SOME ASPECTS OF GLUCOSE METABOLISM IN NORMAL AND ALLOXAN-DIABETIC RATS

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SUMMARY

The *in vivo* incorporation of ^{14}C from orally-administered, uniformly-labelled glucose into expired CO_2, glycogen, and fatty acids has been studied with normal rats, alloxan-diabetic rats and diabetic rats treated with insulin. There was markedly less label in all three products in the diabetic rat. Treatment of the diabetic rat with an amount of insulin just sufficient to normalize the blood-sugar level restored to normal the utilization of glucose for production of CO_2 and synthesis of glycogen, but elevated the synthesis of fatty acids from glucose to considerably above normal.

In all three groups of rats a large part of the ingested ^{14}C was not recovered, after the compounds studied had been taken into account.

INTRODUCTION

Among the innumerable investigations conducted to discover the nature of the metabolic defects in diabetes and the manner in which they are corrected by insulin those using intact animals form a minority. This is understandable, for the intricate interplay of metabolic pathways generally makes the results difficult to interpret correctly. Consequently, our present insight into the derangements caused by diabetes and the action of insulin is in a large measure due to experiments performed with tissue preparations *in vitro*, notably liver, diaphragm and adipose tissue.

On the other hand it is not easy to infer from these most valuable experiments what exactly is taking place in the intact animal. So it seemed of interest to us to approach the problem again, using the whole animal, and armed with our present knowledge. In the investigation reported here we have studied the conversion into glycogen, fatty acids and respiratory CO_2 of uniformly-labelled [^{14}C]glucose, administered orally to normal rats, alloxan-diabetic rats and diabetic rats treated with insulin.

Of course, the idea to study the conversion of glucose along these major metabolic pathways in intact animals is not new. But in practically all experiments in which radioactive glucose was used for this purpose it has been injected in trace doses or in small amounts and never given by mouth. Moreover, whenever insulin has been given to diabetic rats, it has always been injected in large amounts, greatly exceeding that required to stop the urinary excretion of glucose and restore the blood-sugar level to normal. In our experiments we have taken pains to limit the dosage of insulin

to this necessary minimum. The present results are in harmony with those obtained by others, using different experimental techniques: in diabetes, the formation of CO_2, glycogen, and fatty acids from glucose is decreased. Insulin restores the first two processes to normal, but elevates the synthesis of fatty acids to considerably above normal.

MATERIALS AND METHODS

Treatment of animals

Wistar albino rats of the Wu-strain weighing 160–180 g were used (obtained from "Centraal Proefdierenbedrijf T.N.O.", Austerlitz, The Netherlands). They had free access to water and a commercial diet (supplied by the same address as above). Three hours before the start of an experiment the food was taken away.

Rats were made diabetic by a single subcutaneous injection of a 5% solution of alloxan-monohydrate in a citrate–phosphate buffer of pH 4, in a dose of 100 mg/kg body weight. On the day preceding the injection they were only allowed half the average amount of food they daily consumed. The diabetic condition was followed by measuring the volume of the urine excreted in 24 h and the glycosuria, while 2.5 weeks after injection a glucose tolerance test was performed and the response of blood sugar was measured during 2 h following an oral dose of 500 mg glucose. Diabetes was allowed to develop for three weeks and only those animals were used that excreted more than 1 g of glucose daily in a volume of urine exceeding 20 ml.

In the case of treatment with insulin, rats with a diabetes of 3 weeks' standing and a glucose excretion of 2.5 to 6 g/24 h received a first subcutaneous injection of 1 I.U. of protamine-zinc insulin (Organon, Oss, The Netherlands). The injections were repeated daily, increasing the dose by 0.5 I.U. each time, until the urine was permanently free of sugar (controlled with Benedict's test). It took about 10 days to achieve this state. The final daily doses of insulin given ranged from 3.5 to 4.5 I.U. On the day preceding the actual experiment a glucose tolerance test was performed. The rats were given 500 mg of glucose by stomach tube and at the same time 0.1 I.U. of crystalline insulin, free of glucagon, was injected to cope with the transitory hyperglycemia. This treatment proved to be adequate to prevent the blood sugar from rising above 170 mg/100 ml or dropping below 70 mg/100 ml during the two hours following this loading with 500 mg glucose.

Experimental procedure

After fasting for 3 h, the normal rats were given 500 mg glucose containing 20 μC of [^{14}C]glucose (uniformly labelled, obtained from the Radiochemical Centre, Amersham) by stomach tube, dissolved in 2 ml of water. The diabetic rats, also those treated with insulin, received the same amount of sugar, but now with 30 μC of [^{14}C]glucose.

