogen in the liver, has indeed been noted by various observers (Ritzmann, Pollak, Bang), and Markowitz has shown that this hormone causes little or no increase in blood sugar when injected, even in large doses, into rabbits in which only doubtful traces of hepatic glycogen can be found. But, on the other hand, when epinephrin is injected into rabbits in which the liver has previously been robbed of all traces of glycogen, by the combined influence of fasting and strychnine convulsions, glycogen re-accumulates, and when the epinephrin injections are repeated pronounced hyperglycaemia follows each injection (Pollak, Markowitz). Even when this is done on successive days for a week considerable percentages of glycogen may accumulate in the liver, the muscles and the heart (Markowitz). The results of four experiments of this nature are shown in Table III. These results confirm those previously obtained by Pollak, and they show us that epinephrin acts, not only by exciting a rapid breakdown of whatever glycogen may already be present in the liver, but

TABLE III

Glycogen in Rabbits after Repeated Injections of Epinephrin

NO. OF ANIMAL	NO. OF DAYS ON EACH OF WHICH FROM 3-5 CC. 1-1000 ADRENALIN INJECTED IN FREQUENT DOSES	GLYCOGEN			REMARKS
		LIVER	HEART	MUSCLE	
		PERCENT	PERCENT	PERCENT	
7	5	0.331	0.536	0.141	Prior to injection food withheld for 5 days and strychnine injected
28	8	0.161	0.234	0.133	" (4 days)
8	5	0.98	0.44	0.19	" (5 days)
22	9	1.05	0.100	0.265	" (5 days)

also by causing new glycogen to be formed out of other materials. In view of the long-continued nature of these experiments there can be little doubt that fat was the most important source of the glycogen.

That most of those who have studied experimental hyperglycaemia are agreed that the rise in blood sugar is related to the glycogen content of the liver, does not necessarily prove that it is from this same source that sugar is provided to prevent the blood sugar from falling below a certain minimum in the normal animal. We must, therefore, seek for other evidence as to whether a decline in blood sugar below the physiological level can in itself stimulate increased glycogenolysis.

When the blood sugar is being rapidly used up in the muscles the amount of glycogen in the liver can be shown to diminish. This can be satisfactorily demonstrated by comparing the rate at which glycogen disappears from the liver over a period of fasting in one group of animals, with that in another group in which muscular exercise is also performed. Such comparisons can never yield results of any quantitative value, since we cannot be certain that the amounts of glycogen in the liver to start with are the same

in the two groups of animals and it is extremely difficult to induce equal degrees of exercise in the different animals. Nevertheless, from a qualitative standpoint, experiments of this nature clearly demonstrate that liver glycogen is rapidly used up during muscular activity, and it will be sufficient here if we quote only one or two examples.

Bendix withheld food from dogs for several days and then made them perform muscular exercise on a treadmill for some hours, after which they were killed. No glycogen could be detected in the liver although some was found in the muscles. Frentzel injected rabbits, from which food had been withheld for a couple of days, with sufficient strychnine to induce convulsions and found that the liver became free of glycogen, provided the convulsions had lasted for about five hours. Prolonged shivering also has this effect, this being the method which Graham Lusk and his collaborators have used in their studies on the origin of the urinary sugar in phlorhizin diabetes (Lusk: *Science of Nutrition*). When it is remembered that starvation alone does not rid the liver of glycogen, then it is clear from these results that muscular exercise must cause this substance to be broken down into glucose so rapidly, in comparison with its new formation, that none is left over after death. It is not until some hours after the muscular exercise has been discontinued that glycogen reappears in the liver, which indicates that the muscles must continue their demands for fresh supplies of glucose with which to replace the glycogen which was used up during their increased activity (Frentzel).

Evidence of another type, showing that liver glycogen is the source of the blood sugar, is furnished by observing the effects of insulin in relationship to the amount of glycogen in the liver. Thus, it is well known that a certain dose of this hormone causes the blood sugar to fall less, and to remain depressed for a shorter time, in animals which

have been feeding on carbohydrate-rich food up to the time of injection, than in those from which food has been withheld for one or two days, and, as is shown in the curves of Fig. 4, these differences can be related to the percentage

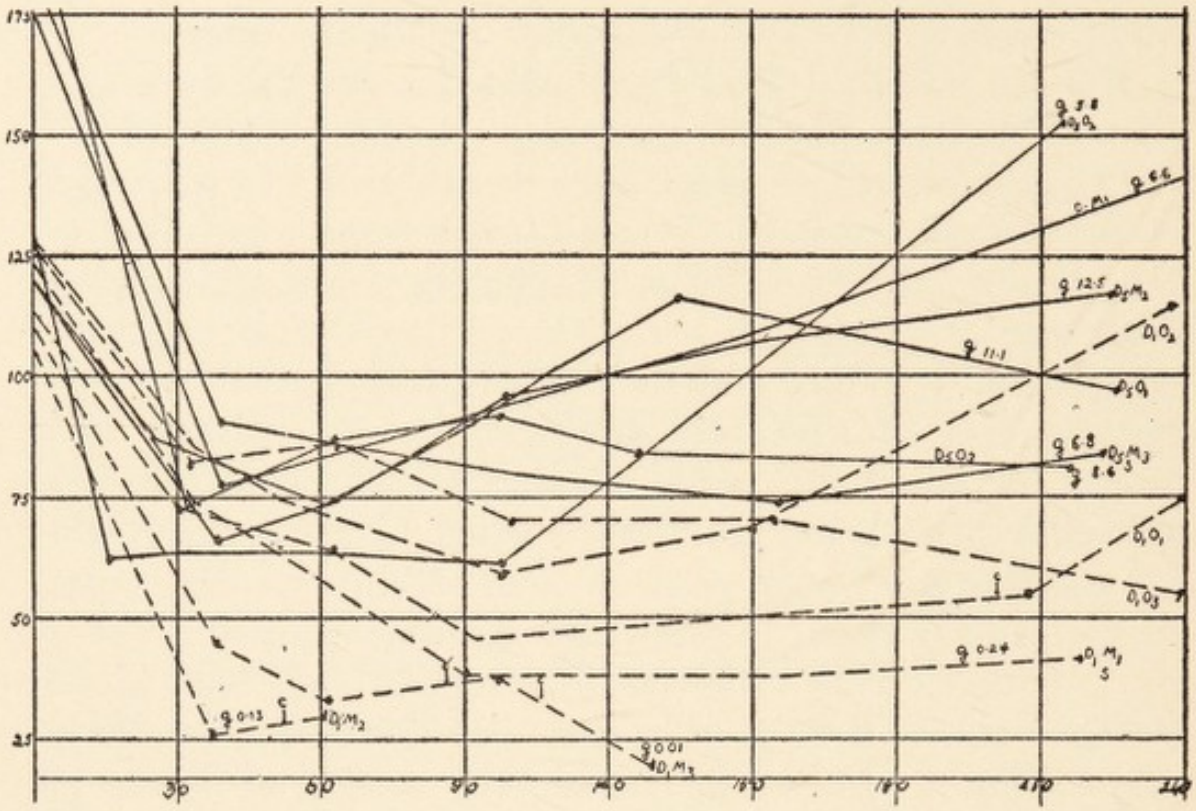


FIGURE 4. Curves showing effect of equal doses of insulin on well-fed (continuous lines) and hungering rabbits (dotted lines). The percentages of glycogen found present in the livers of the various animals are indicated by the figures (g) on the curves, C indicates convulsions. (McCormick, Noble, etc.)

of glycogen in the liver (McCormick and Macleod). It must be pointed out, however, that these effects of feeding are only observed when this is continued up to the time of injection. If food be withheld for a short time prior to the injection (24 hours) in previously well-fed rabbits the fall in blood sugar, in response to equal doses of insulin, is more marked than in rabbits previously starved for several days (Tiitso). This is shown in the curves of Fig. 5. Carbohydrate feeding for some days, therefore, increases the sensitiveness of the animal towards insulin, but at the

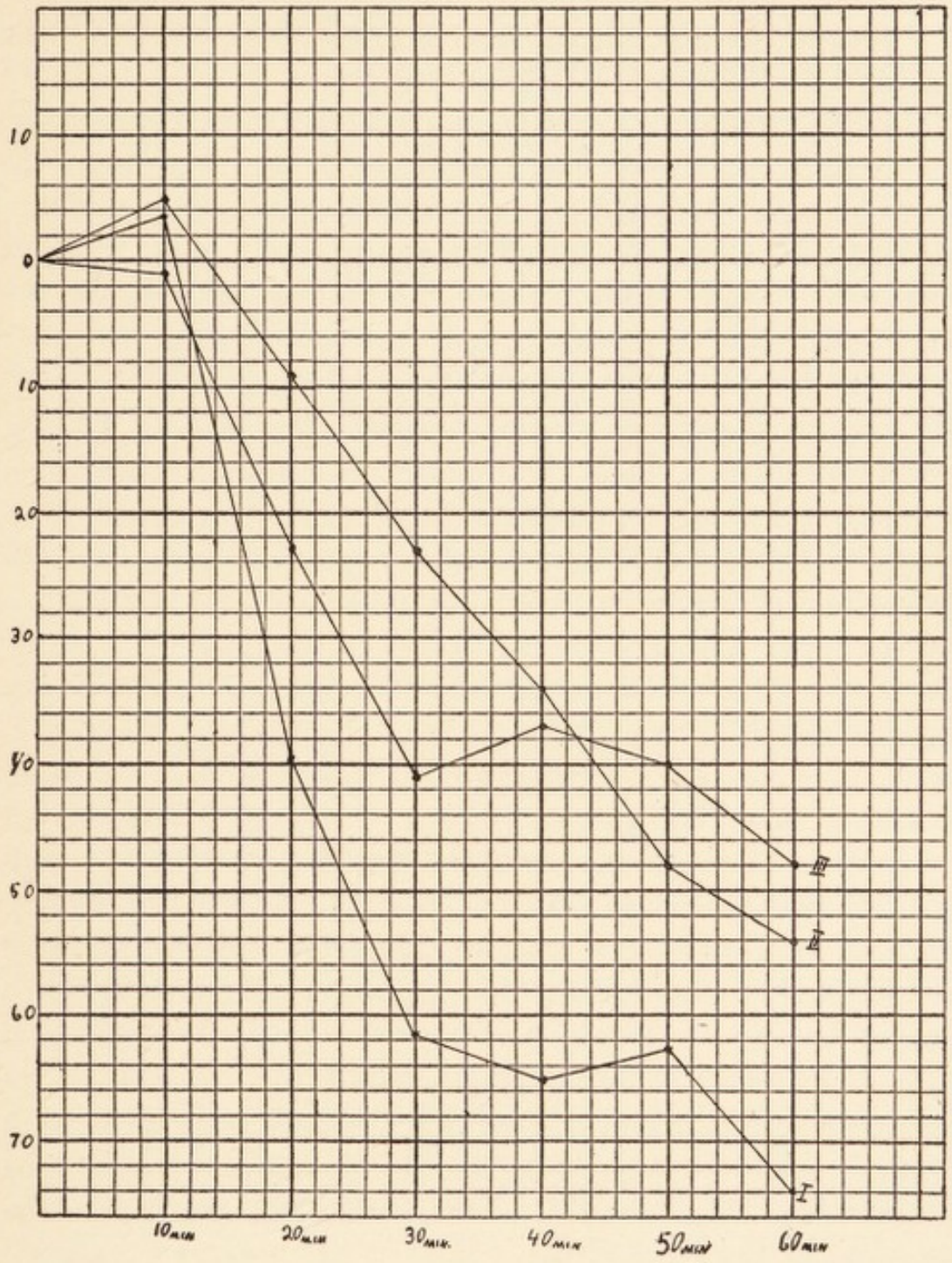


FIGURE 5. The ordinates indicate fall in blood sugar in mg. per 100 cc. blood; the abscissae, time in 10-minute intervals. The curves show the average blood sugar: I, in 7 rabbits fed with carrots; II, in 7 rabbits from which food was withheld for one week; III, in 3 rabbits similarly treated for two weeks. All results are brought to the same value to start with. (Tiitso)

same time the recovery process is more rapid than in starved ones because plenty of glycogen is available. (See also Page and Abderhalden and Wertheimer.)

The hypoglycaemic symptoms also differ in type in the two conditions. In well-fed animals they are ushered in after two or three hours by convulsions which are very violent and last, with intermissions, for several hours, without much fall in body temperature. In fasted animals, on the other hand, the convulsions appear earlier, and although they may be quite violent at first they soon become decidedly feeble, being replaced by coma and quickly falling body temperature. It is clear from these experiments that ample stores of glycogen in the liver, although they do not delay the initial fall in blood sugar, soon counteract the fall by furnishing sugar to take the place of that which has disappeared. When the convulsions continue for some time, as in well-fed animals, the glycogen diminishes in the liver and still more so in the muscles (Dudley and Marrian), but it does not entirely disappear from the latter unless the animal has died in coma (Chaikoff). Insulin also causes a reduction of the liver glycogen without convulsions appearing, as was first of all clearly shown by Cori and Cori, and then by Barbour, Chaikoff, Macleod and Orr, and by others. Curves illustrating this influence of subconvulsive doses of insulin on standard white rats are shown in Fig. 6. Since the insulin was injected while the animals were absorbing carbohydrate, the blood sugar only fell to a moderate extent—not nearly to the convulsive level—and nevertheless it can be seen that much less glycogen was deposited in the liver than in that of the controls, indicating that some of the absorbed sugar was being used to maintain the percentage in the blood.

These researches show that when the blood sugar tends to fall below a certain level, either because of increased demands for sugar by the muscles, or because of the effects

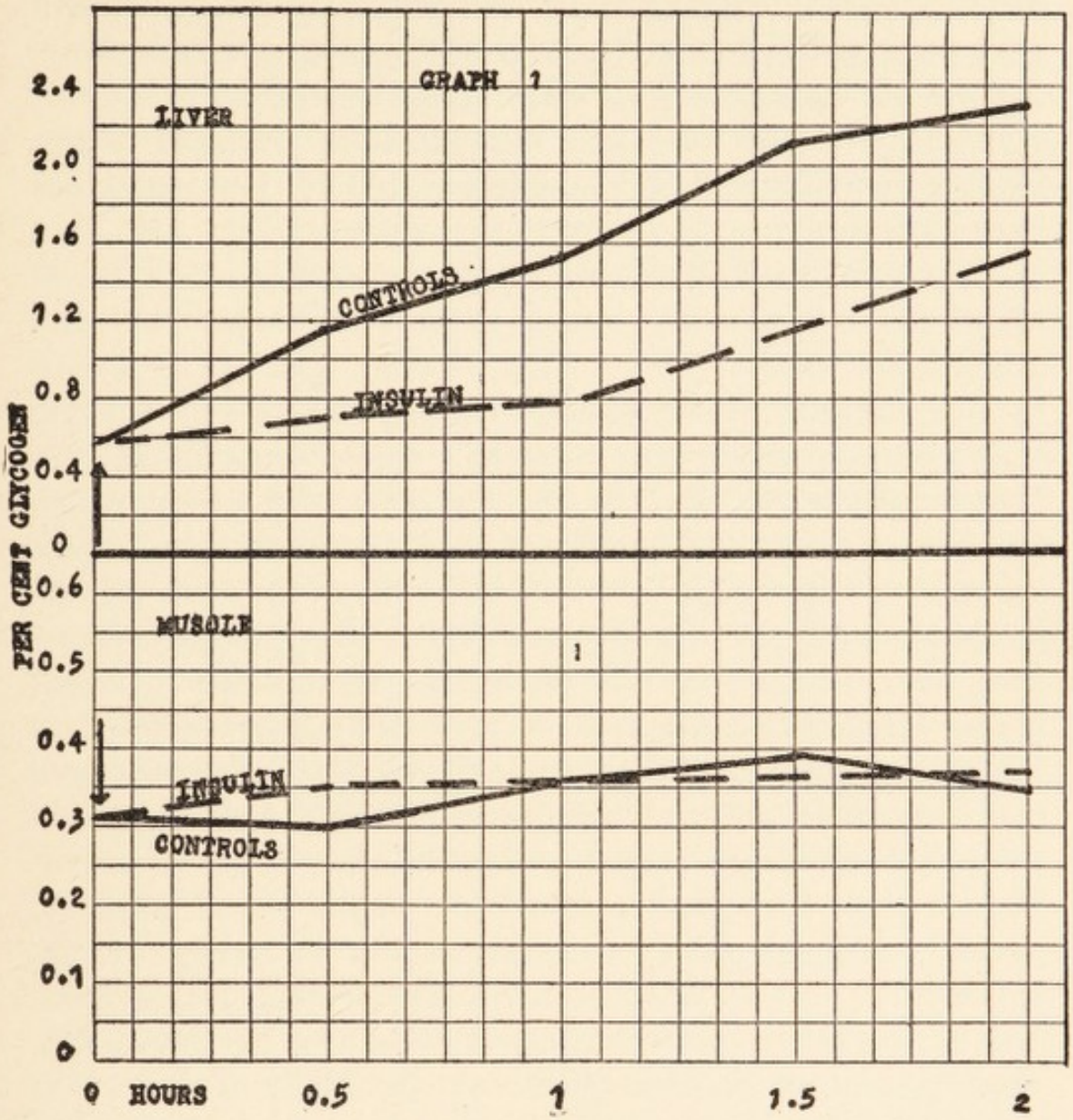


FIGURE 6. A comparison between the deposition of glycogen in fed normal rats and in fed rats which received a dose of insulin of such strength (2 or 3 units per kilogram) as to prevent postprandial hyperglycaemia, but not to produce hypoglycaemia. At zero time, when insulin was injected, as indicated by the arrow, all the animals had been digesting food for one hour.

of insulin, sugar is discharged in large amounts from the liver, where it is produced out of glycogen.

In yet another way may the behavior of animals injected with insulin be used to demonstrate the importance of the liver glycogen in maintaining the blood sugar. On account

of its effect in raising the blood-sugar level, epinephrin may be used to prevent the hypoglycaemia and to antidote the convulsions due to insulin, but it is effective in this latter regard provided only that the liver contains plenty of glycogen. Thus, Soskin has found that epinephrin causes very little recovery in blood sugar when injected into previously fasted rabbits showing hypoglycaemia as a result of insulin, whereas the rise is pronounced when well-fed animals are used.

The stimulus which excites the glycogenolytic process in insulin hypoglycaemia cannot be set up immediately the blood sugar begins to fall, for, if it were, the rate at which this occurs would not be alike in recently fed and in fasting animals. Cannon, McIver and Bliss think that this stimulus takes the form of a hypersecretion of epinephrin, since they have found that the slight temporary rise in blood sugar which is often seen to occur in well-fed rabbits when the lowest point in the curve has been reached, and which can be seen in some of the curves of Fig. 4, is absent in animals from which the adrenal glands have been removed. It is also known that restoration of the blood sugar is greatly delayed after removal of the influence of the splanchnic nerves, which control the glycogenolytic process of the liver. This was first of all shown by Burn, who used ergotamin to block the nerve pathway. In recently fed rabbits under the influence of this drug, the blood sugar following insulin behaves as it does in a starved animal. It fails to rise because glycogenolysis is not stimulated.

And now we come to the central problem of diabetes, namely, where does the excess of sugar come from when the glycogen stores of the liver are empty, or almost empty?