Each animal was immediately placed in a cylindrical glass metabolism cage, which could be hermetically sealed and was equipped with a supply of drinking water and a device for collecting urine (see Fig. 1). Six of these cages were connected to an apparatus* similar to that described by WIENER AND STEYN-PARVÉ[1]. CO_2-free air was drawn through each cage at a rate of 350 ml/min and then into an absorber containing carbonate-free 10% NaOH. Absorbers were switched every 30 min.

* In this case no overpressure was applied.

At the end of 2 h the rat was taken from the cage and killed by decapitation. The gastro-intestinal tract was removed and extracted with boiling water as described by CORI[2]. The combined extracts were made up to a volume of 75 ml. An aliquot was deproteinized with $Ba(OH)_2$ and $ZnSO_4$. Glucose and radioactivity were measured in the protein-free extract to determine the amount of unabsorbed radioactive glucose. The liver and the remainder of the rat ("carcass") were dissolved separately in concentrated KOH by heating for 18 h at 100° (see ref. 1).

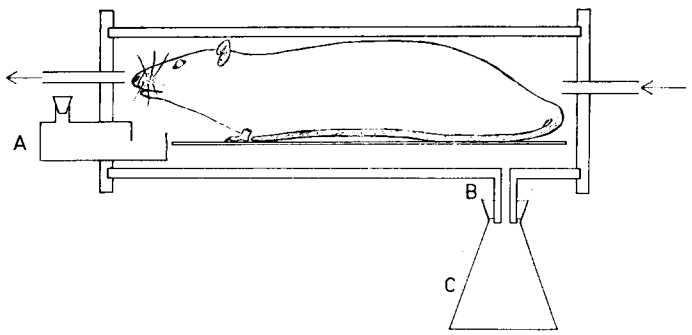

Fig. 1. Rat in metabolism cage. A, supply of drinking water; B and C, device for collecting urine.

The amount of glucose to give the animals and the time of the experiment were chosen after conducting preliminary experiments. The object was to ensure that most of the administered glucose would be absorbed, and that there would be enough glycogen in the tissues and sufficient radioactivity in glycogen and fatty acids for an adequate purification and determination.

Isolation and determination of metabolites

Glucose in the extract of the digestive tract and in the urine was determined with anthrone according to TREVELYAN AND HARRISON[3].

Glycogen and fatty acids in both liver and carcass were isolated, purified and determined as described by WIENER AND STEYN-PARVÉ[1], with a minor modification: after dissolving the carcass in KOH an equal volume of water was added to the hot solution before filtering through glass wool. This aided the subsequent purification of the glycogen.

Expired CO_2 was precipitated from an aliquot of the absorbing alkali as $BaCO_3$ and the dried $BaCO_3$ weighed[4].

Estimation of radioactivity and percentage incorporation of ^{14}C

Glucose in an aliquot of the solution given to the rats, in urine and in the extract of the digestive tract was counted as $BaCO_3$ in an "infinitely-thick" layer, after combustion to CO_2, as described earlier[1].

Glycogen and fatty acids were counted as such after mounting on aluminium planchets. The counted samples were weighed and from the observed counts/min the counting rate was calculated that would have been obtained had the materials been counted as infinitely-thick samples of $BaCO_3$, with the aid of conversion factors, as described by WIENER AND STEYN-PARVÉ[1].

Respiratory CO_2 was counted as an infinitely-thick sample of $BaCO_3$ (see refs. 1, 4).

$BaCO_3$ and glycogen were counted under an end-window Geiger counter. Fatty acids, which had a low activity, were counted in a window-less gas flow counter (Radiation Counter Laboratory, Skokie, Illinois; counting gas: He–isobutane (99:1)). The observed counts/min were converted to the rate that would have been obtained had the fatty acids been counted under the end-window tube. All preparations were counted long enough for the statistical counting error (a few percent) to become negligible as compared to the biological variation.

The percentage incorporation of ^{14}C was calculated by comparing the $BaCO_3$ counting rate of each preparation with that of the $BaCO_3$ prepared from the test dose of glucose. The percentage ^{14}C remaining in the digestive tract, as given in the results, refers to the amount of radioactive glucose administered, that of ^{14}C in expired CO_2, glycogen, fatty acids, and urinary glucose bears reference to the amount of that glucose actually absorbed.

WILCOXON's test[5,6] was used for the statistical evaluation of differences between groups (level of significance: $P \leq 0.05$).