Origin of blood sugar when glycogen is absent from the liver. Pancreatic diabetes. It has been remarked that the

discovery of piqûre diabetes, in 1856, by Claude Bernard marks the beginning of the experimental investigation of the problems of animal metabolism. Prior to this date some rough idea of the general nature of the disturbance of metabolism which causes diabetes mellitus in man had been obtained, by observing that the amount of sugar excreted in the urine became greatly increased when starchy and saccharine foods were taken. This showed that the ability of the organism to utilize carbohydrates had been interfered with, but no useful hypothesis was offered to account for the change. The only method which the physician could adopt, in order to throw light on the problem of the cause of diabetes, was that offered by pathological examination of patients who had succumbed to the disease. One important outcome of this work was the discovery, due largely to Bouchardat, that the pancreas in diabetes is often the seat of conspicuous morbid changes. Realizing, even in those early days of medical research, that progress in the investigation of the causes of disease is best assured when its manifestations can be reproduced in laboratory animals, attempts were made to destroy the pancreas by experimental means, but without any one succeeding in thereby causing the animals to become diabetic.

Although in itself Bernard's discovery of piqûre did not serve to throw any very clear light on the underlying cause of diabetes in man, it drew attention to a possible nervous factor in the etiology of this disease, and a few cases in which glycosuria was the main symptom were found to be associated with recognizable lesions involving the nervous system, such as brain tumors or injuries, while in other cases a family history of neurosis was encountered, or some form of nerve strain could be associated with the onset of more severe symptoms. Otherwise Bernard's discovery was not of any direct assistance to the clinical investigator and both he and others realized that the real cause of diabetes

must be related to the occurrence of morbid changes in the pancreas. But it was not until 1889 that the great discovery was made in Strassbourg by Mehring and Minkowski that complete extirpation of this gland, in dogs, is followed by diabetes of a type more severe in its symptoms and more quickly fatal than anything met with in clinical practice. Pasteur has said that "Fortune favors the mind that is prepared," and so it was in the case of Mehring and Minkowski; they had extirpated the pancreas for the purpose of seeing whether a certain fat, "lipanin," could be absorbed in the absence of the pancreas; polyuria and glycosuria became prominent symptoms after the operation, and they realized that they had discovered the experimental prototype of diabetes in man. This discovery made it possible to transfer the investigation of the underlying cause of the disease, and therefore of its rational treatment, from the limited opportunities of the clinic to the much more controllable ones of the laboratory. During the next few years Minkowski undertook the intensive investigation of the experimental disease and, in general, his problems were of two categories: to discover the reason why removal of the pancreas causes diabetes and to explain the nature of the chemical processes which are responsible for its symptoms. The first of these problems does not concern us here, the second, however, bears directly on the question of our present enquiry.

At the outset it was found that there is a fundamental difference between pancreatic and piqûre diabetes. Following piqûre, as we have seen, the excretion of sugar ceases after a time, presumably when all the glycogen has disappeared from the liver, and it does not occur, or only does so in mild degree when the piqûre is performed on a fasted animal; following pancreatectomy, on the other hand, the excretion of sugar continues after all glycogen has disappeared from the liver, and even when the animal

is deprived of food, or is being fed exclusively on fats and proteins.

In the light of these facts Minkowski recognized that sugar must be formed anew in the diabetic organism, and he sought to determine its source, whether from protein or fat. At first sight it might appear that a direct answer to this question could be furnished by observing the extent to which feeding with these foodstuffs influences the excretion of sugar. When sugar itself was given to the diabetic animals it all reappeared in the urine; that is to say, the excretion of glucose during the hours following the ingestion of the sugar was increased by an amount equal to that which had been given. The sugar had apparently passed through the body unchanged, and Minkowski considered that loss of the power to oxidize carbohydrate must be the fundamental metabolic fault responsible for the diabetes. When fat was given there was no change in the sugar excretion, from which it was concluded that this foodstuff is not changed into sugar, but is directly oxidized. When protein was given the sugar excretion was increased, and although this did not occur to the same extent as with carbohydrate, yet there could be no doubt that the ingested protein furnished at least some of the extra sugar appearing in the urine.

The G:N ratio immediately following pancreatectomy. The question now narrowed itself down as to whether the protein of the tissues of the diabetic animal could be the source of the sugar which continues to be excreted during starvation, and a practical method for finding its solution was furnished by comparing the excretion of nitrogen with that of sugar. The principle of this method is perfectly clear: protein is the only possible source of nitrogen in the body and if it should also be the only source of glucose, then the ratio between glucose and nitrogen in the urine in diabetes, the G:N ratio as it is called, should remain

constant under all degrees of variation in the excretion of nitrogen.

In several depancreatized dogs, which were kept in metabolism cages, so that the total urine voided every 24 hours could be collected, it was found that the G:N ratio fell rather rapidly during each successive day following the pancreatectomy, until after about four days, when it reached a level at which it remained tolerably constant for a week or so, finally again falling rapidly during the days preceding death, which usually occurred in from two to three weeks. The average of the ratios observed during the period when these were fairly constant, in several meat-fed dogs, was 2.8:1, and it was concluded that this represents the extent to which glucose is derived from protein in the diabetic animal. This means that 100 gm. of protein will yield 44.8 gm. of glucose, assuming protein to contain 16 per cent of nitrogen. If, on the other hand, all the carbon of protein except that required to form urea were excreted as glucose, a ratio of 6.61 would be obtained, or 100 gm. of protein would yield 110 gm. of glucose. In accepting a ratio of 2.8 it is therefore assumed, either that glucose is produced from a part only of the protein carbon, and is excreted because it cannot be oxidized by the tissues, or that all this carbon is converted into glucose, but only a constant proportion of that produced is oxidized. The higher ratios of the first few days following the pancreatectomy are no doubt due to the excessive conversion into glucose of the glycogen stored in the body prior to the pancreatectomy, but the low ratios of the terminal stages can not be adequately accounted for. When the depancreatized animals were starved the G:N ratio was only sometimes observed to remain near the 2.8 level.

These results have not until recently been seriously called in question, and the G:N ratio of 2.8 has been accepted as indicating the extent to which the protein, both of

the food and of the tissues, can be converted into sugar in pancreatic diabetes.

As we shall see later, a ratio of 3.65 is obtained in dogs which are repeatedly injected with the glucoside, phlorhizin, and, assuming that in the condition which is thus established the animals are diabetic, it has been customary, in this country especially, to consider that 58 per cent, rather than 45 per cent, of protein is changed by the diabetic animal into glucose and excreted as such. That this also applies in human diabetes has apparently been justified by the fact, observed by S. R. Benedict, that a similar ratio became established in a man (suffering from cancer but not diabetic) who was brought fully under the influence of phlorhizin. We shall return to a fuller discussion of this so-called phlorhizin diabetes later, and we allude to it here only in order that a correct idea may be formed of the evidence upon which is based the present-day doctrine, that sugar can come only from protein in the diabetic animal, a doctrine which forms the basis of the dietetic treatment of this disease in man at the present time.

To return to the ratio in depancreatized animals, it is to be noted that Minkowski's results were not entirely confirmed by other observers. In 1905, both Pflüger and Embden and Salomon obtained lower ratios, and higher ones were sometimes observed when the influence of the pancreas was only gradually removed as occurs when a small portion of the gland is left in the body. Under these conditions, diabetes does not, as a rule, develop until after several months. It then gets more and more severe and for some time the G:N ratio may attain to about Minkowski's level, while the animal is being fed exclusively on meat, although later on it may rise much higher.

Among recent observations may be mentioned those of Falkenhausen, who records the ratios in three depancreatized dogs of varying sizes. On each day, from the third to

the ninth, following pancreatectomy ratios very close to 2.8 were observed in the smallest animal, but on the tenth day the ratio fell to 2.15; in a medium-sized animal ratios approaching Minkowski's were observed on the third, fourth, and fifth post-operative days, and this was also the case in a large dog on the second, third, and fourth days. In the two last mentioned dogs the ratios on later days are not recorded. In two other medium-sized animals, ratios near to 2.8 were observed on the third and fourth days following pancreatectomy. In so far as they go these results confirm Minkowski's, in showing that an average ratio of about 2.8 is to be expected during several days following pancreatectomy in the ordinary run of laboratory dogs. The ratios of the first few days after the operation were much below 2.8 in von Falkenhausen's animals, indicating that there can have been no glycogen reserves and consequently, that the preliminary feeding must have been somewhat scanty. This makes the results of questionable value.

A study of the metabolism of dogs immediately following removal of the pancreas is complicated by the fact that the extensive abdominal wound does not heal. Owing to the diabetic state which becomes established it is difficult to prevent some suppuration and the formation of stitch abscesses, and, partly on this account and partly because of the general after effects of a severe, prolonged abdominal operation, the animal, for some days at least, must be considered as suffering not only from the absence of pancreas but also from the toxic influence of damaged tissue. Another serious objection to the use of recently depancreatized dogs for studies in metabolism, and especially for determining whether carbohydrate may be produced from fatty acid, is that fattened animals seldom survive the operation for more than a few days, so that they are not, as a rule, chosen for the operation.

These objections to considering the value of the G:N ratio of recently depancreatized animals as exclusively dependent on the diabetic state can be circumvented by using depancreatized animals after they have been treated for some time with insulin, but before we consider the behavior of the ratio under these conditions let me refer briefly to our experience with animals treated in this way.

Administration of insulin immediately following pancreatectomy causes rapid healing of the wounds, and repetition of the injections twice daily soon brings the dogs into a condition in every way like that of a normal animal. Sooner or later, however, in animals fed on meat alone it was found that the body weight began to fall, and since this was clearly due to inadequate digestion, because of absence of the pancreatic juice, it was decided to add cane sugar to the daily diet, along with sufficiently more insulin to take care of the extra carbohydrate, with the result that the animals immediately gained in weight.

Although the treated animals now maintained their weight, or even gained somewhat, they did not survive the pancreatectomy for very long. Several died after two months or less, two survived to four months and one to seven. The first symptom of impending disaster was refusal to take food, then vomiting and jaundice (bile in the urine and in one or two cases yellow tinting of the sclera) set in, soon followed by extreme muscular weakness and hyperpyrexia ending usually in death within about four days from the first appearance of the symptoms. Excessive fatty infiltration of the liver was apparently responsible for the jaundice, at least we found so much fat present that very few liver cells could be seen, microscopically, that were not almost completely filled with it.

Now, it is well known that fatty changes in the liver are frequently the result of toxic damage of its cells (as

after poisoning by chloroform, phosphorus or arsenic), and it was considered possible that this might have occurred in our dogs on account of the presence in the blood of imperfectly digested protein split-products. On the supposition that these were produced by the action of putrefactive microorganisms growing excessively in the intestine owing to the absence of the pancreatic ferments, raw pancreas was added to the diet, in the hope that a sufficiency of its ferments might gain the intestine in an active state to restore digestion to its normal course. This has apparently been realized for, since raw pancreas has become a regular ingredient of the diet, the animals have remained in good health, without any symptoms of jaundice and with faeces normal in character, instead of being very putrid, as was the case before pancreas was given. Two dogs that were depancreatized over four years ago (November 1923 and January 1924) and were treated in the manner I have just outlined remained in an excellent condition of bodily nutrition until their death, which occurred in February of this year (1928) as a result of discontinuing the use of insulin. It is of importance to call attention to the fact that these two animals showed no signs of arterial disease or of any other degenerative changes, although, owing to the sugar that was given, hyperglycaemia and glycosuria were persistently present during the four years. This would seem to contradict the view held by some physicians that the arterio sclerosis, the cataract and the gangrene and other degenerative changes, so commonly observed in long-standing cases of diabetes in man, are an effect of the excess of sugar in the body fluids. Notwithstanding the harmless influence of sugar on the tissues it should undoubtedly be kept within normal limits in diabetes in man for another reason, namely, so that the partially diseased pancreatic structures which secrete insulin may not be overtaxed.

Hédon, who has been one of the most prominent and successful investigators of experimental diabetes, has also found that it is necessary to add pancreas to the diet of depancreatized animals treated with insulin, in order to maintain them in health, although Penau and Simmonet deny that this is necessary. Mr. Hershey, working in my laboratory, is investigating the possibility that other substances may be substituted for pancreas in the diet of these animals, but as yet we can draw no conclusions. The question as to whether the presence of the pancreas is necessary for health is an old one, dating back to the time of Conrad Brunner (cf. Michael Foster's *History of Physiology*).

This digression has been necessary so that the exact condition of the depancreatized animals which have been used by us for studying the problems of diabetes may be properly understood. It is important also to note that most depancreatized animals may be brought into any degree of "fatness," by increasing or diminishing the amount of sugar with which they are fed along with corresponding adjustment of the insulin dosage.

The general effects of discontinuing insulin. After discontinuing the use of insulin and food the hyperglycaemia and glycosuria become maximal or, if only a part of the insulin be removed, any desired degree of diabetes can be induced. This form of incomplete diabetes is more suitable for the investigation of certain problems of metabolism, some of which we will consider in another lecture, than that set up by leaving a portion of pancreas in the body, because it is constant in intensity from day to day.

In using these animals for studies in diabetes, the amounts of nitrogen and glucose excreted by the urine in 24 hours and the respiratory exchange are measured. The latter tells us whether the diabetes is complete; when it is so the respiratory quotient which, let me remind you, is the ratio between the volumes of CO_2 exhaled and of O_2 re-

tained by the body, falls to 0.66-0.68 and usually remains at this level after the ingestion of glucose (20-40 gm.), of which practically all that is given can be recovered in the urine.

Every animal used for such investigations should also be carefully examined after death to see whether all of the pancreas has been removed, and in doing this it will often be found that nodules of pancreas occur embedded in the wall of the duodenum near the pancreatic ducts. These cannot be removed by operation, but there is no evidence to indicate that they secrete any insulin. D. J. Bowie, as a result of the most careful searching, of numerous sections of the nodules by microscopic methods, has never been able to detect any islet cells in them. They have been found to occur as frequently, and to be as large, in normal (non-depancreatized) dogs as in those kept alive by insulin for some months after pancreatectomy, and we believe that the claim made by Fisher that regeneration of islet tissue occurs in the walls of the duodenum under these conditions is entirely unwarranted. The question is very important from a practical standpoint since, if Fisher were correct, it would mean that a completely depancreatized animal might recover from diabetes by secreting insulin from regenerated islet tissue. We deny that this can occur, for the two depancreatized dogs that lived for over four years under insulin treatment gave evidence of being just as diabetic at the time of their deaths as during the first year following pancreatectomy. There was an islet-free pancreatic nodule in the duodenum of one of them, but none in the other. This conclusion does not imply that some regeneration of islet tissue may not occur in diabetes mellitus in man, where even in severe cases there must remain *some* undamaged islet tissue from which more may be derived. That regeneration possibly occurs, especially in

young subjects, is supported by the observations of Dr. Gladys Boyd.

The G:N ratio after discontinuing insulin. But this digression has taken us away from the main question, namely, whether the behavior of the G:N ratios of diabetic animals in varying states of bodily nutrition, supports the commonly accepted view that protein is the only source of sugar after all available stores of carbohydrate have been used up. Let us now return to that question. Markowitz and I observed the G:N ratio in 10 dogs each of which had been depancreatized about two months previously. The urinary analyses were not, as a rule, started until the third day after withdrawal of insulin and food, by which time it had been found, in other similarly treated animals, that practically all glycogen has disappeared from the liver and that the respiratory quotient remains at the level of 0.66-0.68, both before and after ingestion of sugar. Table IV shows the results.

TABLE IV

G:N Ratios of Dogs Depancreatized about Two Months Previously

NO.	WT. KG.	DAYS AFTER REMOVAL OF INSULIN. NITROGEN IN GRAMS														AVERAGE BEYOND 2ND DAY
		II		III		IV		V		VI		VII		VIII		
		N	G:N	N	G:N	N	G:N	N	G:N	N	G:N	N	G:N	N	G:N	
1	4.1	.....	.....	4.5	2.4	3.9	1.8	3.0	2.0	3.0	1.8	3.7	1.7	.....	.....	1.94
2	7.3	.....	.....	2.6	8.8	3.8	2.8	.....	.....	.....	.....	.....	.....	.....	.....	5.8
3	7.6	.....	.....	4.2	10.4	6.4	4.9	2.9	5.2	.....	.....	.....	.....	.....	.....	6.8
4	5.9	.....	.....	.....	.....	.....	.....	2.7	3.3	3.8	2.4	4.3	3.1	.....	.....	2.93
5	5.7	.....	.....	.....	.....	.....	.....	2.4	5.1	2.4	2.5	2.5	1.0	.....	.....	2.86
6	6.3	.....	.....	.....	.....	.....	.....	3.9	3.6	4.8	3.2	4.9	2.5	.....	.....	3.1
7	5.4	.....	.....	.....	.....	.....	.....	4.4	5.9	5.3	3.6	5.5	3.3	.....	.....	4.3
8	3.9	.....	.....	2.3	18.1	2.9	3.9	3.4	2.5	.....	.....	.....	.....	.....	.....	8.17
9	9.2	6.9	6.8	8.5	4.9	6.9	3.6	4.7	3.6	5.6	2.8	9.6	2.6	4.6	2.1	3.26
10	4.5	.....	.....	4.6	2.6	5.0	2.7	6.5	2.0	.....	.....	.....	.....	.....	.....	2.4

The excretion of nitrogen is recorded, as well as the G:N ratio, and it shows, in the majority of the animals, that increasing quantities of protein were being metabolized

from day to day. In practically all cases the ratio fell throughout the periods of observation, more rapidly at first than later, and in none of them did it become stabilized around 2.8, although the average for several days might work out at about this value, in perhaps about one-half of the animals. There can be no doubt that between the third and fifth days of the diabetes, in dogs 3, 4, 5, 6, 7, and 9, quantities of glucose were excreted that far exceeded what could have come from protein on the supposition that 2.8 represents the level at which the ratio would stand during such a process. Indeed, even if we assume that this ratio might be 3.65, as observed by Lusk, etc., in phlorhizinized animals fed on meat, many of our results indicate a large excess of sugar.

These high ratios cannot possibly be explained as being due to the excretion of glucose from glycogen previously stored in the body of the animal. All observers are agreed that practically no glycogen remains in the liver by the third day following pancreatectomy, and only traces can be found in three days after withdrawal of insulin from depancreatized animals. In illustration, let me quote the results of observations by Chaikoff in which the glycogen of the liver and muscles was determined in depancreatized animals killed either on the third or the fifth day following withdrawal of insulin, and in which respiratory observations were also made so as to make certain that the animals were completely diabetic. The results are shown in Table V. It can be seen that all the animals were diabetic to the full extent, as evidenced by the low respiratory quotients and the entire absence of pancreatic tissue (except in the duodenal wall in one animal). An average of 0.29 per cent of glycogen was found in the livers of the four animals killed on the third day, and one of 0.11 per cent in those of three killed on the fifth. If we assume that the liver constitutes 5 per cent of the body weight, then, on taking

TABLE V

Respiratory Quotient and Liver and Muscle Glycogen of Depancreatized Dogs

DOG NO.	WEIGHT	DATE DEPANCREATIZED	LAST DAY FED 9 A.M.	DAY KILLED 9 A.M.	DURATION OF FASTING	DATE	R.Q.	O_2 PER KG. AND HR.	GLYCOGEN						TOTAL GLYCOGEN IN MUSCLES PER KG.	OBSERVATION OF DUODENUM	NUTRITIONAL CONDITION OF DOG BEFORE DEATH
									LIVER	MUSCLE							
										RIGHT SARTORIUS	RIGHT GRACILIS	LEFT SARTORIUS	LEFT GRACILIS	AVERAGE			
	kg.				days			cc.	per cent	per cent	per cent	per cent	per cent	per cent			
1		Nov. 18	Dec. 14	Dec. 17	3		not done		0.23	0.41	0.47	0.32	0.28	0.37		No pancreas tissue found	
2	6.7	Dec. 11	Dec. 27	Dec. 30	3	Dec. 29	0.703	614	0.32			.....	.....			No pancreas tissue found	
5	7.4	Dec. 20	Jan. 10	Jan. 13	3	Jan. 12	0.662	648	0.26 0.29 0.33 0.35 0.34	0.36 0.39	0.59 0.40	0.42	0.48	0.47 0.42	1.9 1.7	Pancreas tissue within duodenal wall, but no islet tissue	Medium
8	9.1	Jan. 14	Jan. 25	Jan. 28	3	Jan. 27	0.687	631	0.27 0.29 0.28	0.44	0.50	0.41	0.42	0.44	1.75	No pancreas tissue found	Medium
3	5.4	Dec. 11	Dec. 26	Dec. 31	5	Dec. 30	0.682	871	....	0.24	0.23	0.15	0.28	0.23	0.92	No pancreas tissue found	
4	6.9	Dec. 15	Jan. 7	Jan. 12	5	Jan. 10	0.663	645	0.075 0.079 0.077	0.41	0.35		0.56	0.43	1.7	No pancreas tissue found	Thin
6	5.7	Dec. 24	Jan. 19	Jan. 19	5	Jan. 18	0.687	574	0.186 0.19	0.48	.....	0.35	0.59	0.47	1.9	No pancreas tissue found	Very thin
7	10.0	Jan. 12	Jan. 22	Jan. 27	5	Jan. 26	0.679	545	0.063	0.26	0.32	0.23	0.27	0.26	1.00	No pancreas tissue found	

*Muscles, particularly sartorius, were removed along with small amount of adhering fat. Because of rapid breakdown of glycogen in muscle following removal from body (Macleod and Simpson) it was not thought advisable to remove the fat before treating with KOH.

into consideration the weight of the animals, there was, on an average, 1.2 gm. glycogen in the liver of those killed on the third day and 0.4 gm. in those killed on the fifth. This shows that not more than 0.8 gm. of the glucose excreted during the two days elapsing between the third and fifth could have been derived from the liver glycogen.

But, it will be remarked, some of the glucose might have come from the glycogen of the muscles, for although, as we have seen elsewhere, this does not revert to glucose under normal conditions, it might conceivably do so in the diabetic animal. The lactic acid formed from glycogen might, for example, be carried by the blood to the liver, where it could certainly form sugar. Supposing then, for sake of argument, that this occurred, let us see to what extent the glucose thus derived could account for the high quotients. From the average percentages of glycogen actually found in the muscles (see Table V) it can be computed, on the assumption that these constitute 40 per cent of the body weight, that there was about 1.75 gm. muscle glycogen per Kg. body weight in three of the dogs killed on the third day, and 1.4 gm. in the four killed on the fifth day. At most, not more than 0.35 gm. of glucose per Kg. body weight could have been derived from the muscle glycogen during the 48 hours intervening between the third and fifth days, or, if we make the greatest possible allowance for both liver and muscles, 3.5 gm. of glucose might, as a rough average, be accounted for. If we subtract this amount of glucose from that excreted in a typical case it still leaves a large excess to be accounted for. Thus, in a dog weighing 8.6 Kg., Chaikoff found that 52.7 gm. glucose and 9.71 gm. of nitrogen were excreted between the third and the fifth days, giving a G:N ratio of 5.42; subtracting 3.5 gm. of glucose reduces the ratio to 5.06, which still far exceeds the highest ratio which is possible were protein the only source of the sugar (see p. 53). The only conclusion possible is

that the extra sugar must have come from fat. A certain amount of this must, of course, be derived from the glycerol molecule of the fat, and it becomes of interest to see to what extent the ratio is further reduced by making allowance for this source. When this is done in the above example, by taking into consideration the oxygen consumption and the nitrogen excretion of the animal, the G:N ratio falls to 4.0, which still exceeds that possible from protein. (For details of calculation see Chaikoff's paper.)

When every possible allowance is made, therefore, even to the extent of assuming that muscle glycogen can be excreted as glucose, we are left with a ratio which indicates that fatty acid must be a source of the sugar which is excreted in diabetes.[1]

But quite apart from these computations there is abundant evidence to show that the G:N ratio is far from having the constant value which has been attributed to it. Not only does it progressively decline from day to day in a given animal after pancreatectomy and vary in its level in different animals, but its average level may vary in the same animal on different occasions. The last mentioned fact seems to me a most important one and it can readily be demonstrated by using depancreatized animals treated with insulin, the diabetes being brought on by withdrawal of insulin and food. This may be done when the animal has been well fattened by increasing the sugar and insulin and then, after an interval of treatment with insulin without sugar, when it has become emaciated, or the observations may be made in the reverse order. In several observations of this type we have followed the G:N ratio for as many days following the withdrawal of insulin and food as was possible without running the risk of losing the animal on account of the acute symptoms of acidosis. The only

[1] This conclusion presupposes that there are no considerable stores of some carbohydrate intermediary between glucose and glycogen.

TABLE VI

G:N Ratios of Same Depancreatized Dog on Different Occasions

DOG	DATE	WT. KG.	DAYS AFTER REMOVAL OF INSULIN. NITROGEN IN GRAMS												D:N AVERAGE BEYOND 2ND DAY	REMARKS
			III		IV		V		VI		VII		VIII			
			N	G:N	N	G:N	N	G:N	N	G:N	N	G:N	N	G:N		
I	Mar. 17	3.1	2.9	2.8	.....	.....	.....	.....	3.5	1.9	.....	.....	3.0	1.5	2.7	Excess of fed glucose excreted
	May 10	5.9	3.5	6.6	2.8	3.6	3.1	3.2	3.6	2.6	.....	.....	.....	.....	4.3	Fed glucose recovered quantitatively
	June 22	5.5	4.0	5.5	2.6	5.5	5.2	2.8	4.0	3.0	.....	.....	.....	.....	4.2	Died on seventh day after withdrawal of insulin (*Macleod and Markowitz*)
II	Aug. 21	6.4	3.6	4.2	5.5	2.9	5.8	2.9	.....	.....	.....	.....	.....	.....	3.3	
	Sept. 20	8.6	3.8	6.2	5.9	5.0	6.0	6.0	.....	.....	.....	.....	.....	.....	5.7	
III	Oct. 22	5.9	5.7	4.4	6.9	2.9	6.4	2.3	.....	.....	.....	.....	.....	.....	3.2	
	Nov. 14	4.8	3.0	2.8	3.6	3.1	3.4	2.8	.....	.....	.....	.....	.....	.....	2.9	(*Chaikoff*)

difficulty in this experiment is that the animal does not usually survive removal of insulin for more than four or five days when it is in the fattened condition, but we have successfully carried through several observations, the results being shown in Table VI, above. It can be seen that ratios averaging 2.8: 1 were obtained only when the animals were of reduced body weight; when they were fattened the average ratios in 3 out of 4 observations exceeded 3.65.

Sufficient evidence has I think been presented to show that the G:N ratio, in pancreatic diabetes, instead of supporting the view that new sugar can be formed only from protein, proves on the contrary that it must also be derived from other sources. It will be observed in Table VI that the daily excretion of nitrogen was decidedly higher when the animals were fat than when they were thin. This is contrary to expectation—for when less fat is available it would be expected that more protein would be used to furnish sugar—and may possibly be explained by a raising of the basal metabolism, due to the larger quantities of food and insulin with which the fattened animals had previously been treated.

CHAPTER III

EVIDENCE *of gluconeogenesis from fat obtained by studying the energy balance and the R.Q. during muscular work.* In discussing the work of Terroine and his co-workers we saw that when seeds sprout in the darkness those rich in fat lose more energy than those rich in carbohydrate, which is interpreted as indicating that energy is lost in converting fat into carbohydrate for the growing seedling. It will also be recalled that this loss of energy amounted to 23 per cent, which corresponds with the value arrived at by Zuntz, on the assumption that 100 gm. fat forms 191.25 gm. sugar. Although there is some doubt as to the validity of such calculations, it nevertheless becomes of interest, in connection with our present problem, to see if a similar loss of energy occurs in fat-fed animals when extra demands are made for muscular fuel. This can be done by comparing the energy expenditure for the same amount of work performed either on a diet rich in fat or on one rich in carbohydrate. The energy cost of the work should be greater on the fatty diet.

The most suitable animal for such observations is man, and Krogh and Linhard, along with Liljestrand and Andresen, have carried out the necessary observations with very great care. Each of six observed persons lived for some time on a diet composed of a minimum of protein and an excess of either fat or carbohydrate, after which the metabolism was measured, first of all with the subject resting and then while working at a uniform speed on a calibrated bicycle ergometer. This was mounted in a respiratory

cabinet through which a constant current of air was drawn by means of a motor driven meter (Jaquet apparatus), proportionate samples of air being removed for analysis. A known and constant amount of work was performed in each experiment, so that the O_2 consumption and the R.Q. and, therefore, the energy expenditure could be calculated per calorie of work. The net expenditure of energy was obtained by subtracting the resting metabolism from the total metabolism during work, and it was found that the amount expended per unit of work was, on an average, 11 per cent greater on the fat than on the carbohydrate diet. Thus, the net expenditure of energy per calorie of work averaged about 4.6 calories on the fat diet (with an R.Q. of 0.71), and about 4.1 calories on one of carbohydrate (with an R.Q. of 1.00).

In the majority of the subjects fatigue came on earlier and was more intense when the work was performed on the fatty diet. Important differences were also observed in the behavior of the R.Q. during the transition from rest to exercise. With a resting quotient of between 0.8 and 0.9, the change was slight, but when it was below 0.8 the quotient rose, and when above 0.9 it fell, as a result of work.

While the authors are very careful not to draw final conclusions from their results, they nevertheless suggest that these indicate that the proportion of fat to carbohydrate which is catabolized during both rest and work is dependent upon the available supplies of these substances. When fat is abundant it is converted into carbohydrate (giving a resting R.Q. below 0.8), when carbohydrate is abundant it is converted into fat (giving an R.Q. above 0.9), and during exercise these anabolic quotients do not become changed in the same proportion as the quotient of the increased catabolism (i.e., the amount of carbohydrate metabolism of the muscles), this becoming relatively greater with initially low quotients and relatively less with

high ones. But the most important conclusion is "that the experiments cannot be used as evidence to prove that fat must be converted into sugar before being utilized for muscular work." While this is no doubt the proper conclusion to come to, the results certainly do show that fat metabolism is attended by an energy loss which is much greater than in carbohydrate metabolism. Although only 11 per cent of energy was lost when muscular work was performed on fat, instead of 23 per cent as demanded by the Zuntz equation, it must be remembered, in the first place, that feeding with fat does not primarily determine the availability of this substance in metabolism and, in the second place, that the equation used by Zuntz may not really represent the chemical process by which glucose is formed from fat. It seems to me that it might be interesting to repeat these observations on subjects having either a large or a small amount of body fat (Krogh, Linhard, Liljestrand and Andresen).

The value of the respiratory quotient as an indicator of metabolism. When diabetes is fully developed the respiratory quotient remains at a low level, which, after allowance is made for the amount of protein undergoing metabolism, is at, or below, that for the oxidation of fat. This has been considered to show: (1) that the tissues have lost the power to oxidize carbohydrate and, (2) that fatty acid is not converted into carbohydrate. By those who believe that sugar is derived exclusively from protein in diabetes the respiratory quotient is considered to rank with the G:N ratio as supporting evidence.

But, first of all, let us briefly review the facts which are taken to warrant the use of the quotient as a qualitative index of resting metabolism. In normal animals in the fasting condition the quotient is between 0.70 and 0.75. Thus, it stands about this level, both in winter frogs that have been kept in the laboratory without food for several

months and in rabbits and dogs from which food has been withheld for several days (Olmsted and Harvey, Shafer). In man it rapidly falls after food is withheld, reaching to about 0.75 within two days, and then more gradually, with some fluctuations, until by the second week it has declined to about 0.70 (Benedict). Its behavior indicates that the chemical processes, of which the quotient is an expression, must be proceeding with tolerable uniformity. When food is being assimilated the quotient rises, and this is most pronounced with carbohydrates, all forms of which do not, however, have equal effects. Thus, Cathcart and Markowitz found, by analysis of samples of expired air collected in Douglas bags at half-hour intervals, that the rise in the quotient, after taking 50 gm. of various sugars the first thing in the morning (basal conditions), varied according to the particular sugar that was ingested. Since varying quantities of food had been taken on the day preceding each experiment, the basal quotient was not constant, but ranged between 0.78 and 0.85 on different days. The sugars which caused it to rise most markedly, and most promptly (end of first half hour), were the monosaccharides fructose (laevulose), galactose, and the disaccharide, cane sugar. Glucose and maltose had conspicuously less decided effects. Higgins, and Benedict and Carpenter have reported similar observations. It may be of significance that the sugars having the greatest influence were precisely those which Folin and Berglund had found, under similar conditions of administration, to cause the least rise in blood sugar and the most marked increase in the reducing power of the urine. These relationships are very suggestive.

Quotients standing practically at unity are obtained in rabbits and other herbivora when these animals are fed on carbohydrate-rich food. It is clear, however, that the quotient cannot constantly remain at unity, even when nothing but carbohydrates are being fed, because protein metabol-

ism, which gives a quotient of about 0.80, is always going on to a certain extent. In the herbivora this influence of protein is relatively small, but in other types of animals it is sufficient to keep the average quotient below unity, even when the diet is composed exclusively of carbohydrate. To allow for oxidation of protein it is usual, in metabolism experiments, to subtract from the quantities of O_2 and CO_2 actually found to be retained and excreted by the animal, the quantities of each derived from protein, this being ascertained by measuring the nitrogen excretion in the urine. This correction is only of value when the quotient and the nitrogen excretion are simultaneously measured over long periods of time, since otherwise the protein undergoing metabolism during the period when the respiratory exchange was being measured cannot be accurately known.

When protein is given to a previously starving animal the quotient rises to about 0.80, which corresponds with the theoretical quotient for a protein having the composition $C_{72}H_{112}N_{18}O_{22}$. When fat is given the fasting animal the quotient is not affected, but after making allowance for protein metabolized, either in starvation or after feeding with fat, it works out at 0.707, which is therefore considered to be indicative of exclusive fat oxidation. Based on these results, Zuntz and Schumburg, Rubner and Lusk have constructed tables which show, for each quotient between 0.707 and 1.00, the proportion of fat and carbohydrate used by an animal after allowance is made for the protein which has meanwhile been metabolized.

The quotient after pancreatectomy. In diabetes the quotient stands at, or just below, 0.70 during starvation and it is not raised when carbohydrate food is ingested. Thus, in depancreatized dogs previously treated with insulin, as described on page 57, the quotients (uncorrected for protein), which are shown in Table VII were obtained:

TABLE VII

EXPERIMENT	WT. (KG.)	DAYS AFTER DISCONTINUANCE OF FOOD AND INSULIN	R.Q.	REMARKS
M(1)	4.25	2nd	0.67	
		3rd during forenoon	0.66	Dog restless
		during afternoon	0.73	36 gm. sugar at noon
	4.01	5th during forenoon	0.66	Dog restless
		during afternoon	0.67	36 gm. sugar at noon
M(2)	3.63	2nd during forenoon	0.67	Following 17 days of food and insulin
		during afternoon	0.72	36 gm. sugar, dog restless
	3.34	3rd during forenoon	0.66	
		during afternoon	0.67	36. gm. sugar at noon dog restless
L	5.05	2nd	0.74	
		3rd during forenoon	0.67	
	5.00	during afternoon	0.66	36 gm. sugar at noon
1 C & M[1]	4.46	5th	0.69	2 hours' observation
		4th	0.72	2 hours' observation
6 C & M	5.34	5th	0.70	2 hours' observation
		6th	0.70	2 hours' observation
		7th	0.70	2 hours' observation

[1]C & M refer to results taken from paper by Campbell and Markowitz: *Am. Jour. Physiol.*, 1927, LXXX, 561.

It can be seen that the quotients fell to between 0.66 and 0.74 by the second day after withdrawal of insulin and food and did not rise after large amounts of sugar. A very large proportion of the ingested sugar was also recovered in the urine, and we will allude to the significance of this in another connection.

Before we give our reasons for believing that the interpretation which is usually given for these results is unsound, there is one characteristic of the diabetic quotient which requires explanation, namely that it often stands between 0.66 and 0.68, even without allowing for protein, instead of being at least 0.707, the quotient for fat. This is considered to depend on the fact that some of the fatty acid, in place of being completely oxidized, is converted into, and excreted, as the so-called ketone bodies (b-oxybutyric acid, acetoacetic acid and acetone). Chaikoff found that

about 0.3 gm. of ketone bodies per kilo body weight might be excreted during each 24-hour period from the third to the sixth days following withdrawal of insulin and food from depancreatized dogs, the amount rising to 0.46 gm. on the fifth day in one animal. This is only somewhat less than the amounts of these bodies sometimes excreted by patients suffering from acute diabetes, viz., 0.5 to 1.0 gm. As a result of such excretion, a certain proportion of the O_2 which is retained by the animal will not reappear as CO_2 in the expired air, so that the quotient will be lower. Although it is possible to calculate the extent to which the quotient will be thus influenced, it is not so easy in practice to apply the correction, since the ketone excretion, in depancreatized dogs after discontinuing insulin, is not uniform from day to day, but increases up to about the fifth day and then decreases. Supposing that we take the average excretion to be 0.5 gm. per kilo body weight in dogs, the quotient would be depressed by about 0.01, on the basis that an excretion of 40 gm. ketone bodies will depress the quotient by 0.012 in a man of average body weight, 70 Kg. (Magnus-Levy).

Further evidence that the formation of ketone bodies is a factor of little consequence, in accounting for the low quotients, is furnished by the fact that a level of 0.67 may be reached in two days after withdrawal of insulin and food, by which time, however, the ketonuria is only slight; neither does the quotient become depressed from the third to the fifth days in proportion as the ketonuria is rapidly becoming more pronounced.

But of still greater significance than the actual level reached by the quotient is the fact that the ingestion of glucose does not cause it to become raised, at least after the third or fourth days following withdrawal of insulin and food. The interpretation which is usually given for this result, namely that it indicates an inability of the *tissues*

to oxidize carbohydrate, is entirely unwarranted. As we have seen elsewhere, it is often possible in diabetic animals to recover practically all of the ingested sugar in the excreta, indicating that it has not been used by the body, but this does not necessarily mean that failure of the tissues *per se* to oxidize the carbohydrate is the primary cause. It is much more in accord with other known facts of carbohydrate metabolism to suppose, that the ingested glucose fails to be utilized because it is suddenly added to an organism in which sugar production out of protein and fat is already proceeding beyond the powers of the tissues to utilize the sugar. In diabetes there is probably a sluggishness in the formation of glycogen in the muscles out of blood sugar, because this is not suitably prepared for condensation, owing to the lack of insulin. The latter may act either directly on the sugar, or indirectly, in that it facilitates glycogen formation in the liver, the glucose derived from this glycogen being then transformed into muscle glycogen independently of insulin. To account for the hyperglycaemia in diabetes it may be supposed that the form of glucose capable of forming muscle glycogen can be provided only out of certain parts of the molecules of fatty acid or protein, the other forms of glucose, derived from the remaining parts of these molecules, appearing in the blood as unusable glucose. But this is all mere speculation, for every attempt to demonstrate the presence of a physiologically more active form of glucose has ended in failure, as will be shown in the last lecture.

It should here be noted that it is only when the sugar is given to a fasting diabetic animal that practically all of it reappears in the urine. When given along with food, the proportion which reappears in the urine is very variable, in our experience.