EXPERIMENTS AND RESULTS

Two separate experiments were conducted with normal rats (six animals each time), two with alloxan-diabetic rats (six and five animals), and one with diabetic rats treated with insulin (six animals). In the results presented in Tables I to IV the two groups of normal and of diabetic rats were both taken together because they showed no marked differences. The following observations can be made:

Absorption of glucose from the digestive tract (see Table I)

This is never complete in the 2-h period of study. There would seem to be a tendency towards improved absorption, progressing from normal through diabetic to insulin-treated diabetic rats, but only the difference between normal and insulin-treated animals is significant (P-values: 0.021 and 0.003 for amounts of glucose and percentage ^{14}C, respectively).

TABLE I

ABSORPTION OF RADIOACTIVE GLUCOSE FROM THE DIGESTIVE TRACT OF RATS

Contents of digestive tract examined 2 h after administration by stomach tube of 500 mg glucose, containing 20 or 30 μC [^{14}C]glucose (see also text). Percent ^{14}C, ^{14}C expressed as percentage of ^{14}C administered.

State of rats	*Number*	*Glucose remaining in digestive tract*			
		mg		*Percent ^{14}C*	
		Median	*Range*	*Median*	*Range*
Normal	12	80	20–151	17	7–25
Diabetic	11	58	10–243	9	7–26
Diabetic + insulin	6	27	9–78	7	3–9

Respiratory CO_2 (see Table II)

All three groups of rats produce the same amount of CO_2 in 2 h, indicating that the metabolic oxidation rate is unaffected by the state of the animal. However, the

TABLE II

CONVERSION OF ORALLY ADMINISTERED [^{14}C]GLUCOSE INTO RESPIRATORY CO_2

Total amount of CO_2 and total radioactivity refer to expired CO_2 collected in 2 h after administration by stomach tube of 500 mg glucose, containing 20 or 30 μC [^{14}C]glucose. Values given are median values; range between parentheses. Percent ^{14}C, ^{14}C in CO_2 expressed as percentage of ^{14}C absorbed. Relative specific activity = (specific activity of sample/specific activity of glucose) × 100

State of rats	*Number*	*Respiratory CO_2*					
		mg (total)	*Percent ^{14}C (total)*	*Relative specific activity*			
				0–30 min	*30–60 min*	*60–90 min*	*90–120 min*
Normal	12	1010 (940–1275)	35 (29–40)	3.7 (2.7–4.6)	19.2 (15.0–21.5)	29.1 (23.3–35.2)	31.7 (27.2–35.4)
Diabetic	11	961 (813–1153)	16 (6–37)	2.1 (1.0–5.1)	9.3 (4.4–23.6)	13.6 (7.7–29.4)	17.9 (8.1–29.5)
Diabetic + insulin	6	1052 (965–1200)	31 (24–41)	3.6 (2.6–4.7)	22.4 (17.5–25.2)	28.8 (22.0–38.1)	25.9 (17.2–35.6)

CO_2 expired by the diabetic rats contains less than half the amount of ^{14}C recovered in the CO_2 of the normal rats (median: 16% *vs.* 35%, $P < 0.003$). The relative specific activity of the CO_2 expired in subsequent 0.5-h periods by the diabetic rats also drops significantly behind normal (P-values: 0–30 min = 0.005; 30–60 min = < 0.003; 60–90 min = < 0.003; 90–120 min = < 0.003). Treatment with insulin brings the amount of ^{14}C recovered as CO_2 back to normal.

Liver glycogen (see Table III)

The livers of the diabetic rats contain significantly less glycogen than normal (median: 150 *vs.* 242 mg; $P = 0.012$). The amount of ^{14}C incorporated in this glycogen and its relative specific activity are also markedly reduced (P-values: 0.016 and 0.009). After treatment with insulin the amount of liver glycogen rises slightly, but not significantly. The amount of ^{14}C incorporated, however, is sharply increased (median: 2.5% *vs.* 0.14%; $P = 0.014$) and so is the relative specific activity. There are no significant differences between the insulin-treated and the normal animals.

Carcass glycogen (see Table III)

The diabetic rats also have significantly less glycogen in the remainder of their body than the normal ones (median: 98 mg *vs.* 143 mg; $P < 0.003$). The amount of ^{14}C incorporated in this glycogen and its relative specific activity have dropped significantly (P-values < 0.003). Here also, administration of insulin has no pronounced effect on the amount of glycogen, but it does restore the amount of ^{14}C incorporated to normal. As this amount of ^{14}C is now present in less glycogen, the relative specific activity is higher than normal in the insulin-treated rats.