Observations on the respiratory metabolism have recently also been made by L. Hédon, who used the method

of Haldane and Pembrey for measurement of the respiratory exchange. In this method the oxygen absorption is determined by subtracting, from the combined weights of water and CO_2 exhaled, the loss of weight of the animal during the period of collection of the respired air. The method is an excellent one in principle, and entirely satisfactory in its practical application to small animals, since in them the changes in body weight can be accurately and quickly measured; but with large ones, such as dogs, this cannot so readily be done without chances of serious error. Nevertheless, Hédon, by using a balance weighing from 0.01 gm. to 10,000 gm. and dogs of relatively small size (5 Kg.), has placed on record some important results.

In normal animals the quotients averaged 0.735 between the fourth and sixth days after withdrawal of food; after pancreatectomy (by the two-stage method elaborated by the author's father, E. Hédon) they fell during the first two or three days following the entire removal of the gland, to 0.72, and then rose slowly, from day to day, until they stood at or above the level observed in normal, starved animals, finally rising very decidedly for a day or two preceding death. In an observation in which the dog was treated for some time with insulin after complete pancreatectomy, the quotients, after discontinuing the insulin were:

0.740	(Food withheld	18	hrs.	and	insulin,	11	hours)
0.712	("	"	18	"	"	"	42	")
0.737	("	"	17	"	"	"	22	")
0.717	("	"	17	"	"	"	45	")
0.736	("	"	18	"	"	"	2	days)
0.716	("	"	26	"	"	"	3	")
0.717	("	"	18	"	"	"	4	")
0.688[1]	("	"	25	"	"	"	4	")

[1]Severe acidosis, alkaline reserve of blood being 11%.

These quotients are in general somewhat higher than those observed in my laboratory, but it will be observed that most of them were taken earlier in the diabetes. In one ob-

servation on the fourth day after withdrawal of insulin a value of 0.688 was obtained, which agrees closely with our findings. The author considers the lower quotients observed by us, as well as by himself, in animals made diabetic by withdrawal of insulin, as contrasted with those made diabetic by removal of a graft, to be dependent on the greater suddenness of onset of the diabetic state and the consequently more pronounced acidosis.

In addition to the two series of observations which we have just considered several others of older date may be briefly alluded to. Lafon measured the respiratory exchange of depancreatized dogs, by keeping them for considerable periods of time in a cabinet, the air of which was then analyzed volumetrically. In his most complete observation the quotients, on four successive days, were 0.745, 0.727, 0.710, and 0.731 respectively, as compared with 0.758 in this animal previous to pancreatectomy. As a result of his observations on four animals, he concludes that pancreatectomy causes the quotient to fall only slightly below the normal. Falta, Grote, and Staehelin observed the quotient to range between 0.683 and 0.711 after pancreatectomy, as compared with 0.707 to 0.736 in the normal animal (Jacquet respiratory apparatus). Moorehouse, Patterson and Stephenson found, as a rule, in 6 dogs depancreatized in one operation, that the quotient fell at first to 0.68 and then, in certain of the observations, that it rose somewhat in subsequent days. In one animal which was depancreatized in two stages, the initial quotient was not so low, but in another that was very fat, a quotient of 0.670 was observed.

Taking the above evidence as a whole, we may with safety conclude that the respiratory quotient observed during fasting stands at a somewhat lower level in completely depancreatized dogs than in normal animals. This cannot be attributed entirely to the excretion of ketone bodies and

may depend on a slow steady gluconeogenesis from fatty acid.

The interpretation of the respiratory quotient in diabetes. It is considered by those who deny that sugar can be formed out of fatty acid that if such a process were taking place in diabetes, the quotient should stand much lower than it actually does, it being incorrectly assumed that carbohydrate itself cannot be oxidized by the tissues. We consider that the depression of the quotient which occurs is explained by the excess of oxygen required to convert fats and proteins into carbohydrate. In normal animals the liver forms its glycogen readily out of absorbed carbohydrate, and, no oxygen being required for gluconeogenesis, the quotient of the animal as a whole may be the same as that of the muscles, namely unity. In diabetes, on the other hand, the liver cannot form glycogen out of carbohydrate and must form it out of protein and fatty acid, consequently large quantities of oxygen have to be retained and the quotient of the entire animal becomes very low.

Objection to this interpretation may be raised on the score that the diabetic quotient does not vary beyond rather narrow limits. In our experience with fasting depancreatized dogs it is never below 0.66 and it seldom rises above 0.70. But is this really surprising? Is it not rather to be expected, in view of the fact that the conditions under which the quotient is being observed are constant? Thus, in depancreatized animals, which are at present under consideration, gluconeogenesis, since it is uncontrolled owing to the absence of insulin, is proceeding at a maximum, and a constant proportion of the glucose thus formed is being converted into glycogen in the muscles, and the remainder excreted in the urine.

The effect of muscular exercise on the quotient in diabetes. If our view be the correct one, then the R.Q. of the excess metabolism of a short bout of moderate exercise per-

formed by a diabetic animal should be about the same as that following exercise in a normal one. Since diabetes is never complete in man (see p. 60) this possibility cannot be put to the test on him, although interesting results have been obtained by Hetzel and Long. Depancreatized dogs must be used, but here the technical difficulty enters in of getting the animals to do work. They could be made to do so by means of a treadmill installed in the respiratory cabinet, or as Chaikoff and I have done, by inducing shivering. This does not fulfil the conditions required to give a quotient of unity for the excess metabolism, because shivering is of the nature of prolonged exercise so that a steady state becomes established (cf. p. 10), but the results are of importance, since they show that the quotient rises at first, and then falls. Although the details of these experiments have not as yet been published, it may be of interest to refer briefly to them here.

Each animal was first of all observed for several hours while resting and with the cabinet at room temperature, and on the next day it was given a cold bath and then placed in the cabinet which was kept at a temperature of 10° to 13° C., by means of cold water circulating between its double walls. This caused most of the animals to shiver and greatly increased the energy output. In the first observations normal dogs were used. The results are shown in Table VIII.

It will be seen that the oxygen consumption of all the animals was increased greatly by the shivering, especially during the first two hours, the falling off during the third hour being probably due to a fall in body temperature. The R.Q. rose very decidedly during the first half hour of shivering in three of the four observations on normal dogs and then fell gradually. In eight of the nine observations on diabetic dogs similar, although perhaps less marked changes, occurred. When the quotient of the excess meta-

TABLE VIII

DOG NO. / WT.	RESTING[1] RESP. EXCHANGE		SHIVERING RESPIRATORY EXCHANGE								REMARKS
			1ST ½ HR.		2ND ½ HR.		2ND HR.		3RD HR.		
	O_2 CC. PER HOUR	R.Q.	O_2 CC. PER HOUR	R.Q.	O_2 CC. PER HOUR	R.Q.	O_2 CC. PER HOUR	R.Q.	O_2 CC. PER HOUR	R.Q.	
16	5125	0.71	12478	0.70	7900	0.73	13958	0.69	10704	0.69	Excess R.Q. of 1st ½ hr. 0.68
6.6 Kg.											
17											
6.0 Kg.	3116	0.71	5706	0.93	6088	0.76	.....	.....	5409	0.75	" 1.2
17A											
5.4 Kg.	3742	0.69	5742	0.83	6508	0.86	5473	0.72	5530	0.69	" 1.09
19											
11.5 Kg.	5030	0.70	7484	0.79	8708	0.71	9048	0.67	8726	0.73	" 0.97
						DIABETIC					
1(1)											Excess R.Q. of 1st
4.9 Kg.	3583	0.69	7484	0.76	8112	0.70	7351	0.71	6633	0.69	½ hr. 0.83
1(2)											
4.9 Kg.	3794	0.70	8796	0.77	8784	0.72	7722	0.71	7859	0.69	" 0.82
1(3)											
5.6 Kg.	4292	0.68	11028	0.73	10658	0.67	8649	0.69	7238	0.69	" 0.75
9(1)											
4.0 Kg.	2391	0.70	4094	0.85	4144	0.83	3931	0.84	3664	0.83	" 1.05
9(2)											
3.9 Kg.	2653	0.68	3852	0.66	3844	0.72	4211	0.70	4173	0.70	
11											
8.0 Kg.	3957	0.68	9201	0.83*	.....	.....	9882	0.76	8136	0.70	*1 hr. observation
14											Excess R.Q. of 1st
5.6 Kg.	4851	0.70	8382	0.77	6688	0.72	6183	0.71	6270	0.69	½ hr. 0.85
18(1)											
7.6 Kg.	4057	0.69	5904	0.82	7666	0.65	7635	0.66	5782	0.75	" 1.09
18(2)											
7.0 Kg.	3730	0.68	6338	0.73	5788	0.72	5945	0.69	5663	0.70	" 0.80

[1]Average of 4 hour observations.

bolism is calculated for the first half hour of shivering, by the principles which were explained in a previous chapter (p. 8), quotients of unity, or over, are seen from the table to have been obtained in three of the observations on normal dogs, but only in two of those on diabetic ones. It seems unlikely that the high quotients can have been due

to a blowing off of CO_2 from the blood, although, in the light of Kilborn's results on the eviscerated cat preparation, this possibility will have to be investigated. Taking the results as they stand it must be concluded that shivering causes a rise in the R.Q. in diabetic fasting animals, which is not much less marked than that observed under the same conditions in normal ones. It could not be expected to be as marked, because of the lesser reserves of carbohydrate in the former. Finally, it will be seen that the quotient had fallen back practically to the normal level by the second hour in all but one of the observations, which was on a diabetic animal. We cannot explain this exceptional result, but we interpret the fall of the quotient in the others, as due to the setting in of more active gluconeogenesis. Examination of the excretion of nitrogen in two of the animals showed no change in protein metabolism, presumably therefore fatty acid was the source of the new sugar.

That the R.Q. is as constant in diabetes as it is in the normal condition, is no more surprising than that the body temperature should be constant, either at a normal level or at a high one, as in continued fevers, or that the percentage of various constituents of the blood should be practically constant. These are all balanced reactions and the R.Q. is of a similar nature; it represents the constant of a balanced reaction in which one part is the quotient of gluconeogenesis and the other that of carbohydrate combustion.

The quotient in human diabetes. In diabetic patients maintained on carbohydrate-free food, many of the results are inadmissible as evidence in the present discussion, because the periods during which the expired air was collected have not been of sufficient duration to rule out the sources of error referred to earlier in this chapter. Some of the results are, however, of interest; thus Joslin and Benedict, who collected the expired air over periods of one hour, sometimes obtained quotients that were lower than

0.66, but they are careful to point out that such short-period quotients are not of much value, as an index of the type of metabolism. Lusk, on the other hand, failed to observe a quotient lower than 0.699 in a patient in which the G:N ratio was 1 to 3.97, and it was 0.70 in another in which the G:N ratio was 1 to 3.5. Numerous other observers, especially of the German School (Leo, Magnus-Levy, Leimdörfer, etc.) have observed quotients lower than 0.66 but they are of uncertain value as evidence, because of the too brief periods of observation.

On the whole, therefore, the clinical evidence agrees with that of the laboratory in showing that the diabetic quotient does not go below 0.66, when sufficiently long observations are made.

The lack of constancy which is a feature of the clinical quotients is no doubt to be explained by the fact that some insular tissue capable of secreting insulin still remains in the pancreas. So long as this is so, there will be opportunity for glycogen to be stored up in the liver, and when oxidation becomes increased in the muscles, as during exercise, the replenishment of their stores of glycogen will be accomplished by mobilization of that available in the liver, without there being any immediate increase in gluconeogenesis. This will raise the quotient. Even if a diabetic patient were properly observed, by keeping him for several hours in a respiratory cabinet while on a strictly protein diet, the R.Q. would, therefore, probably vary from time to time, depending on the extent to which his pancreas was secreting insulin.

Phlorhizin diabetes. Although we consider the assumption unwarranted, that the metabolism of animals completely under the influence of phlorhizin is analogous with that in diabetes, it is nevertheless important to review some of the observations which have been made on phlorhizinized animals, since they have so largely influenced present-day

teaching in this field. They are usually considered to prove the hypotheses to which we have referred before, namely, (1) that the tissues in diabetes have lost the power to oxidize carbohydrate and (2) that the sugar which continues to be excreted after the original stores of carbohydrate have been used up comes exclusively from protein. The observations made on phlorhizinized animals which are offered in support of these conclusions are: (1) that the R.Q. falls to the level which represents the combustion of a fat, and fails to rise when glucose is ingested, (2) that administered glucose can be recovered quantitatively in the urine, (3) that the G:N ratio remains fixed at 3.65 however greatly the amount of protein, or of amino acids, may vary in the diet, and (4) that ingestion of fatty acid does not influence either the R.Q. or the G:N ratio.

The respiratory quotient in phlorhizinized animals. First of all let us consider the behavior of the R.Q. In a fasting dog fully intoxicated with phlorhizin, Lusk found it to be 0.716, changing from 0.711 during the second and third hours following ingestion of 10 gm. of glucose. After 70 gm. glucose the quotient rose to 0.724, but this authority nevertheless concluded that "the respiratory quotient indicates that glucose could have been oxidized only in minimal quantities." M. Ringer, a pupil of Lusk, has more recently found that 40 gm. of glucose does not affect the quotient, and this has also been confirmed by Gaebler and Murlin. But from the same laboratory have recently appeared several reports showing results of an entirely different nature: namely, that the quotient may rise quite decidedly in phlorhizinized animals after glucose, especially when this is given at intervals, instead of in one quantity.

The first of these reports was published by Wierzuchowski and it will suffice here to give his most striking result. It was obtained by giving to a fasting phlorhizinized dog

20 gm. glucose in two quantities 4 hours apart, when the quotients during each hour following the last dose were, respectively, 0.771, 0.802, 0.702, and 0.702 showing, according to the usual interpretation, that more carbohydrate was being oxidized over a period of about 2 hours. Wierzuchowski attributes the positive character of his result to the fact that the first 20 gm. removes ketone substances from the organism, by some of it being oxidized, so that no acidosis is present when glucose is given a second time. He had previously demonstrated that glucose ingestion has an antiketogenic effect in phlorhizin diabetes, so that failure of the R.Q. to rise, when comparatively small amounts of glucose are given at one time, may be due to retention of the CO_2 by the blood, to take the place of the ketone acids which are disappearing. In support of this explanation, it was found that the CO_2-combining power of the blood became raised towards the normal after giving glucose to phlorhizinized animals, but was unchanged when it was given a second time.

But a moderate quantity of glucose given at one time may also raise the quotient, as has been shown by Deuel, Wilson, and Milhorat. Thus, in one phlorhizinized animal (No. 41), in which the non-protein R.Q. was 0.707, ingestion of 16 gm. glucose raised it to 0.790 and 0.793 in 2 and 3 hours, respectively; and even with 10 gm. of sugar the quotient rose to 0.740 and 0.742. Corresponding results were obtained in numerous other similar experiments by the same workers.

Recovery of glucose in phlorhizinized animals. We see then that one of the chief pieces of evidence which has been brought forward in defence of the claim that no glucose can be oxidized by the tissues in diabetes has to be withdrawn. What of the others? Is it the case, for example, that administered glucose can be recovered *in toto* in the excreta in phlorhizin intoxication? Reilly, Nolan, and Lusk, and

later Lusk's pupil A. I. Ringer, succeeded in accounting for about 93 per cent of ingested glucose in the urine. Ringer's result is particularly interesting, since the nitrogen excretion on the day on which the sugar was given declined markedly, indicating apparently that the glucose, although it was practically all excreted, was nevertheless capable of exercising a protein-sparing action. Michael Ringer, Gaebler, and Murlin obtained similar results. Sansum and Woodyatt, on the other hand, could only account for from 50 to 86 per cent of the sugar, when from 8 to 16 gm. was ingested, and Wierzuchowski (*loc. cit.*) was able to recover no more than 67 per cent when 40 gm. was given in two quantities 4 hours apart. Still other results from Lusk's laboratory, by Deuel, Wilson and Milhorat (*loc. cit.*), indicate a recovery of 90 per cent when 16 gm. of glucose was given, and the usual method is used for calculating the extra sugar excretion but, when the extra sugar excreted (i.e., unoxidized) is added to the extra sugar which was meanwhile oxidized, as judged from the rise in R.Q., the total comes to 19.3 gm., that is, 3.3 gm. *more* than the amount actually given. This result shows that when sugar is administered to phlorhizinized animals it sets up metabolic changes which do not conform with the interpretation that loss of the power to oxidize carbohydrate is an outstanding feature even in this supposed form of diabetes. We have also observed that considerably more sugar than was administered may appear in the urine when sugar is given to depancreatized dogs.

But quite apart from these criticisms of the results obtained on phlorhizinized animals, we are very doubtful whether they should be admitted as evidence in the judgment of the case at issue, because the calculations are based on the assumption that the endogenous sugar production on the day of the observation is the same as that of the pre-

ceding day. On this basis, the "extra sugar" excreted is computed by subtracting, from the total sugar of the urine, an amount obtained by multiplying the grams of nitrogen simultaneously excreted, by the G:N ratio of the day preceding that of the sugar ingestion. This is done even when, as is usually the case, the nitrogen excretion falls decidedly, indicating that fundamental changes in the nature of the metabolism of the organism must have occurred. The rather absurd conclusion is therefore arrived at, that when sugar is ingested in diabetes it is excreted unused and yet exercises an influence on the metabolism of what is commonly believed to be the most stable of the tissue constituents, the proteins.

The G:N ratio in phlorhizinized animals. The most outstanding feature of phlorhizin intoxication is the constancy in the G:N ratio, both when the animal is starved and when fed on protein, provided, in the latter case, that a sufficient period of time is allowed, after the meal, for both sugar and nitrogen to be excreted. But the ratio is not the same in phlorhizinized animals of different species. In rabbits, goats, and cats, it stands at the same level as after pancreatectomy in dogs, viz., 2.8, whereas in the dog and in man it averages 3.65 (Lusk). These differences have never been satisfactorily explained, although it is thought that the condition of the kidney, on which organ phlorhizin primarily acts, may have something to do with them, since phlorhizin gives a ratio of 2.8, instead of 3.65, in the dog when this organ is previously damaged by administering camphor (Jackson).

There can be no question as to the constancy of the G:N ratio in phlorhizinized dogs, however greatly the protein of the diet may vary in amount. It also remains unchanged during severe muscular exercise (Lusk). Now, in these two conditions, changes in opposite directions occur in the

metabolism of fat, protein feeding causing it to become lessened, and exercise causing it to become increased, so that the constancy of the ratio would seem to contradict the view that sugar can be derived from the fat of the body. Let us, therefore, examine the facts more closely. With regard to protein feeding, Mandel and Lusk obtained the following results in a phlorhizinized dog, observed both in the fasting condition and after feeding with meat:

	G:N RATIO	CALORIES FROM PROTEIN	CALORIES FROM FAT	CALORIES TOTAL
Fasting	3.69	80.2	274.4	354.6
300 gm. meat	3.55	161.9	261.7	423.6

It can be seen, that although twice as much protein was metabolized on the meat diet (with a corresponding increase in the excretion of glucose), there was only a trivial decrease (less than 5 per cent) in the fat metabolized. Indeed, in face of the greatly increased sugar-formation which must have occurred from protein, it is not surprising that the slight decrease in fat breakdown should fail to make a perceptible difference in the excreted sugar, which we suppose was derived from this source.

Lusk's experiment on the effect of muscular exercise was carried out on a phlorhizinized dog from which all glycogen had been removed, by shivering. When the animal was made to perform an amount of exercise which would have more than doubled the metabolism of fat during the hour when the exercise was taken, no change was noted in the excretion of either nitrogen or glucose. Thus:

	GLUCOSE	NITROGEN	G:N RATIO
Rest	4.57	1.26	3.63
Work, 1500 metres during the first hour	4.62	1.26	3.67

Now, we cannot see why these results should be considered to disprove the possible derivation of sugar from

fat. We interpret them rather as showing that the glycogen which was furnished to the active muscles was being manufactured out of fat at a rate corresponding to the extra demands. In other words, all the new carbohydrate produced out of fat was immediately used, so that no excess of glucose was excreted by the kidneys. It is well known that exercise in normal animals does not occur at the expense of protein (Cathcart), and we believe that it occurs at the expense of carbohydrate, which is renewed by gluconeogenesis from fat when the available stores become depleted. The rate of this latter process is conditioned upon the demands for glycogen, primarily of the muscles and secondarily of the liver, and we can see no reason for considering that this adjustment becomes upset in diabetes.

Direct evidence that fat can form carbohydrate in animals. It may be remarked, that the evidence which I have so far brought forward furnishes no direct proof for the view[1] that carbohydrate can be formed out of fatty acid in the animal body, but only contradicts what has been regarded as proof for the opposite view, that such formation cannot occur. In attacking such a well-established stronghold of belief, as that held by the negativists, our first manœuver has of necessity been to reduce its defences, so that the false doctrines may be displaced. One of the leading investigators of metabolism in recent years has written thus: "One by one the bulwarks of the doctrine of the conversion of fat into glucose have been shattered, and it may now be relegated to the realm of scientific superstition" (Lusk), and the majority have contented themselves by following the leader. They have felt secure in their new position but now the searchlight of scientific fact shows that

[1] This older view was first clearly stated by Chauveau.

its defences are unreliable. Can we, then, furnish evidence which directly supports the older view?

It has to be admitted at the outset that this is no easy task, mainly because we know very little regarding the history of fat in the animal body. Important discoveries have indeed been made, such as that the fatty acid chain may become progressively oxidized at the β carbon atom, and that the ketone bodies may be derived from the residue of the chain containing four carbon atoms, which is left after this process has gone as far as it can. But many of the details of the process are unknown and it is improbable that we can form any clear picture of how carbohydrate is formed out of fatty acid until these have been worked out.

Apart from the chemical difficulties there is also the physiological one, to which we have already alluded, that fat, being preeminently the energy-storing material of the body, follows a roundabout path during the early stages of its metabolism. The fat absorbed from the intestine is not immediately utilized for the production of energy, but some of it becomes deposited in the fat dépôts, and some is built into the essential structures of the most active cells, by entering into the formation of very complex molecules, such as those of lecithin, cerebrosin, etc., or by changing into other substances, such as the cholesterolesters.

Is it, therefore, to be wondered at, that it should so far have been impossible to detect any immediate increase in the excretion of sugar in the urine of depancreatized dogs when fat is fed to them, for not only does the fat run the risk of being very imperfectly absorbed, because of the absence of the lipolytic enzymes of the pancreatic juice, but what is absorbed may not be immediately used. The fact that feeding with fat does not affect the sugar excretion in animals intoxicated with phlorhizin, may be ex-

plained by supposing that it is the demands of the organism for carbohydrate to be used as fuel, rather than the availability of raw material out of which it may be formed, that determines the intensity of the gluconeogenic process. Assuming that gluconeogenesis is mainly a function of the liver, we may say that it is the call of the liver for raw material out of which to form glycogen that is the dominating factor regulating the intensity of that process. Protein can furnish some of this material, but fat is its chief source. This does not imply that the magnitude of the fat reserves of the body has no influence on gluconeogenesis. On the contrary, we have seen that more sugar accumulates in the blood and more sugar is excreted by fat, as compared with lean depancreatized dogs, and it is well known in clinical practice that severe diabetes in a fat patient is a greater risk than in one that is thin. Although this risk is mainly due to the more rapid development of ketosis and coma, the symptoms of hyperglycaemia and glycosuria are also more marked. There are also several clinical investigators who believe that fat-feeding raises the sugar excretion in diabetes.[1]

Sugar formation by the liver. Attempts to furnish direct proof that fat forms sugar have been made from time to time, but it would serve no useful purpose to review all of them here. Such a review will be found in the recent essays of Geelmuyden. We would, however, refer to the researches published nearly thirty years ago by Seegen whose experiments were of two general types. In one of these, he compared the percentage of sugar in blood drawn from the portal vein, with that in blood drawn, at the same time, from the hepatic veins, or carotid artery, and he always found higher percentages in the latter, indicating

[1] A review of these investigations will be found in Geelmuyden's essays (cf. *Ergebnisse der Physiologie*) and in *Carbohydrate Metabolism and Insulin*, by the author.

that the liver was discharging sugar. This surplus persisted when the liver, through previous fasting of the animal, was deprived of glycogen, indicating that the sugar must have come from non-carbohydrate sources. Seegen went so far as to conclude that this extra sugar was derived from other substances than glycogen even when abundance of this was present in the liver, but it is almost certain that he erred in this regard because he had to use the inaccurate methods then available for determination of the glycogen. The other series of experiments consisted in incubating suspensions of cut-up liver, with oxygenated blood, when it was found that the amount of sugar which accumulated in the mixture after some hours was increased when fats, or glycerol or fatty acids were added to the mixture. Repetition of Seegen's experiments by numerous other workers failed, however, to confirm his results and they are scarcely ever referred to in modern literature.

Even had the chemical methods employed been flawless, the conditions chosen by Seegen were not entirely suitable for demonstrating gluconeogenesis, since considerable amounts of glycogen were usually present in the liver. To ascertain whether sugar can be formed out of fat in this organ it is obviously much simpler to free the organ of glycogen to start with, and Embden has reported experiments in which this precaution was taken. Embden rendered dogs glycogen-free, by starvation, muscular exercise and injection of strychnine, and immediately after death he removed a lobe of liver in which the free sugar was determined and the absence of glycogen verified by analysis. The remainder of the organ was then perfused at body temperature through its vessels, with defibrinated blood in which the percentage of sugar was known, and it was found, after about one hour, that much more sugar accumulated in the perfused blood than could be accounted for by the free sugar in the organ to start with. Lattes repeated

these observations on dogs that were either depancreatized or had been injected with phlorhizin, and obtained similar results to those of Embden, and so also have Bornstein and Griesbach who, however, attribute the increase in sugar to lactic acid, which, it is known, becomes less in the blood as the liver perfusion proceeds.

Burn and Marks have recently reported similar experiments in which the liver of the cat, rather than the dog, was used, the advantage being that oedema does not set in so quickly in the former as in the latter. For several days preceding the experiment the animals were fed on fat, and their own blood (defibrinated), diluted with saline, was used as the perfusion fluid. Determinations were made of glycogen and free sugar in a lobe of liver removed before the perfusion started, and of sugar in the perfusion fluid. These were repeated at intervals during the perfusion and the total carbohydrate of liver and perfusion fluid was computed from the results. This increased rapidly at first, and then more slowly until oedema became evident, after which no further increase occurred. In a series of 22 experiments considerable production of new sugar was readily demonstrable; thus, in two experiments, which are reported in detail, the total initial carbohydrates were, respectively, 358 mg. and 373 mg., and the final, 722 mg. and 764 mg. Some glycogen was also formed in the livers. Pancreatectomy did not seem to alter the magnitude of the results (1 cat). Determination of lactic acid showed that the new sugar was not derived from this source, neither could it be accounted for by the protein undergoing breakdown in the course of the perfusion, as was shown by calculating the G:N ratio after measurement of the urea and ammonia in the perfusion fluid. The ratio was always far above the limits which are considered to indicate gluconeogenesis from protein, so that only a small fraction of the sugar could have come from this source, leaving fat as the only

one remaining. As the authors point out, so much fat is present in the liver, of starved and depancreatized animals, and it is so unevenly distributed over the different lobes as to make it impossible, by chemical methods, to compare the rate of fat disappearance with that of sugar formation. But, nevertheless, the results of Burn and Marks, taken along with those of Seegen and Embden, leave no doubt that sugar is formed in the liver out of something that is neither carbohydrate nor protein in nature. Thomas and I have attempted to demonstrate a similar increase of sugar in thin slices of freshly excised liver (cat) suspended in blood, in an atmosphere of either oxygen or nitrogen, but have failed to find any increase which could not be accounted for by glycogen. The sectioning of the liver had probably destroyed its glyconeogenic powers.

The effect of feeding with fat on the sugar excretion in phlorhizinized rats. The difficulty in proving that the sugar which is formed in the perfused liver comes from fat, may be due to the impossibility of maintaining the vital functions of this organ for a sufficient period of time after the natural circulation of blood through it has been interrupted, and this possibility leads us to return to experiments in which the circulation is undisturbed, a condition which is only fulfilled by using intact animals. Earlier in this chapter it was pointed out that the metabolic process through which fat must pass in the organism, before it can become available to form sugar, is too drawn out to make it possible to demonstrate a relationship between fat ingestion and sugar excretion in diabetic, or phlorhizinized dogs. This difficulty might be overcome by feeding fat to animals in which metabolism is much more rapid than in dogs, after having depleted the stores of glycogen and reduced those of body fat to a minimum. Such experiments have been attempted by Calvo-Criado, working in Asher's laboratory. White

rats were fed for some days (3-6) with lean meat, peptone and thyroid gland, it having previously been shown that glycogen formation in the liver becomes reduced to a minimum by this treatment (Takahashi). The peptone, but not the thyroid gland, was withdrawn on the fifth or seventh day, after which phlorhizin was given (subcutaneously in oil) every two days.

When the glycosuria had become established fat was added to the diet, for periods of a few days each, and the behavior of the excretion of sugar and nitrogen compared with that of equal periods in which no fat was given. In most of the experiments, of which the results of eleven are reported, the fat caused the sugar excretion to become increased, without any decided change in that of nitrogen, the most striking results being obtained in two rats in which a small, constant quantity of cane sugar was included in the diet, with the object in view of maintaining the liver in a condition more nearly approaching the normal than in the other experiments. The results of these experiments are not entirely convincing, on account of the very large variations which were observed from day to day, independently of giving fat. On the whole, however, we may consider them as affording support to the view that sugar can come from fat in the animal body. Calvo's results have been more or less confirmed by Okumura and by Kojima, although the latter considers that it is the glycerol, rather than the fatty acid of fat, which is responsible for the increase in sugar excretion. The results themselves have been challenged by Takao.

The effect of epinephrin on the sugar excretion in diabetes. Experiments recently performed in my laboratory by Chaikoff and Weber have yielded results which seem to me to prove irrefutably the derivation of sugar from fatty acid, at least in diabetic animals. Epinephrin was injected at 3 hourly intervals, during 12 hours, into well-fattened,

but meanwhile fasting, depancreatized dogs, previously treated with insulin, with the result that much more sugar was excreted in the urine than could be accounted for as coming from the glycogen stores of the body, at the time of injection, and the protein and glycerol metabolized.

But before we proceed to examine these results it may be well briefly to review those of previous workers who have used a similar method. The earliest experiments were those reported by Eppinger, Falta, and Rudinger, in which a single dose of epinephrin was injected into recently depancreatized dogs, with the result that more sugar was excreted than, it was considered, could have come from the meager stores of glycogen and the protein metabolized. These results have not been universally accepted, because A. I. Ringer in particular failed, in 1910, to corroborate them, by injecting epinephrin into fasting dogs brought fully under the influence of phlorhizin. Ringer found that although epinephrin may at first cause an excess of glucose to be excreted, this did not occur following subsequent injections into the same animal, indicating that the extra sugar excreted after the first injection had come from the glycogen stores of the body. Ringer also observed that the excretion of nitrogen remained unchanged as a result of the epinephrin injections.

There can, of course, be no doubt with regard to the accuracy of these results from Lusk's laboratory, but I do not consider that they are applicable in the present connection, since the animals used were only diabetic in the sense that they were under the influence of phlorhizin. Although many of the symptoms of intoxication by this drug are the same as those of true diabetes, such as that which follows pancreatectomy, there are, as we have already seen, striking differences between the two conditions, the most notable of which, to recapitulate, are: (1) that ingestion of sugar causes the R.Q. to rise, and the ketonuria to

disappear, after phlorhizin, whereas, as a rule, it has no such effects after pancreatectomy, (2) that the blood sugar remains practically normal after phlorhizin, but is very high after pancreatectomy, (3) that the islet tissue is intact and the internal secretion of insulin therefore still possible after phlorhizin. The most important of these differences in the present connection is the last mentioned, for we may suppose that when epinephrin is injected into phlorhizinized animals the internal secretion of insulin is stimulated, perhaps through the tendency for the blood sugar to rise, with the result that gluconeogenesis becomes inhibited. There is good reason to believe that insulin has this effect (cf. Laufberger and Best, Dale, Hoet and Marks), so that when it is absent from the body, as in true diabetes, gluconeogenesis becomes excessive, although it can be still further stimulated by epinephrin. In consideration of these facts it is clear that animals intoxicated with phlorhizin are not suitable test objects in the present connection, because they can still secrete insulin internally.

We may now return to Chaikoff and Weber's experiments, a typical example of which is shown in Table IX. It will be observed that the G:N ratio was falling during the third day after removal of insulin and food. Epinephrin was injected, at 3 hourly intervals, during the first 12-hour period of the fourth day, with the result that 31.3 gm. of glucose were excreted in excess of that of the preceding 12 hours. We have to consider whether this can be attributed to a sweeping out of the bodily stores of glycogen, and to sugar arising from the increased metabolism of protein which occurred. We are certain it cannot, for the following reasons: Only a small proportion of the sugar could have come from the meager stores of glycogen remaining in the liver on the fourth day after withdrawal of food and insulin. Thus, Chaikoff, as a result of the examination of several depancreatized animals killed on the third day

TABLE IX

Metabolism of Depancreatized Dog Weighing 6.8 Kg. Food and Insulin Withdrawn, May 23

DATE	PERIOD AFTER WITHDRAWAL OF FOOD AND INSULIN	URINE			RESPIRED AIR			REMARKS
		GLUCOSE GM.	NITROGEN GM.	G:N RATIO	O_2 CONSUMED PER KG. PER HOUR	R.Q.	CAL. PER KG. PER 12 HRS.	
May 25	First 12 hours, 3rd day	16.80	2.62	6.4	604*	0.683	34	*Measured over first 3 hours of period
May 25	Second 12 hours, 3rd day	11.05	2.46	4.5	.....	.....	....	
May 26	First 12 hours, 4th day	42.41	3.16	13.4	874*	0.747	50	*First 3 hours. 1 cc. adrenalin (1:1000) injected every 3 hours throughout period
May 26	Second 12 hours, 4th day	14.33	2.56	5.6	.....	.....	....	
	Difference	31.36	0.7					

Liver	1.2 gms. glycogen	
Muscles	13.6 gms. to start with	
Protein	2.5 (0.7×3.65)	
	17.3	
Glycerol	3.5 (340)	
	9.4	
	20.8	

after insulin, found a maximum of 0.35 per cent of glycogen in the liver. The liver of the present animal weighed 340 gm. and might therefore have contributed 1.2 gm. of sugar.

There remains the glycogen of the muscles; may this not have supplied a large part of the extra sugar? We think not, since Bollman, Mann, and Magath, as well as Soskin have shown that no increase occurs on the percentage of blood sugar when epinephrin is injected into hepatectomized animals, and Choi has observed epinephrin to cause a reduction in the muscle glycogen in decapitated eviscerated cats only when such large amounts are injected as to cause twitching. But even if, for the sake of argument, we should suppose that the muscle glycogen could be excreted as sugar, the amount thus derived would fall far short of that required to account for all the extra sugar. Thus, if we consider that the muscles contained 0.5 per cent of glycogen—which exceeds the highest value actually found by Chaikoff in those of several depancreatized dogs killed on the *third day* after withdrawal of insulin—and that they constitute 40 per cent of the body weight, then, in the present animal, weighing 6.8 Kg., a total of

$$\frac{(40 \times 6.800 \times 0.50)}{(100 \times 1 \times 100)}$$

13.6 gm. of glycogen was present. Even if all of this were converted into glucose, which is utterly impossible, the amount would still fall far short of that required.

We have yet to allow for some of the extra sugar coming from protein. In this particular experiment the excretion of nitrogen rose by 0.7 gm. during the epinephrin period. It is held by Lusk that not more than 58 per cent of protein may be converted into sugar, giving a G:N ratio of 3.65, so that only 2.555 gm. could have been so derived. Adding these possible sources for the sugar, accounts for

17.3 gm. (1.2 + 13.6 + 2.5), leaving to be derived from fat, 31.3 — 17.3 = 14.0 gm. Some of this, no doubt, was derived from the glycerol which is well-known to be quantitatively converted into glucose in diabetes, and we can compute the maximum extent to which such conversion could have occurred in our experiment, from the respiratory exchange during the epinephrin period. Thus, 340 calories were expended; this corresponds to $\frac{340}{9.4} = 35$ gm. of fat of which 10 per cent or 3.5 gm. was glycerol. As a final result we are therefore left with over 10 gm. of extra sugar to account for, the only source for which is fatty acid. Observations of a similar nature were made on six other depancreatized dogs with the final results shown in Table X.

In only one of these observations (H) can the extra glucose eliminated, as a result of epinephrin, be accounted for by glucose coming from liver glycogen, protein and glycerol, and in two of them J and C (second experiment) there is an excess, even when the assumption is made that all of the muscle glycogen was also excreted as glucose. I have already called attention to the observations by Choi, in which it was found that epinephrin did not cause the muscle glycogen to become diminished in decapitated cats, unless when sufficiently large doses were given to cause muscular tremors. When given to dehepatized dogs Soskin and Bollman, Mann and Magath also found that epinephrin did not cause any increase in blood sugar. In view of these facts it seems incredible that muscle glycogen could be converted into glucose and excreted as such.

In case further proof be demanded let me offer one other result obtained in an experiment in which the urine of each six hours of the epinephrin day was examined. In the first of these periods, 18 gm. of sugar was excreted in excess of that possible from liver glycogen, protein and glycerol.

TABLE X

Effects of Repeated Large Doses of Epinephrin on Carbohydrate Balance in Depancreatized Dogs

DOG WEIGHT	GLUCOSE EXCRETION IN 12 HOURS			GLUCOSE WHICH CAN BE ACCOUNTED FOR FROM LIVER GLYCOGEN PROTEIN AND GLYCEROL[1]	TOTAL GLYCOGEN IN MUSCLES	REMARKS
	PRECEDING EPINEPHRIN	DURING EPINEPHRIN	DIFF'CE			
	GM.	GM.	GM.	GM.	GM.	
J						
9.0 Kg.	12.9	52.7	39.8	7.65	16.9	
C						
6.3 Kg.	24.2	36.8	12.6	5.30	11.8	Small dose of epinephrin
	13.6	36.4	22.8	4.17	11.8	
B						
6.2 Kg.	6.7	23.0	16.3	7.86	11.6	
I						
14.0 Kg.	23.2	38.4	15.2	10.0	26.3	
H						
6.8 Kg.	18.02*	28.8	10.8	18.84	12.8	*24 hours
	47.80*	74.8	27.0	21.3	12.8	*24 hours
K						
6.7 Kg.	9.7	24.7	15.0	7.06	12.6	

[1]Calculated as follows: Sugar from protein = excess of N. excreted × 3.65
Sugar from liver = 0.35 (maximal percentage found in livers of several animals on 3rd day of diabetes) × wt. liver.
Sugar from glycerol = calorie expenditure divided by 9.4 calories to give fat consumed. One-tenth of this gives glycerol.