Liver fatty acids (see Table III)

The diabetic rat livers contain significantly less fatty acids than normal ($P = 0.035$). The percentage ^{14}C incorporated and the relative specific activity are also significantly lower (P-values < 0.003 and 0.004). Treatment with insulin brings about a dramatic increase in all three properties, to levels significantly above

TABLE III

CONVERSION OF ORALLY ADMINISTERED [^{14}C]GLUCOSE INTO GLYCOGEN AND FATTY ACIDS

All values were obtained 2 h after administration by stomach tube of 500 mg glucose, containing 20 or 30 μC [^{14}C]glucose. Values given are median values; range between parentheses. Percent ^{14}C, ^{14}C incorporated expressed as percentage of ^{14}C absorbed. Relative specific activity, see Table II.

State of rats	No.	Glycogen					
		Liver			Carcass		
		mg	Percent ^{14}C	Relative specific activity	mg	Percent ^{14}C	Relative specific activity
Normal	12*	242 (200–263)	1.6 (1.1–2.3)	2.3 (2.1–3.1)	143 (117–200)	0.6 (0.2–2.2)	1.5 (0.9–4.7)
Diabetic	11	150 (27–255)	0.14 (0.03–2.30)	0.75 (0.09–2.80)	98 (58–157)	0.14 (0.03–0.41)	0.46 (0.10–1.80)
Diabetic + insulin	6	179 (96–304)	2.5 (0.3–6.7)	6.5 (0.6–10.5)	112 (55–144)	0.85 (0.54–1.50)	3.9 (2.4–6.8)

State of rats	No.	Fatty acids					
		Liver			Carcass		
		mg	Percent ^{14}C	Relative specific activity	g	Percent ^{14}C	Relative specific activity
Normal	12*	215 (190–250)	0.04 (0.03–0.09)	0.04 (0.03–0.10)	6.7 (3.9–8.9)	1.0 (0.48–2.3)	0.03 (0.02–0.05)
Diabetic	11	189 (133–226)	0.01 (< 0.01–0.03)	0.01 (< 0.01–0.03)	6.6 (3.1–7.8)	0.23 (0.12–0.32)	0.01 (0.01–0.01)
Diabetic + insulin	6	297 (244–331)	0.14 (0.13–0.67)	0.12 (0.11–0.56)	9.5 (7.3–11.5)	6.0 (2.8–8.8)	0.15 (0.09–0.22)

* Values for liver glycogen and liver fatty acids are based on 5 rats only; the other normal livers were used for an examination of liver protein, which proved to contain only traces of ^{14}C.

normal: the median amount of fatty acids is now 297 mg as against 215 mg in the normal rats; the percentage of ^{14}C incorporated 0.14 *vs.* 0.04 and the relative specific activity 0.12 *vs.* 0.04.

Carcass fatty acids (see Table III)

The amount of fatty acids in the remainder of the body of the diabetic rat is normal, but the amount of ^{14}C incorporated and the relative specific activity are far below normal (P-values < 0.003). After administration of insulin the amount of fatty acids rises to 9.5 g, significantly above the normal 6.7 g ($P = 0.012$), and the increase in incorporation of ^{14}C is even more dramatic: 6.0% *vs.* 1.0% in the normal animal; relative specific activity 0.15 *vs.* 0.03.

Glucose in urine (see Table IV)

The diabetic rats excrete a median amount of 225 mg glucose in 2 h, containing 16% of the absorbed radioactivity. The diabetic rats treated with insulin still excrete a median amount of 36 mg glucose, and 1.5% of the absorbed radioactivity. So the amount of insulin injected has not been quite sufficient to suppress completely the overflow of glucose into the urine.

TABLE IV

EXCRETION OF GLUCOSE INTO THE URINE

Urine collected during 2 h after administration by stomach tube of 500 mg glucose, containing 20 or 30 μC [^{14}C]glucose. Values given are median values; range between parentheses. Percent ^{14}C, ^{14}C in urine expressed as percentage of ^{14}C absorbed. Relative specific activity, see Table II.

State of rats	*Number*	*Glucose in urine*		
		mg	*Percent ^{14}C*	*Relative specific activity*
Diabetic	6*	225 (16–371)	16 (2–18)	39.3 (8.8–49.1)
Diabetic + insulin	6	36 (0.2–52.0)	1.5 (< 0.01–3.8)	6.1 (0.2–13.9)

* Values for relative specific activity are based on 11 rats.