There might possibly have been 17 gm. glycogen in the muscles of this animal at the start of the period, so that we may, for the present, consider that all extra sugar was accounted for. But during the second 6-hour period (when epinephrin continued to be given) 21.2 gm. of glucose was

excreted, none of which could have come from glycogen and certainly not more than half of it from protein and glycerol. (The excess N. during these 6 hours was 1.7 gm.)

When fatty acid is converted into glucose oxygen is of course absorbed thus:

$$3C_{16}H_{32}O_2 + 21O_2 = 8C_6H_{12}O_6$$

which would lead one to expect that the R.Q. of the animal would be lowered. But, on the contrary, in two of the foregoing experiments, in which the respiratory exchange was measured, the R.Q., as well as the O_2 consumption, increased during the hour or so following the first injection of epinephrin. In the second of these animals this was obviously due to excitement, as indicated by barking and restlessness while in the respiratory cabinet. This, no doubt, caused a blowing off of CO_2, and thus raised the respiratory quotient.

On the other hand, neither the R.Q. nor the O_2 consumption became raised, after the subsequent injections of epinephrin; indeed the former fell to 0.682 which is about the lowest level met with in diabetic animals.

The amount of sugar coming from fatty acid in this (second) respiratory experiment, without making any deduction for glycogen of muscles, was 5.9 gm. This process would require 2.79 gm. O_2 or 1.6 liters. Subtracting this from the O_2 actually retained by the animal raises the quotient to 0.704. (Further details of these calculations will be found in Chaikoff and Weber's paper.) The results of the respiratory observations do not, therefore, contradict the conclusion that carbohydrate was being formed out of fatty acid.

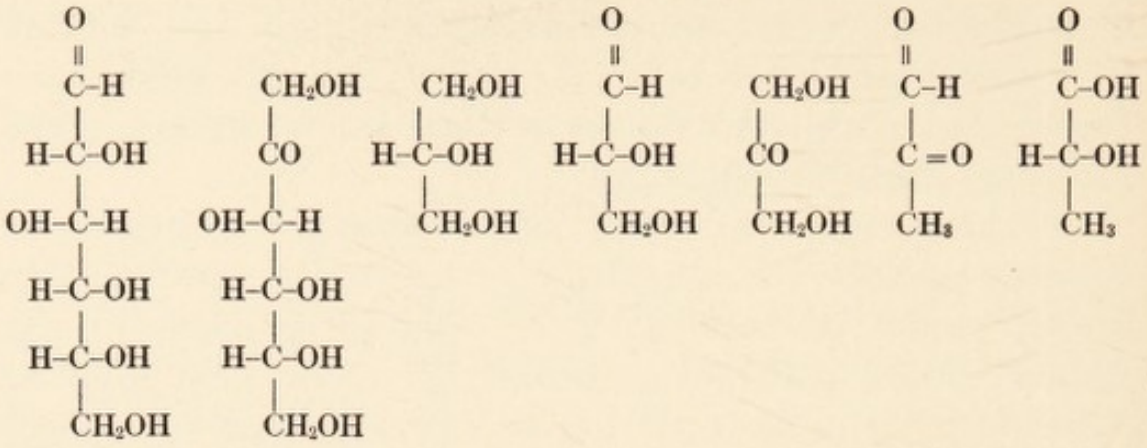
CHAPTER IV

SOME *possible intermediary substances of carbohydrate metabolism.* It will be recalled that the energy changes which are responsible for muscular contraction are considered, in their totality, to depend on: (1) a sudden production of lactic acid out of glycogen and (2) oxidation of a part of the resulting lactic acid, or some derivative, to yield the energy necessary to bring about a resynthesis into glycogen of the remainder. Little is known with regard to what intermediary substances may be formed in these processes, and the supposition that part of the lactic acid is oxidized is only based on the observation that the R.Q. of contracting isolated frog muscle is unity. The last mentioned result does not necessarily indicate the combustion of lactic acid as such, since any substance having the general formula $C_x(H_2O)_y$ would give a similar quotient, and it is possible, therefore, that the lactic acid which disappears becomes changed into some other substance prior to its oxidation. In addition it must not be forgotten that other chemical processes may occur during the recovery phase of contraction, to furnish at least a part of the energy necessary for resynthesis of glycogen, if not indeed also to furnish some of that which appears as movement or heat. This possibility has recently been strengthened by the discovery by Fiske and Subarrow (see also Eggleton and Eggleton) of a highly labile compound of creatin and phosphoric acid in muscle, which is capable of yielding energy when it falls apart into its two constituents. The inability of various workers to obtain respiratory quotients of unity after hepatectomy in mammals also indicates that other types

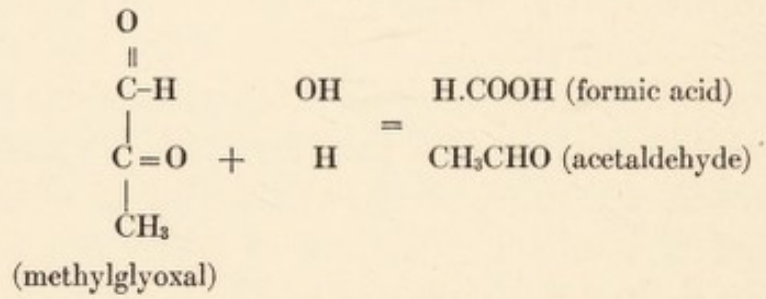
of oxidation process are occurring in muscle and it must be remembered that both Meyerhof and Embden have found that chemical changes setting free ammonia come into play during contraction.

Of the various problems yet awaiting investigation, in connection with the chemistry of muscular activity, two are of particular interest to us, and these are: (1) the nature of the substances into which lactic acid may be changed prior to its oxidation and (2) whether substances not related to lactic acid, or to preformed carbohydrates, may be simultaneously oxidized during the recovery phase, if not also during that of contraction itself. Finally, it must not be forgotten that a very considerable metabolism is going on in muscle and other tissues while in a condition of rest, and it is a question whether this is of the same type as that responsible for contraction, or other activity.

Information obtained by the direct chemical analysis of muscle is not sufficient to tell us what substances are formed as intermediary stages in the preparation of the fuel used for energy production, so that we are compelled to attack the problem by less direct methods. But first of all, we must know what the intermediary substances are likely to be, and the chemist has furnished us with this information, as a result of various *in vitro* reactions and changes. We do not here propose to consider all the substances of this list, but will rather choose, as examples, one or two which have recently received the most attention by students of animal metabolism. These are acetaldehyde, dihydroxyacetone, methylglyoxal and glyceric aldehyde. Each of the last three substances contains three carbon atoms, being thus more or less related to lactic acid and glycerol, and capable of production by a simple splitting into two parts, of the molecule of glucose or fructose. It may be of assistance if we place the structural formulae of these substances side by side, thus:

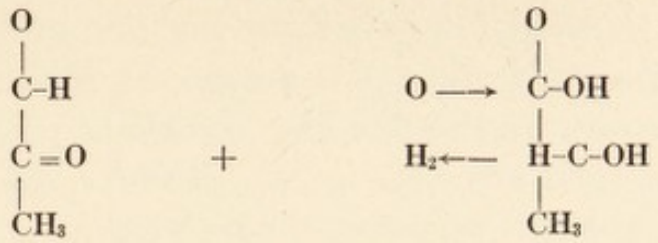


Now it is significant, as shown by Lusk and others, that every one of the above "trioses" is completely converted into glucose when administered to phlorhizinized dogs, which strongly suggests their close relationship to carbohydrate metabolism. This is also the case with acetaldehyde, CH_3-CHO, which may be formed out of methylglyoxal thus:



Methylglyoxal occupies an important position in most of the schemes which have been suggested for intermediary metabolism, especially because it may be formed from such amino acids as alanin, and because enzymes, called glyoxalases, capable of converting it into lactic acid exist in various tissues. (See structural formula at top of the opposite page.) It may, therefore, be a most important intermediary metabolite, not of carbohydrate alone but also of protein and, possibly also, of fatty acid.

The fact that glucose is formed out of all of these



(methylglyoxal) (d-lactic acid)

substances in the diabetic organism does not in itself entitle us to conclude that they arise in the course of normal metabolism. To determine this, several methods of investigation are open. One of these is to see whether the substance in question is present as such in the tissues, or fluids, of normal or diabetic animals. If it is found, the evidence is strongly in favor of its being an intermediary substance in metabolism, although its absence does not necessarily indicate the contrary, for this may merely depend on the fact that the substance is so rapidly transformed into something else, that no balance remains over to be detected by chemical means. Metabolism is a dynamic and not a static process, a process of constant change, and if our methods of chemical analysis of dead tissue should reveal one substance to be present in large amount and some other to be absent, this does not indicate which is the more important intermediary substance. Indeed, the more important substance may be the one we fail to detect, rapid transformation preventing its accumulation. The substance which we do detect may, moreover, be no intermediary in the strict sense, but only a by-product of the main reaction. On account of these limitations, attempts have been made to cause transient metabolites to combine with foreign substances to form compounds of a less destructible nature. The principle of this fixation method is well exemplified in the union of benzoic acid with glycine to form hippuric acid, which is then excreted in the urine, and also in the union of sulphite with acetaldehyde which, as we shall see, may occur in the tissues.

Another method for investigating the problem depends on determining whether the supposed intermediary substance is utilized by the diabetic animal in place of glucose, thus causing the glycosuria to decrease, the respiratory quotient to become raised and the ketonuria to disappear (see p. 75). It is theoretically possible, for example, that failure of the diabetic organism to make full use of glucose is dependent on its having difficulty in transforming this sugar into some intermediary, which itself can readily be dealt with. If such a substance could be found it would obviously be advantageous to give it as a substitute for sugar in diabetes and this, as we shall see, has been recommended in the case of dihydroxyacetone.

Turning now to the actually observed facts, we find most attention has been given in recent years to acetaldehyde and dihydroxyacetone. Little attention has been paid to glyceric aldehyde, because of its highly toxic nature (Woodyatt), or to methylglyoxal or its acid, pyruvic. This narrows our list down to acetaldehyde, dihydroxyacetone, and, for reasons which will be apparent later, we will add fructose. We may take the behavior of these as test cases, selected on the basis that they are at present considered by many to be the most likely intermediaries of carbohydrate metabolism.

Acetaldehyde. This substance is undoubtedly produced in the fermentative breakdown of glucose by yeast, where it is considered to serve as the mother substance of alcohol, into which it is transformed by reduction $CH_3CHO + 2H = CH_3CH_2OH$. Its oxidation, on the other hand, may lead to the formation of acetic acid, $CH_3CHO + O = CH_3COOH$, which occurs in the tissues of plants and in the excreta of animals. Acetic acid is considered to be readily oxidized in the animal body, and to be incapable of forming sugar. It may be formed when the fatty acid chain breaks down in the process of beta oxidation (see

p. 89), and if acetaldehyde should then be formed, it is easy to see how sugar might arise from fat. This latter possibility makes the search, as to whether acetaldehyde is present in the tissues of animals, a very exciting one since, if it should prove successful a step in the intermediary processes by which fatty acids are transformed into carbohydrates would be revealed.

The detection of acetaldehyde in animal tissues and fluids is a very difficult matter, for not only is it a highly volatile and unstable substance, but it is readily formed during microbal growth. A few words may not therefore be inappropriate regarding the methods used for its identification and estimation. The first step consists in distilling the animal fluid or tissue extracts, from which proteins have been removed, usually in the presence of nitrogen and at a low temperature, until about one-tenth of the fluid has distilled over, the aldehyde, on account of its highly volatile nature, being all contained in this fraction. The identification of the acetaldehyde is based on the fact that it combines with a reagent, known as dimedon (dimethylhydroresorcinol), to form a highly insoluble crystalline compound with a sharp and characteristic melting point. This reagent reacts with neither sugar nor ketone bodies, and its compound with aldehyde (aldomedon) is about seven times the weight of the combining aldehyde. For the quantitative estimation of aldehyde its reaction either with hydroxylamine or with Nessler's solution may be used. In the latter case, the aldehyde becomes oxidized and the mercuric salts of the Nessler correspondingly reduced, the extent to which this occurs being then determined iodometrically.

A careful critical examination of these methods, by Gee and Chaikoff, has established their dependability when properly used, but these workers have also pointed out a possible source of error, especially when diabetic blood,

or urine, is being investigated, due to the presence of acetone. Discounting those observations in which there may have been obvious technical errors of one kind or another the following results are worthy of consideration.

Acetaldehyde is present in the blood of normal animals, about 3 mg. per liter having been recovered, as aldemedon, from perfectly fresh ox blood (Fabre, Supniewski), an amount which corresponds with that found in dog blood, by using Nessler's solution (Gee and Chaikoff). A certain proportion of the acetaldehyde apparently exists in a free state, since some reduction occurs when Nessler's solution is suspended in a flat dish for two days, at 37° C., over blood filtrate, precautions being taken, by the addition of sulphuric acid, to prevent any growth of microorganisms in the filtrate.

There can, therefore, be no doubt of the presence of acetaldehyde in normal blood. The quantities are far below those which could develop any toxic effects, and this may depend either on combination of acetaldehyde with amino groups in the blood (Neuberg), or on its becoming changed into acetic acid and alcohol, by what is known as the Canizarro reaction for which an enzyme (aldehyde mutase), having an accelerating influence, has been found present in the liver (Battelli and Stern).

$$\begin{array}{ll} \mathrm{CHO} & \mathrm{O} \\ | & \\ \mathrm{CH_3} & = \mathrm{CH_3COOH\ (acetic\ acid)} \end{array}$$

$$\begin{array}{ll} \mathrm{CHO} & \mathrm{H_2} \\ | & \\ \mathrm{CH_3} & = \mathrm{CH_3CH_2OH\ (ethyl\ alcohol)} \end{array}$$

Opinions are divided as to whether acetaldehyde accumulates in the blood when metabolism is upset, as in pancreatic diabetes or after the injection of insulin. Both Fabre and Supniewski have claimed that it accumulates in diabetes, but Gee and Chaikoff, working with better con-

trolled methods have failed to confirm their results. Supniewski maintains that aldehyde increases in the blood of normal dogs injected with insulin and glucose, but the errors dependent on his having had to work with relatively small quantities of blood make this doubtful.

Acetaldehyde is also present in minute quantities, in the tissues and in the urine. Hirsch found it in protein-free extracts of frog muscle, and Supniewski in the liver and muscles of the dog and rabbit. But the most interesting observations in this direction are those of Neuberg and Gottschalk, who studied the production of aldehyde in incubated suspensions of muscle and liver, by the method of sulphite fixation, already alluded to, in which the aldehyde, as quickly as it is formed, is prevented from changing into other substances. They found that aldehyde is formed under these conditions and that the addition of insulin to the tissue suspension augments the yield, especially when fructose is added along with the insulin. In order to prevent the growth of organisms in these experiments an antiseptic called optochin was used, but Gee and Chaikoff, by careful bacteriological examination of blood filtrates containing this antiseptic, did not find that it really has the desired effect. Finally Stepp and his collaborators have demonstrated the presence of aldehyde in normal human urine, and they state that it is greatly increased in that of diabetic patients.

Taking the evidence as a whole, there can be no doubt of the presence of acetaldehyde in the animal tissues and fluids and there is, therefore, a possibility that it serves as a stepping stone in the processes of metabolism. Since it can be derived from any one of the three proximate principles of food (carbohydrates, proteins, and fats) the significance of these results will be evident.

Dihydroxyacetone. Of all the substances which might occur as intermediaries in the metabolism of sugar in the

animal body, dihydroxyacetone is perhaps the most interesting. Unlike the other trioses this substance has no toxic effects when administered to animals, and partly on this account and partly because it can now be readily obtained in quantity, its exact behavior in metabolism has recently been very thoroughly investigated. In neutral phosphate solution it takes up oxygen rapidly, whereas glucose scarcely does so at all (Wind). It decolorizes permanganate solution at 37° C. more rapidly than glucose, and it can reduce methylene blue anaerobically, which glucose cannot do (cf. Lambie and Redhead).

It has been known for some time that dihydroxyacetone is among the products of the fermentation of sugar by yeast, that it causes glucose, as well as d-lactic acid, to increase in nutrient fluids after they have been perfused through the excised mammalian liver (Embden, Baldes, and Schmitz) and that it is converted into glucose, and excreted as such, in dogs kept fully under the influence of phlorhizin (Ringer and Frankel). It can also cause glycogen to be formed in the liver, as Mostkowski found by feeding it to chickens. But, as has already been pointed out, facts such as these do not in themselves necessarily indicate that dihydroxyacetone is formed as an intermediate step in normal metabolism. They merely show that this is possible, and it has become especially important to investigate the possibility, since it has been claimed, that dihydroxyacetone can be oxidized in the tissues without first of all forming glucose, both in normal and diabetic animals, which would mean that its utilization is independent of the presence of insulin. If this should prove to be the case it is clear that feeding with dihydroxyacetone in diabetes, as was first proposed by Emil Fisher, would circumvent the necessity for routine injections of insulin and would prevent development of ketosis.

Isaac and Adler have found that administration of dihydroxyacetone to normal persons, or to diabetic patients under treatment with insulin, causes lactic acid to appear in excess in the blood and urine, whereas when given to diabetic patients untreated with insulin extra sugar is formed instead. Rabinowitch recommends that dihydroxyacetone should be used in the treatment of severe diabetic coma, and he considers that the beneficial effects which he observed were due to direct oxidation of the dihydroxyacetone, the evidence being that the R.Q. rose from 0.706 to 0.817 in a patient to which it was given. Mason also found that the R.Q. rose more markedly both in normal persons and diabetic patients after the ingestion of dihydroxyacetone, than after glucose, and Lambie and Redhead have confirmed this, both the R.Q. and the total oxygen consumption attaining a maximum in from 20 to 30 minutes after feeding with dihydroxyacetone whereas after glucose 45 minutes was required. It may be remarked in passing, that the results recorded by the latter authors (6-minute observations by the Douglas bag method) are decidedly irregular, the R.Q. in certain of the diabetic cases, prior to giving sugar or dihydroxyacetone, being as low as 0.50, and after ingestion of the latter substance rising sometimes to 1.10.

Lambie and Redhead quite properly point out that the more rapid response of the metabolism to dihydroxyacetone than to glucose, in these observations, may be due to its being more quickly absorbed from the alimentary canal. They have accordingly repeated the observations following slow (5-minute) intravenous injection, of 20 gm. of either glucose or dihydroxyacetone. Both the R.Q. and the O_2-consumption rose gradually after glucose, coinciding with the passing off of the hyperglycaemia, and maximal values were reached in about 30 minutes, when the metabolism was 16 per cent above the basal level in a

healthy person, and somewhat less than this in a diabetic. After dihydroxyacetone, on the other hand, the above values rose immediately, along with the blood sugar, the maxima being reached at the time the injection was completed, when the metabolism was 57 per cent above the basal level. While admitting that dihydroxyacetone can be converted into glucose in diabetes, these authors consider that a certain amount is directly oxidized when it is suddenly added to the blood. They have also found that the inorganic phosphates of the blood fall more rapidly after administration of dihydroxyacetone, than after glucose. It is possible that the rise in R.Q. and the changes in phosphates in these experiments were dependent upon a driving out of carbonic acid from the blood as a result of the conversion of some of the dihydroxyacetone into lactic acid. This, we know, occurs in normal persons and it may also occur, along with the formation of glucose, in patients with incomplete diabetes.