DISCUSSION

Let us first give our attention to the recovery of ^{14}C, administered as glucose, in expired CO_2, glycogen, and fatty acids of normal rats. The percentage of ^{14}C present in CO_2 after 2 h (Table II, 29–40%) seems to fit in well with comparable observations by others. ZILVERSMIT *et al.*[7] find that of 100 mg radioglucose, injected intraperitoneally into a rat, 27.6% is converted to CO_2 at the end of 2 h; from the paper by FELLER *et al.*[8] one can calculate an average recovery of 32.7% in expired CO_2, 2 h after intravenous injection of 12–18 mg radioactive glucose into rats. The amounts of ^{14}C recovered in glycogen and fatty acids of liver and carcass at first sight seem to be rather small, in particular as these substances are commonly regarded as terminal products of major pathways of glucose metabolism. But STETTEN AND BOXER[9], as far back as 1944, have estimated that only about 3% of the glucose consumed by rats in 24 h is handled by way of glycogen. They regard glycogen formation as a quantitatively minor pathway of glucose metabolism. Our recovery of 1.6% of ^{14}C, derived from glucose, in liver glycogen and 0.6% in carcass glycogen is in keeping with this view. LOURAU-PITRES[10] recovers not more than 0.2% of the dose in liver glycogen, 2 h after subcutaneous injection of radioactive glucose in mice. Only LEVIN AND WEINHOUSE[11] present higher figures for liver glycogen and muscle glycogen, but neither their glucose-fed nor their postabsorptive rats were in a state comparable to ours, and these only received trace doses of labelled glucose by intraperitoneal injection.

As regards the conversion of glucose into fatty acids one often encounters the statement that a major part of the glucose not oxidized to CO_2 is transformed into fatty acids. Reference is invariably made to STETTEN AND BOXER[9], who calculate that of the 14 g glucose consumed by their rats in 24 h, about 5 g are needed to replace fatty acids. However, two points should be mentioned that sometimes seem to have been overlooked. Firstly, only two-thirds of the carbon atoms of glucose actually appear as carbon atoms in fatty acids. Thus of ^{14}C-glucose not about 30%, but about 20% of the ^{14}C could be expected to appear in fatty acids, if STETTEN AND BOXER's calculations and the assumptions on which they are based are correct. This brings us to the second point. These authors calculate the fraction of total fatty acids replaced per day from results published by BERNHARD AND BULLET[12]. Unfortunately, these latter results have been derived from experiments with very few rats, and the figures

obtained vary so widely that it appears difficult to use them for a reliable calculation. So it would seem possible that the amount of glucose converted to fatty acids might well be lower than estimated by STETTEN AND BOXER. Indeed, other workers report lower figures[13], and we have come to the conclusion that the weak point in STETTEN AND BOXER's argument is their assumption that all the fatty acids synthesized each day are derived from dietary glucose. If one calculates the rate of renewal of fatty acids from the rate of incorporation of deuterium derived from body water, as STETTEN AND BOXER do, it is impossible to distinguish between acetyl-coenzyme A building blocks derived from glucose and those arising from the breakdown of body fatty acids. It seems most likely that part of the latter acetyl-coenzyme A will be used again for fatty acid synthesis and thus recycled. This inference is drawn from the following considerations: MASORO *et al.*[13], feeding mice *ad libitum* a high carbohydrate diet labelled with [^{14}C]glucose, find that after 24 h 10.6% of the ingested ^{14}C is incorporated into fatty acids, and 14.5% after 48 h. Applying STETTEN AND BOXER's method to these figures, we can calculate the fraction of the fatty acids replaced per day (we may presume that the level of ^{14}C in the body is reasonably constant during these 48 h). If all these newly-synthesized fatty acids were derived solely from glucose further calculation shows that this would require more than three times as much glucose as the mice actually consume, which is manifestly impossible.

Turning back to our own results, we can calculate that the mice of MASORO *et al.* would have incorporated 1.3% of the ^{14}C into fatty acids after 2 h. This value is well within our range of 0.48 to 2.3% for incorporation of ^{14}C into carcass fatty acids (Table III), while our rats were in the relatively unfavourable position of receiving one dose of [^{14}C]glucose only, instead of a continuous supply.

Summarizing, we are confident that our values for the recovery of ^{14}C—ingested as glucose—in CO_2, glycogen, and fatty acids reflect the true situation in normal rats. They may therefore be used as a basis for studying the influence of diabetes and treatment with insulin upon these pathways of glucose metabolism.

A definite drawback in our experiments with alloxan-diabetic rats is the very large variation in the results. This indicates an inadequacy in our criteria for the selection of the diabetic animals. Naturally, the aim is to use a group of rats that are diseased to about the same degree. The problem of how to select such a group does not always seem to have received the attention it deserves. Those workers who have given it their attention concede that it is not easy to solve[14]. Experiments not reported here have later shown us that each of the criteria usually applied: urinary glucose excretion, 24-h volume of urine, fasting blood sugar, varies considerably from day to day in any one animal. Perhaps a more satisfactory selection can be made after recording these criteria for a number of days preceding an experiment, and basing the selection upon a combination of criteria. Fortunately for us, the changes in the utilization of glucose to be discussed here are so marked, that their significance stands out in spite of the large variation within our diabetic group.

Our diabetic rats produce as much CO_2 as their normal counterparts, but the amount of ^{14}C therein falls to less than half. Similar observations have been made by CHAIKOFF *et al.*[7,8] after intraperitoneal or intravenous injection of labelled glucose. They believe, however, that the rate of oxidation of body glucose to CO_2 is only

slightly reduced in the diabetic rat; the decrease of label in CO_2 is attributed to the loss of labelled glucose with the urine. On the other hand, impairment of glucose oxidation by tissues of diabetic rats (*e.g.*, liver slices, diaphragm, adipose tissue) is well established[15–17]. According to the prevalent view in the tissue of diabetics less glucose is available for oxidation and consequently there is a more rapid oxidation of long-chain fatty acids[18].

The impairment of glucose utilization for glycogenesis and lipogenesis, already concluded to from experiments *in vivo* and *in vitro*[15–17, 19], is confirmed by our results. The nature of this impairment is not yet understood. It seems likely that there will be a broad spectrum of defects, rather than a single metabolic block[20].

Insulin, injected into the diabetic rats in amounts that preliminary experiments show to be just sufficient to normalize the blood sugar level, also normalizes the oxidation of glucose to CO_2 and its conversion into glycogen. Although in experiments *in vitro* with tissues of insulin-treated rats the response of glycogenesis is delayed in liver as compared to muscle (diaphragm), a normal state only being attained after 24 h[16], glycogenesis in the livers of our insulinized rats is quite normal in the first 2 h after feeding glucose.

The influence of insulin upon the conversion of glucose into fatty acids is very interesting: lipogenesis, so severely depressed in diabetes, is not just restored to normal, but elevated considerably above this level. Similar observations have been made in studies with rat-liver slices[15,16] and "eviscerated" rabbits[21], but have received little comment. In these investigations the amount of insulin injected into the diabetic or eviscerated animals was always considerably in excess of what could be deemed necessary to normalize the blood sugar level, which may explain the lack of interest.

In our experiments we have endeavoured to limit the amount of insulin to this necessary minimum. Indeed, we have probably fallen short of this minimum, as our rats treated with insulin still excreted a little glucose in the urine. But even these sub-minimal amounts of insulin still have this pronounced effect on lipogenesis. According to the current view lipogenesis would mainly take place in the extra-hepatic tissues, in particular in adipose tissue[18,22]. Insulin has been shown to stimulate very markedly the utilization of glucose for fat synthesis in adipose tissue[17]. So it would seem that, as regards circulating insulin, the state achieved by giving diabetic rats just enough insulin to normalize the blood sugar level is not the same as that prevailing in the normal rat, where the need for insulin is supplied by the pancreas in a natural manner.

Adding together the ^{14}C recovered in CO_2, glycogen, and fatty acids, we see that a considerable amount of the radioactivity absorbed as glucose is still unaccounted for in all three groups of rats (missing, in normal rats, 63%; diabetic, 67%; diabetic + insulin, 58%). A similar observation has been made before in this laboratory, after feeding radioactive glucose to pigeons[1]. Subsequent investigations, now in progress, indicate that most of the missing ^{14}C will probably be found in a number of intermediate products of carbohydrate metabolism. Protein probably will not contain much label, as only traces of ^{14}C were found in protein isolated from the livers of a number of normal rats. The question arises whether this distribution of carbon from absorbed glucose over CO_2, glycogen, fatty acids, and some unknown intermediates may not to

some extent be affected by the manner in which this glucose is ingested. The pattern of distribution may well be different when a single, fairly large, amount of the sugar is brought into the stomach all at once, as compared to the situation in which the rat is free to consume a diet rich in carbohydrate at its own pace.

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