Reference must also be made to an experiment by Kermack, Lambie, and Slater, in which an eviscerated, decerebrated cat was first of all injected for a period of 45 minutes with glucose at the rate of 0.15 gm. per Kg. an hour and then, for a period of 40 minutes, with similar amounts of dihydroxyacetone. Since no fall occurred in the percentage of blood sugar during the injection of glucose, but did occur to a certain extent during the injection of dihydroxyacetone, without any of this substance being recognizable by chemical means in the blood, the authors conclude that the latter must have been directly utilized. But the conclusion is unwarranted, since, apart from the fact that only one experiment is recorded, the influence of the liver was not entirely eliminated, some circulation of blood through it being still possible, by way of diaphragmatic and other anastomatic veins.

It will be evident that these results cannot be accepted

as proof that dihydroxyacetone is directly utilized by the tissues. On the contrary it has, in my judgment, been conclusively shown by Campbell, Fletcher, Hepburn, and Markowitz that this substance, like others chemically related to it, is converted promptly and completely into glucose in diabetic animals and less completely so in normal animals (some lactic acid being also formed). Its apparent antiketogenic influence, its power to form glycogen and its effect on the respiratory exchange are entirely dependent on this conversion. We will now examine the evidence of these workers.

In the first place, no dihydroxyacetone could be detected in either normal or diabetic blood by a method elaborated by Campbell, which depends on the fact that dihydroxyacetone at boiling temperature reduces acid phosphate molybdate solutions directly, forming a blue color. The amount of reduction may be determined either colorimetrically or, preferably, by a titrametric method, in which the reduced blue solution is reoxidized with standard permanganate. Glucose also reduces the above reagent to a very slight degree (1-180 as great), and this is allowed for in calculating the results. The accuracy of this method has recently been confirmed by McClellan. After giving dihydroxyacetone by mouth considerable amounts appear in the blood without any escaping with the urine, unless large amounts are ingested, and as the dihydroxyacetone disappears from the blood, the percentage of blood sugar increases. This relationship has been clearly shown (Campbell and Hepburn, Kermack, Lambie and Slater) both in rabbits and in patients while under the influence of large doses of insulin. When dihydroxyacetone was given the blood sugar rose and the hypoglycaemic symptoms disappeared. Now, since it is known that glucose is conspicuously more efficient in the relief of these symptoms than any other sugar, or sugar derivative, with the possible exception of mannose, it may

be concluded that the dihydroxyacetone was promptly converted into it. In agreement with this view, it was found, by Campbell and Hepburn, that recovery from insulin convulsions in rabbits did not occur so promptly when dihydroxyacetone was injected intravenously as when glucose was injected, although Lambie and his co-workers deny that this difference exists. Subcutaneously injected, the two substances have similar effects.

It will be noted that the evidence, which has been considered to show that the tissue can directly oxidize dihydroxyacetone in diabetes, is based on observations made on clinical cases of the disease. Now, quite apart from the possible fallacies in the observations themselves, to which we have already alluded, there is a serious objection to accepting such evidence, on the ground that diabetes in man is seldom if ever complete. A much more reliable test object is a depancreatized dog, in which there can be no doubt on this score. When given to such animals, Campbell and Markowitz found that dihydroxyacetone appears almost quantitatively in the urine as glucose. Thus, in each of five dogs which had been depancreatized some weeks previously and kept on insulin until a few days prior to the experiment, the following quantities of *extra* glucose appeared in the urine, passed during the 24 hours following the ingestion of 20 gm. of dihydroxyacetone: *1.* 17.8 gm., *2.* 17.3 gm., *3.* 16.0 gm., *4.* 20.2 gm., *5.* 23.5 gm. These results were obtained by subtracting from the total glucose excretion the glucose derived from body protein, which, following the usual practice, was calculated from the G:N ratio of the day, or days, preceding that of the observation. Since more urine was usually excreted on the test day than on those preceding it (owing to the diuretic effect of the extra glucose), it is possible that some nitrogen previously retained in the tissues, on account of their dehydration, which is common in diabetes, may have been washed out,

so that less glucose than that allowed for would come from protein. In any case, the correspondence between intake and output, is as close as that observed after ingestion of glucose itself.

If dihydroxyacetone were directly utilized by depancreatized animals it should raise the respiratory quotient, since its general formula is $C_3H_6O_3$. This, however, it does not do, on the contrary, if anything, it depresses the quotient. The animals, on the third, fourth or fifth days after removal of insulin and food, were kept under observation in the respiratory cabinet for 3 hours in the forenoon and the respiratory exchange measured during the second and third hours. Dihydroxyacetone (20 gm.) was given in solution by stomach tube about noon, and the respiratory exchange measured from hour to hour during the afternoon. The results of a typical experiment are shown in Table XI.

TABLE XI

Dog 1. *Weight* 4.46 *Kg. Fifth Day after Insulin*

TIME	O_2 CC.	CO_2 CC.	R.Q.
10:30–11:30 a.m.	3.675	2.489	0.677
11:30–12:30 a.m.	3.430	2.402	0.700
	20 gm. dihydroxyacetone		
1:10–2:10 p.m.	4.485	2.973	0.663
2:10–3:10 p.m.	4.260	2.754	0.646
3:10–4:10 p.m.	3.542	2.428	0.686
4:10–5:10 p.m.	3.754	2.494	0.664

Corresponding results were obtained on three other dogs in one of which the observations were repeated on four successive days, beginning with the fifth after removal of insulin and food. Since vomiting was troublesome in this animal small doses of insulin (2.4) had to be injected on

three of the days. It is of significance that this did not affect the nature of the results.

The reputed influence of dihydroxyacetone in decreasing the excretion of ketone bodies in clinical diabetes (Rabinowitch) cannot be demonstrated to occur in the case of depancreatized dogs. This will be evident from the results shown in Table XII.

TABLE XII

Milligrams Ketone Bodies Excreted in Urine on Days Indicated after Removal of Food and Insulin

DOG	2ND	3RD	4TH	5TH (20 GM. DIHYDROXY-ACETONE)	6TH	7TH	8TH	9TH
1	....	139	741	1896				
3	....	28	245	290				
5	60	1338	2715	2735				
6[1]	....	....	1836	3693[1] (no dihydroxy-acetone)	2.010[1] (20 gm. dihydroxy-acetone)	2.220	2.371	2.358

[1] 2-4 units of insulin had to be given on these days to control vomiting.

The high excretion of ketone bodies in dog 6 is probably owing to this animal having been unusually fat at the time the observations were made. It also gave evidence of being dehydrated, and it vomited frequently.

It is well known that the inorganic phosphates of the blood and urine undergo remarkable changes during various disturbances of carbohydrate metabolism. Administration of excess of glucose to normal fasting animals causes the phosphates of the blood to decrease, whereas those of the urine, although decreased at first, subsequently rise above the normal level, so that in the 24 hours there is, if anything, somewhat more than usual. In depancreatized dogs, however, glucose has no effect on the phosphates,

either of blood or urine. Rabinowitch found that glucose caused no change, whereas dihydroxyacetone caused depression in the blood phosphates in diabetic patients, and he claims this as evidence of a predilection of the diabetic organism for dihydroxyacetone. Lambie and Redhead have confirmed these results, with the exception that glucose depressed the blood phosphates to a certain degree. Campbell and Markowitz on the other hand, using depancreatized dogs, observed an increase in the urinary output of phosphates on the day dihydroxyacetone was fed. All three groups of observers agree that in normal persons the blood phosphates are similarly depressed by glucose and dihydroxyacetone. In view of the mystery which surrounds the behavior of the phosphates during disturbances of carbohydrate metabolism it is inadmissible to conclude that because dihydroxyacetone causes the blood phosphates to become more depressed than does glucose, the former is directly utilized. Perhaps the disturbance is occasioned by disturbances in the ionic equilibrium of the blood, due to the production of lactic acid. Investigation of the effect of other substances which are known to be transformed into glucose in diabetes, such as glycerol or lactic acid, might have thrown some light on this question.

All the evidence obtainable from observations on completely depancreatized dogs shows, therefore, that dihydroxyacetone is not used for combustion in the tissues, until after it has been converted into glucose. But objection may be raised to considering the results of experiments made on completely depancreatized animals as applicable to diabetes in man, on the score that the islet tissue in this disease is never so far destroyed but that some insulin is still present in the body. In order to obtain an experimental form of diabetes comparable with the clinical, various observers, such as Sandmeyer, Allen and Hédon, have used partially depancreatized dogs, but a better method is to

give a fractional dose of insulin to one from which the pancreas has been totally removed. The animal is then in a state of constant partial diabetes, the intensity of which can be controlled, by altering the dosage of insulin and the amount of carbohydrate in the food. This is a much more suitable experimental condition on which to test the influence of various factors on diabetes than either a partially depancreatized dog or a diabetic patient, for the reason that the amount of insulin in the body does not vary from day to day, as it must do when some pancreas is left. In the latter case various conditions, such as nervous excitement, muscular exercise, ingestion of foods and the administration of drugs may stimulate the secretion of endogenous insulin, thus causing changes to occur in metabolism which cannot be controlled or accounted for.

In order to determine whether a substance, such as dihydroxyacetone, can be utilized in preference to glucose the sugar of the daily ration is replaced by an equivalent amount of the substance. If no change occurs in the excretion of sugar it can be concluded, with certainty, that there has been no preferential utilization.

In observations of this type Campbell and Markowitz found that the substitution of dihydroxyacetone for glucose, during a period of four days, did not affect the daily average of sugar excreted, as shown in Table XIII, opposite. In two other similar observations a slight decrease occurred during the dihydroxyacetone period, but this was almost certainly due to some of this substance being retained as such in the tissues, so that it was not converted into glucose until after administration of dihydroxyacetone had been discontinued. This probably accounts for the greater sugar excretion which was observed to occur during the first few days after returning to sugar.

Having shown that dihydroxyacetone is converted into glucose before it is used, both in the diabetic and the normal

TABLE XIII

DAY	CARBOHYDRATE FED	EXCRETION		AVERAGE OF SUGAR
		NITROGEN	SUGAR	
1	glucose 40 gm.	13.4	(27.8[1])	
2	"	13.3	19.5	
3	"	13.9	16.7	18.1
4	dihydroxyacetone 4 gm.	13.8	18.3	
5	"	13.6	22.7	
6	"	13.6	19.3	
7	"	13.4	18.2	19.6
8	glucose 40 gm.	14.7	23.1	
9	"	12.5	23.9	
10	"	12.4	17.8	
11	"	14.4	21.8	21.4

[1]This high excretion is omitted from the average since the animal was only placed on the weighed diet on the previous day.

animal, it remains to consider in which organ of the body this conversion occurs. When dihydroxyacetone is added to the blood of depancreatized dogs deprived of insulin, the concentration of blood sugar rises, as that of dihydroxyacetone falls. This is illustrated in the results shown in Table XIV taken from an experiment in which the animal had been deprived of insulin for four days prior to the experiment. The dihydroxyacetone was determined by the method which we have already outlined, and the blood

TABLE XIV

TIME	DIHYDROXYACETONE PER CENT	GLUCOSE PER CENT	
2:25 p.m.	0.0	0.278	
2:32 p.m.			20 gm. dihydroxyacetone by mouth
2:54 p.m.	0.123	0.312	
3:23 p.m.	0.180	0.388	
3:50 p.m.	0.121	0.450	
4:17 p.m.	0.077	0.440	
4:44 p.m.	0.040	0.460	
5:27 p.m.	0.007	0.470	

sugar, by subtracting from the total reducing power that of the dihydroxyacetone. These results corroborate those obtained on rabbits exhibiting insulin hypoglycaemia. They leave no doubt that glucose is formed out of dihydroxyacetone and the question arises as to where in the body this conversion occurs.

It has already been shown by Embden and his co-workers that some sugar is formed when dihydroxyacetone is added to blood which is perfused through an excised liver. Although this does not prove that the liver is the only place in the body in which the transformation can occur, it indicates that this could be ascertained by seeing whether sugar is still formed by giving dihydroxyacetone to animals in which this organ is removed from the circulation. Campbell and Markowitz have essayed to do this in various ways. In the first series of experiments they anastomosed the portal vein with the inferior vena cava (Eck fistula) and then tied these vessels, as well as the hepatic arteries, thus depriving the liver of its main circulation (functional hepatectomy). After the animal had recovered from the anaesthetic the blood sugar steadily fell, and when it had reached 0.040 per cent and the symptoms of hypoglycaemia were pronounced, 5 gm. of dihydroxyacetone were injected intravenously. Dihydroxyacetone then declined gradually in the blood, accompanied by a slight increase in sugar (to 0.060), and recovery from the symptoms was slow and imperfect, in striking contrast with the prompt effect which dihydroxyacetone has in raising the blood sugar and in removing the symptoms of hypoglycaemia in animals in which the liver is intact.

These results indicate that the liver must have something to do with the transformation of dihydroxyacetone into glucose, but they nevertheless leave undecided the main question, as to whether it is exclusively responsible. The

possibility that sufficient circulation of blood remained in the liver, through vessels passing between it and the diaphragm, to permit of its still having some influence made it necessary to repeat the experiments after complete hepatectomy. The principle of the methods for doing this have been outlined elsewhere and two experiments in which dihydroxyacetone was injected were successfully carried through by Campbell and Markowitz, with the results shown in Table XV.

The first animal was allowed to become hypoglycaemic, but this was prevented in the second one, by injecting considerable quantities of glucose along with the dihydroxyacetone. At the beginning and end of each observation, pieces of muscle were excised, and free sugar and dihydroxyacetone were determined in watery extracts of them. In both experiments the percentage of dihydroxyacetone in the blood fell somewhat during the 30 minutes immediately following its injection, but thereafter the percentage remained practically unchanged. This initial fall was no doubt due to diffusion of dihydroxyacetone into the tissues, in which a percentage not much inferior to that present in the blood was found to exist. Indeed, if we assume that the same percentage as that found in the sample of muscle also existed in 75 per cent of the animal (Bollmann, Mann, and Magath) then 7.1 and 5.3 gm. of dihydroxyacetone was accounted for in the two animals, respectively, and if we also make allowance for that remaining in the blood these figures become 10 gm. and 8.2 gm. practically agreeing with the amounts injected. Of course, the assumptions on which these calculations are based may not be warranted, but nevertheless there is nothing in either observation to indicate that any of the administered dihydroxyacetone was utilized or changed.

Because of the assertion by Rabinowitch, and others, that dihydroxyacetone can be directly used in diabetes, Camp-

TABLE XV

TIME	CONDITION AND INJECTIONS	BLOOD		MUSCLE	
		SUGAR PER CENT	DIHYDROXY-ACETONE PER CENT	SUGAR PER CENT	DIHYDROXY-ACETONE PER CENT
I					
11:00 a.m.	Evisceration complete				
1:45 p.m.	Convulsions. 0.75 gm. glucose intravenously[1]	0.030	0.0	.....	.....
2:00 p.m.	Unconscious. Piece muscle removed			0.154	0.0
2:35 p.m.	Convulsions. 9 gm. dihydroxyacetone and 2 gm. glucose intravenously	.....	.....	.....	.....
3:10 p.m.		0.027	0.136	.....	.....
3:40 p.m.	Symptoms less	0.014	0.127	.....	.....
3:50 p.m.	Breathing irregular. 20 gm. glucose intravenously	.....	.....	.....	.....
4:05 p.m.	Breathing normal	0.210	0.127	.....	.....
4:30 p.m.		0.236	0.130	.....	.....
4:53 p.m.		0.170	0.127	.....	.....
5:10 p.m.	Condition satisfactory. Piece muscle removed	.....	.....	0.133	0.090
II					
11:00 a.m.	Evisceration complete. Muscle removed	.....	.....	0.387	0.0
11:40 a.m.		0.10	0.0		
11:45 a.m.	3.5 gm. glucose and 9 gm. dihydroxyacetone				
12:00 m.		0.205	0.167		
12:35 p.m.	Fully conscious	0.106	0.134		
12:40 p.m.	5 gm. glucose				
1:10 p.m.		0.304	0.121		
1:35 p.m.	2 gm. glucose[1]	0.275	0.120		
1:45 p.m.	Unconscious. Muscle removed	.....	.....	0.308	0.078

[1]The glucose in these cases was injected after removal of blood for sugar examination.

bell and Markowitz also conducted an observation similar to the foregoing on a depancreatized dog. In two hours after hepatectomy the percentage of blood sugar was 0.050 and there were convulsions. Injection of 10 gm. of dihy-

droxyacetone caused 0.217 per cent of this substance to appear in the blood, in which, however, the percentage of sugar continued to fall until arrested by injection of glucose. In an hour and a half after the injection, the percentage of dihydroxyacetone was still 0.180.

Even at risk of repetition I wish to point out that these observations on hepatectomized dogs show beyond all doubt that *dihydroxyacetone cannot be used by the tissues directly, but must first of all be transformed into glucose in the liver*. The apparent predilection of diabetic patients for this triose, as claimed by Rabinowitch, Mason, and Lambie and his co-workers, is possibly due to its having some influence in stimulating the internal secretion of insulin, but until other similar substances have been tried one cannot be certain.

Many substances besides dihydroxyacetone have been shown to have an influence on the hyperglycaemia and other symptoms in clinical forms of diabetes, such as guanidin compounds and certain vegetable extracts, but no one has suggested that this depends on their being directly used by the tissues. If dihydroxyacetone may appear to show some effect in certain cases of diabetes, which cannot be adequately accounted for by its being transformed into glucose, may it not be because of some action similar to that of guanidin compounds?

Fructose. There is an impression in clinical circles that fructose, or laevulose, is better tolerated by the diabetic patient than glucose. It has accordingly been a common practice to use this sugar in preference to sucrose when it is considered advisable to allow some preformed carbohydrate in the diabetic diet. The justification for this practice, which was instituted by Külz, has been most thoroughly and carefully investigated by metabolism experiments conducted by Joslin, and his final conclusion is that fructose raises both the respiratory quotient and the heat

production more than glucose, indicating, perhaps, that it is more readily converted into fat. Joslin, however, is not emphatic in recommending the use of this sugar in diabetes.

Observations on normal persons and laboratory animals also indicate that fructose does not behave like glucose in metabolism. When given by mouth practically no rise occurs in the blood sugar level, which shows that fructose cannot be immediately converted into glucose in the organism, neither does sugar appear in the urine provided chemically pure preparations are used (Folin and Berglund). The respiratory quotient also rises more markedly after ingestion of fructose than after glucose, indeed the former sugar may cause the quotient to rise to over unity, at least for short periods.

Minkowski considered that fructose caused glycogen to be deposited in the liver when it was fed to depancreatized dogs, whereas glucose could not do so, but Cruickshank has been unable to confirm Minkowski's results. Our results in this regard are contradictory; in one depancreatized dog the liver was found to contain 1.23 per cent of glycogen after the animal had been given large quantities of sucrose during each of the three days preceding death, while in another similarly treated animal only a trace was detectable.

Perhaps the most convincing evidence showing that fructose runs a different course in metabolism from that of glucose, is furnished by comparison of the effects of the two sugars in antidoting the symptoms of hypoglycaemia due to insulin, or to hepatectomy. Fructose, given subcutaneously or intravenously is decidedly inferior to glucose in this regard (Noble and Macleod, Mann and Magath), although it causes the blood sugar (reducing power) to rise, which, as we have seen, is not the case when this sugar is given in moderate amounts to normal animals. The slight improvement in the symptoms, with frequent

remissions, which may follow the administration of fructose suggests the possibility that some may be converted into glucose although too slowly to make it an effective antidote. These relationships of fructose and glucose with regard to their effects on hypoglycaemia correspond to the relative fermentability of the two sugars by yeast.

But the question, as to whether fructose can be utilized for the production of energy in the diabetic animal in preference to glucose is not answered by these findings. On theoretical grounds it does not seem likely that there is any difference, since the glycogen which is formed in the liver when fructose is fed to normal animals is apparently of the same nature as that formed from glucose, or from protein, or from fat; thus, it yields glucose, and glucose alone, on hydrolysis, whatever may have been its source.

How, then, may we obtain a definite answer to our question? It seems to us that the only way is by comparing the carbohydrate balance in completely depancreatized dogs during different periods in which one or other of these sugars is given. This has been done by Campbell and Markowitz, and, in order that the diabetes might be comparable with that met with clinically, the method was adopted of giving a certain fixed amount of insulin, 10 units, twice daily at the time the animals were fed. The observations were divided into three periods, each of three or four days duration, and 40 gm. of either sugar was given daily. A typical result is shown in Table XVI. Similar observations were made on two other dogs and the grand averages of the excretion of glucose for all the days on which glucose was fed were: Dog 1 (including another series of observations like those shown in the table) glucose 21.56, fructose 21.35. Dog 2 (two series of observations), glucose 26.5, fructose 21.5. Dog 3 (one observa-

TABLE XVI

	DAY	EXCRETION IN URINE		AVERAGE GLUCOSE EXCRETION FOR PERIOD
		NITROGEN	GLUCOSE	
Period 1. Glucose given	1	14.7	23.1	
	2	12.5	23.9	
	3	12.4	17.8	
	4	21.8	21.8	21.4
Period 2. Fructose given	5	13.6	27.7	
	6	13.8	26.5	
	7	13.8	25.9	
	8	14.1	22.5	25.1
Period 3. Glucose given	9	14.5	29.0	
	10	12.9	13.2	
	11	14.0	20.2	20.8

tion) glucose 10.85, fructose 13.0.[1] These results offer no evidence of a preferential utilization of fructose. It is nevertheless worthy of note that considerable fluctuations occurred from day to day in the amounts of glucose excreted, although the nitrogen was remarkably constant. These fluctuations can not be attributed to technical errors, for every possible care was taken both in the collection and the analysis of the urines. They are the rule, in experiments of this type and may depend on variations from day to day in the amount of muscular exercise and excitement (barking) of the animals. We can see no justification for giving fructose to diabetic patients.

I have discussed in some detail the metabolic significance of three substances, acetaldehyde, dihydroxyacetone and fructose, because I consider them the most suitable to use for testing the hypothesis, that formation of d-glucose is an essential stage in carbohydrate metabolism. This, I think, is proved beyond all peradventure of doubt in the case of the last two substances and, although it cannot

[1] Only traces of fructose were found present in the blood and urine during the periods in which this sugar was fed.

be proved in the case of acetaldehyde, on account of its toxic nature, it is at least probable, in view of the fact that small amounts are always present in the blood and tissues, that this substance is formed as an intermediate stage in the transformation of fatty and of amino acids into glucose.

It would serve no useful purpose if I were to tell you of the observations which have been made with the other possible intermediaries of carbohydrate metabolism, to which I referred at the beginning of the chapter. Suffice it to say that when tested by the method of preferential utilization in depancreatized dogs, Campbell and Markowitz found that glycerol is utilized to the same extent as glucose, thus leaving little doubt that the fairly satisfactory restorative action which this substance sometimes exhibits in hypoglycaemic convulsions, is to be attributed to its conversion into glucose.

We have not investigated the behavior of pyruvic acid or methylglyoxal, but there is nothing to indicate that either substance can replace glucose in metabolism; they probably form lactic acid. Apart from the difficulty in obtaining adequate quantities of these substances, there is little encouragement to study their behavior when given to diabetic animals since they do not alleviate the symptoms of hypoglycaemia (Lambie *et al*).

The initial changes in glucose in metabolism. All the evidence which we have just reviewed points to glucose formation as an essential step towards the utilization, not only of preformed carbohydrates, but also of the various smaller molecules into which it may be split. There is no doubt that it is through the formation of these smaller molecules that amino acids can form carbohydrate, and there does not seem to be any chemical reason why a similar fate should be considered impossible in the case of the higher fatty acids. The well known oxidation of these at the β-carbon atom

causes molecules of two carbon atoms each to be split off; usually it is supposed that these are directly oxidized, but is it not more likely, in the light of the evidence which we have just considered, that three such molecules, perhaps as acetaldehyde, are condensed to form glucose, and then glycogen?

But glucose, as we know it, seems too stable a substance to be used immediately in metabolism and this fact is responsible for the prevailing belief that it must be changed into some more reactive form, as the first step in its metabolism. When we proceed to investigate its immediate fate in the animal body, by the chemical means at our disposal, we find that, instead of being broken down, much (if not all) of the glucose which is added to the circulating blood becomes built up into glycogen, partly in the liver and partly in the muscles. It has been customary to regard this as a process having the same physiological significance as that by which starch is formed in plant life; that is to say, as one for the storage of carbohydrate which is to be subsequently used when no more glucose is available in the body. To a certain extent this is, no doubt, a function of glycogen, but, as I suggested some years ago, and as Cramer has more recently insisted upon, glycogen may have a far greater significance than that implied in considering it merely as a storage material. Cramer has done well to point out that Claude Bernard, the discoverer of glycogen, considered this substance as an internal secretion of the liver derived, not from preformed carbohydrates alone, but from other foodstuffs as well, thus suggesting that it has a higher function than that of being merely a storage material.

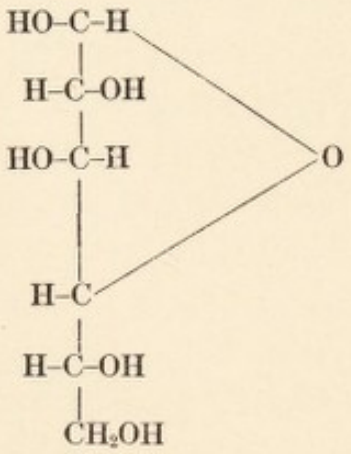
In a previous chapter we saw that when the glycogen of the muscles breaks down, either *post mortem* or as a result of exercise, lactic acid is formed instead of glucose. This would seem to show that although it is originally derived

from the sugar supplied by the blood, glycogen never again returns to sugar within the muscle itself, but is converted into lactic acid. To this extent it has entered an irreversible reaction, thus: Glucose → glycogen ⇆ lactic acid. On the other hand when the glycogen of the liver breaks down glucose rather than lactic acid is formed, and the question arises as to whether this glucose is exactly of the same nature as that out of which the glycogen was formed.

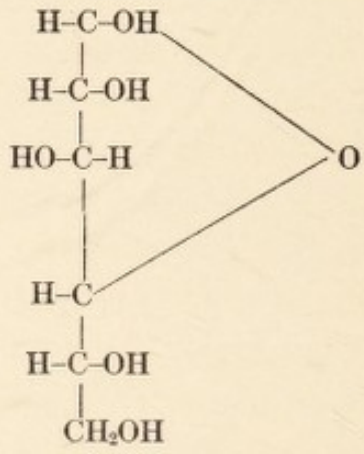
That the muscles can use glucose which has not gone through a glycogen stage in the liver is shown by numerous facts, among which the following may be mentioned: (1) the immediate effect of injections of glucose in removing the symptoms of hypoglycaemia even in the absence of the liver; (2) the increased respiratory exchange (O_2 consumption) in the isolated heart, in eviscerated cats and in hepatectomized dogs when glucose is injected. It may be in all these cases that the foreign glucose passes through a glycogen stage in the muscles themselves before it is actually used, a view which receives some support in the fact that glycogen is formed in the muscles when glucose is injected into eviscerated cats along with insulin (Best, Hoet, and Marks) and also, to a less extent, in intact animals without the injection of insulin (Choi). In any case, the possibility has to be borne in mind that some definite change may occur in the glucose molecule itself preceding its entry into any one of these processes, and it is with a consideration of this possibility that I bring the present chapter to a close.

As is well known, glucose when dissolved in water exists at first as α-glucose with a high rotatory power $\alpha_{[D]}$ 110° but soon changes in part into another variety, β-glucose, with a low rotatory power, $\alpha_{[D]}$ + 19°. This causes the rotatory power of the solution gradually to fall until a stable mixture of the two varieties, giving a $\alpha_{[D]}$ of 52.5°, is formed. The addition of weak alkali greatly accel-

erates this change. The possibility has been considered that one of these glucoses may be more readily used in animal metabolism than the other, but the only evidence of any such difference is that more lactic acid accumulates in excised frog muscles when α-glucose is added than with β-glucose (Laquer and Griebel). In recent years it has been shown, through the work of J. C. Irvine and his school, that there must exist still a third variety of glucose, called gamma (γ), which is characterized by being less stable than α- and β-glucose and by rotating polarized light to the left, instead of to the right. The structural differences of the α and β varieties are expressed in the following formulae:



α-glucose



β-glucose

from which it will be noted that they differ from each other in the relative positions of the H and OH groups attached to the terminal C atom, which again is linked in a ring formation with the fourth C atom. In γ-glucose, on the other hand, this linkage occurs with some other C atom, perhaps the third, thus accounting for its lesser stability. All three varieties have the same reducing power when this is measured in the usual way (by heating the sugar in alkaline solution in the presence of metallic hydroxides such as cupric hydrate), and the only method by which the presence of γ-glucose can be detected is by comparing the

rotating, with the reducing power of solutions supposed to contain it.

Hewitt and Pryde were the first to investigate the possibility that the highly reactive γ-glucose might occur in the animal body, and they claimed that it was formed through the agency of the intestinal epithelium. Exact repetition of their experiments, both by Stiven and Waymouth Reid and by Eadie, failed to corroborate the results. Shortly after insulin became available, Winter and Smith announced that the rotatory power of protein-free filtrates of the blood of normal animals, and still more so of those that were under the influence of insulin, was lower than what would be expected from the reducing power, thus revealing the presence of γ-glucose, whereas the two values were in a reversed relationship in the blood of diabetic animals and patients, showing not only that this form of glucose was absent but that others with lower reducing power (disaccharides) occurred. They claimed further that the presence of insulin was necessary to produce γ-glucose. Repetition of Winter and Smith's experiments in my laboratory, by Eadie, failed to support their hypothesis, for although some slight discrepancies between the rotatory and the reducing powers were sometimes found to occur in protein-free blood filtrates, these could be attributed to various other causes than the presence of γ-glucose.

The Winter and Smith hypothesis seemed to have passed into oblivion, as many hypotheses do, but it has been resurrected in a new guise by Lundsgaard and Holbøll. These workers stated that the addition of insulin to solutions of glucose (1-2 per cent) in the presence of freshly excised mammalian tissue caused, after 2 hours incubation at body temperature, a lessening of the rotatory power without any corresponding change in reducing power. To rid them of proteins the incubated solutions were dialyzed

through collodion sacs, which was a decided improvement over the complicated method used by Winter and Smith. As far as one could judge from the first published communications of Lundsgaard and Holbøll, the observed changes were quite large, although no actual polariscopic readings were reported, but only percentile changes.

In view of the great importance which such results as these would have, Lundsgaard and Holbøll's chief experiments were repeated exactly according to the published directions by A. D. Barbour, but with entirely negative results. Using a Hilger polarimeter and a monochromatic light source (mercury vapor lamp) it was impossible, in incubated mixtures of glucose and freshly excised muscle, to demonstrate that the least discrepancy ever occurred between the rotatory and reducing powers of glucose, after making allowance for experimental errors. Since experimental conditions were chosen which, according to the Danish observers, should have given the most striking results, Barbour did not examine their numerous other claims with regard to this supposedly new form of glucose. Hagedorn and others have also obtained negative results.

As this matter stands at present, there is no evidence that glucose is changed into any new form, as a first step in its metabolism in the animal body.

Tissue reductase. It would take us beyond the limits set for this discussion to consider, in any detail, the long series of experiments which, in recent years, have been carried out by Ahlgren, with reference to the possibility that insulin and other hormones, or drugs, affecting carbohydrate metabolism may have some influence on tissue oxidation. Brief reference to some of the results is necessary, however, since their interpretation is based on the assumption that loss of the power of the tissues to utilize glucose is the underlying cause for the hyperglycaemia and glycosuria in diabetes (Ahlgren). To investigate the oxidative powers

of the isolated tissues, Ahlgren has used two methods: (1) the rate of decolorization of methylene blue *in vacuo* and (2) measurement of the respiratory exchange, by the use of a microrespirometer. In both methods portions of tissue, such as muscle, are cut up into small pieces and then examined immediately, so that any changes which are observed to occur in them may be assumed to be of the same nature as those taking place during life, instead of being merely *post mortem* changes.

The methylene blue method depends on the following hypothesis: When oxidation occurs in tissues there is a transfer of hydrogen from the substance which is oxidized to the substance which yields the oxygen. The former is therefore called a hydrogen donator and the latter, a hydrogen acceptor, and it is supposed that the transfer is dependent on a factor which is present in the tissues itself, called hydrogen transportase, or mutase. Methylene blue acts as an acceptor, and it is rapidly decolorized by the donators present in unwashed tissue, but not by tissue from which these have been removed by washing. We can therefore determine what substances may act as donators, by seeing whether their addition to well-washed tissue suspensions accelerates bleaching, and Thunberg has found that many substances which are possible intermediaries of carbohydrate metabolism act in this way. The important point for us is that glucose itself cannot act as a donator, but becomes capable of doing so when insulin is also present, from which it is concluded that the insulin, in some way, prepares glucose for oxidation (Ahlgren).

Were this conclusion dependent merely on these experiments we might hesitate in accepting it as applicable to the living animal, but it is supported by observations on the respiratory exchange of excised tissue. In this method three separate portions of the cut-up tissue are first of all stirred in buffered solutions, one containing glucose, a

second, insulin and a third, both glucose and insulin. The tissue is then removed from each vessel and spread out on a piece of gauze which is placed in the chamber of the microrespirometer containing an atmosphere of oxygen (Ahlgren, Brahme). After two hours the amounts of O_2 used and of CO_2 are computed from the changes in the levels in the microrespirometer. In a typical experiment the following results were obtained:

Gas Exchange in Cubic mm. per gm. Muscle per hour

	CONTROL	GLUCOSE	INSULIN	GLUCOSE + INSULIN
O_2	142	133	141	207
CO_2	145	130	138	216

It is again evident that oxidation has occurred more actively when both glucose and insulin were present.

I must confess that my mind is not made up, as to the exact significance of these interesting experiments, and although lack of experience with work in the field of tissue oxidations disqualifies me as a competent critic, it may not be out of place to offer some comments on the far-reaching conclusions which are drawn from the results.

I may say, at the outset, that Markowitz and I have repeatedly been able to show, with washed portions of the heart of the rabbit, that decolorization of methylene blue is accelerated by the addition of insulin. Ahlgren's technique was rigidly followed, and it did not vary in different experiments, unless with regard to the amount of insulin which was added to the different tubes. We often obtained entirely negative results and this may have depended on the, now known, fact that the rate of decolorization becomes progressively accelerated and then retarded as the concentration of insulin is increased. Ahlgren has since shown that similar relationships exist when the respiratory exchange of the tissue is measured. Now we venture to suggest that such a result detracts from the significance of the

findings, for there is no evidence that insulin acts in this manner when progressively increasing doses are injected into different animals. That massive doses may suppress instead of raising the respiratory exchange, especially in small animals, is no evidence that its effect on tissue oxidation has been reversed, as Ahlgren seems to think.

Various other workers have failed to obtain the same results as Ahlgren. Thus, Cloedt and Canneyt, using the dinitrobenzol method of Lipschitz, could detect no accelerating influence of insulin, although Ahlgren obtained positive results with this substance, by varying the concentration of insulin. The essential experiments by the methylene blue method have, however, also been confirmed by Gigon and by von Bliz.

It is more particularly with regard to the respiratory experiments that the results of different observers are at variance. Thus Grafe, who measured the exchange by means of Barcroft's apparatus, and who used very pure preparations of insulin, could not demonstrate that they had any influence on the rate of tissue oxidation. Negative results were also obtained by Heymans and Matton, and Azuma and Hartree did not find that the addition of insulin (without glucose) to the solutions in which the excised sartorius muscle of the frog was suspended had any influence on the heat production.

To explain all of these negative results, Ahlgren points out that the concentrations of insulin used were too large, thus causing its inhibitory, rather than its accelerating, influence to come into play. To such a rebuttal there is at present no reply.

But there seems to me to be another general objection to the experiments, which is, that numerous other substances besides insulin have also been found to have an accelerating influence on the rate of oxidation in tissue suspensions. Thus, to take a few examples from among the 40 substances

(hormones and pharmaceutical agents) examined by Ahlgren, acceleration has been observed with cocain, ergotamin, pilocarpine, and with morphine and its derivatives. There is what Ahlgren considers to be a significant difference between the action of these substances and that of insulin, namely, that their accelerating influence is exhibited in the absence of glucose, depression actually occurring when this sugar is present.

Epinephrin also accelerates the process, and, like insulin, its concentration has a marked influence in that both very small and very large amounts retard it. Here again the mechanism of action is supposed to differ from that of insulin, not only because the presence of glucose is not essential, and even retards the process, but also because the effect of epinephrin persists in tissue for considerably longer after excision than is the case with insulin (Ahlgren). Other observers, quoted by Ahlgren, have also found this hormone to have an augmenting influence on the oxidative changes in isolated tissues (Adler and Lipschitz, Grafe and Martin, and Armitstead), but this has been denied by Hutchinson and Griffith. Of course, positive results in experiments of this type are more important than negative ones, but, nevertheless, I doubt whether they are really of significance.

These results are considered to be related to the calorigenic influence of epinephrin, which is manifested when it is injected into living animals, and yet Soskin has found in my laboratory that epinephrin does not have this effect when injected into hepatectomized dogs. Caskey has also been unable to show that epinephrin influences the heat output of the muscles after removal of the liver from the circulation.

Time and space will not permit of a further discussion of these interesting observations. The results appear to me to be significant but they are too bewildering in their mul-

titude, and the explanations which are offered for many of those which do not conform with others are often more ingenious than convincing.

In summing up the evidence which I have attempted to bring forward in this chapter, regarding the nature of the intermediary substances of carbohydrate metabolism, I would place particular emphasis on the significance of acetaldehyde and dihydroxyacetone. The former is certainly present in the blood and tissues of normal animals, and the fact that only traces are present does not detract from its importance, indeed may enhance it, by indicating that it rapidly changes into something else immediately it is formed. Is it impossible that acetaldehyde may be formed when the two carbon atoms of fatty acid are thrown off in the process of beta oxidation, and that three such molecules then condense to form glucose?

For dihydroxyacetone, on the other hand, I can see no useful rôle in metabolism. It cannot be formed during the breakdown of the muscular glycogen, and there is no reason to expect that it can occur as a step in the resynthesis of lactic acid into glycogen. Like many other substances including glycerol, lactic acid and several of the amino acids it can readily be shown to be converted into glucose in the diabetic organism, and no doubt it follows the same course in normal metabolism. If the metabolic significance of glyceric aldehyde and methylglyoxal could be investigated by methods similar to those which we have reviewed I have not the slightest doubt but that they would give corresponding results, but this cannot be done in the case of the former substance because of its toxic nature, or in that of the latter because of difficulty in preparing a sufficient amount of it.

We have also studied the behavior of fructose without being able to find for it a place in metabolism of any greater

significance than that of a precursor of glycogen. And finally, we have seen that it has so far been impossible to demonstrate that glucose undergoes any particular change preceding its condensation to form glycogen, which must therefore be considered as the first step in its metabolism.

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