OXIDATION OF GLUCOSE LABELED WITH RADIOACTIVE CARBON BY NORMAL AND ALLOXAN-DIABETIC RATS*

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The nature of the metabolic defect in diabetes has been vigorously debated for well over 30 years, but despite the interesting new lights shed on it during the past few years (1, 2) *rigid* proof as to whether the derangement in this disease results from an impaired capacity of the diabetic animal to convert glucose to CO_2 is still lacking. With the introduction of isotopic carbon, it became possible for the first time to study the direct conversion of carbon-containing compounds to CO_2. We wish to report here observations dealing with the fate of the carbon of administered radioglucose in normal and alloxan-diabetic rats. The evidence obtained indicates that the over-all rate of oxidation of glucose by the alloxan-diabetic rat need not differ significantly from that in the normal rat.

EXPERIMENTAL

Production and Care of Diabetic Rats—The first five rats (Table I) were injected intraperitoneally with a 2 per cent aqueous solution of alloxan. A single injection of 200 mg. per kilo of body weight was found effective in producing glycosuria and at the same time kept mortality at a minimum. However, the intraperitoneal route of administration produced adhesions in the abdominal cavity, and for this reason the alloxan was injected intravenously in the other animals. A dose of 50 mg. of alloxan per kilo of body weight, administered as a 5 per cent solution in isotonic saline, was used for intravenous purposes. Higher dosages resulted in undue mortality, whereas lower doses frequently failed to induce diabetes.

The animals were kept in individual cages in a warm room. The stock diet (which consisted of 68.5 parts of wheat, 5 of casein, 10 of fish meal, 1.5 of salt, 5 of a fish oil, and 10 of alfalfa) was supplemented twice weekly with lettuce.

Urine was collected daily and preserved with toluene. Glucose in urine was determined on the day of collection by oxidation with potassium ferri-

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cyanide and subsequent titration with ceric sulfate (3, 4). Blood glucose was determined by the same method on an aliquot of a protein-free filtrate prepared with $ZnSO_4$ and NaOH (5). The last traces of zinc were removed by the addition of sodium carbonate.[1]

The duration of diabetes, degree of glycosuria, and weight changes that occurred in the nine diabetic rats used in this study are recorded in Table I.

Preparation of C^{14}-Labeled Glucose[2]—C^{14}-labeled starch and glucose were isolated from tobacco leaves which had been illuminated in the presence of $C^{14}O_2$. Labeled glucose was then obtained by acid hydrolysis of the starch and by fractional crystallization of the soluble sugars as described by

TABLE I

History of Diabetic Rats

Rat No.	Route of alloxan administration	Duration of diabetes before experiment	Sugar excretion			Weight			Average volume of urine per day
			Maximum per 24 hrs.	During experiment		Before alloxan injection	On day of experiment	Minimum during diabetic state	
		days	*gm.*	*hrs.*	*gm.*	*gm.*	*gm.*	*gm.*	*cc.*
D3	Intraperitoneal	22	6.8	6	1.13	195	158	124	60
D6	"	16	5.6	6	0.36	150	150	132	50
D20	"	10	10	48	18.2	300	270	228	120
D23	"	48	11	12	2.45		163	160	70
D28	"	70	7.6		7.6*	218	212	208	50
D40	Intravenous	14	8.4	12	4.20	190	174	158	50
D48	"	25	6.8		6.6*	168	160	152	60
D53	"	20	11.3		11.3*	262	186	186	50
DJ2	"	14	11.4	46	7.0	200	164	129	65

* Rats so designated were nephrectomized; the amounts recorded were excreted during the 24 hours preceding nephrectomy.

Putman *et al.* (6). The purified radioglucose was dissolved in an isotonic NaCl solution and kept frozen (−18°) until just before it was injected.

Collection and Determination of Exhaled CO_2—During the experiment the rat was kept in an all-glass cage which was ventilated continuously with CO_2-free air at 27–28°. The air collected from the cage was passed through a column of carbonate-free NaOH (40 milliequivalents were used for each hour of CO_2 collection per rat). A porous glass disk at the bottom of the column served to break the stream of air into fine bubbles. The apparatus was tested for complete recovery of the expired CO_2. The rats had access to food and water at all times while in the cage.

[1] Kaplan, A., unpublished observations.

[2] We are indebted to Dr. W. Z. Hassid for the samples of photosynthetically prepared radioglucose used in this study.

The $NaOH$-Na_2CO_3 solution was made to volume and the amount of carbonate determined by titration of two different aliquots with 0.1 N HCl. One aliquot was titrated to a brom-cresol green end-point. The value so obtained is a measure of the amounts of NaOH and Na_2CO_3 present. To the other aliquot an excess of $BaCl_2$ was added before titrating it to the phenolphthalein end-point; this titration value represents the amount of unused NaOH. The difference between the two titration values, therefore, gives the amount of Na_2CO_3 that was formed during the collection of CO_2. After centrifugation, the $BaCO_3$ precipitate was washed twice with distilled water and suspended in alcohol. The precipitate was ground in a glass homogenizing tube, mounted on an aluminum disk, and its radioactivity determined after the manner described by Dauben *et al.* (7). In general, each sample was counted for more than 3000 counts; the error of the counting was less than 2 per cent.

Determination of Radioglucose in Urine. Fermentation—A sample of urine buffered with phosphate at about pH 5 was incubated at 37° with yeast (*Torula monosa*). The yield of CO_2 evolved was improved by the presence of 10^{-3} M sodium azide (8). The carbon dioxide was collected in 0.25 N barium hydroxide solution containing 2 per cent $BaCl_2$ according to Van Slyke and Folch (9). The precipitate of barium carbonate which formed was treated as described for respiratory CO_2. The total amount of C^{14}-labeled glucose excreted in urine was obtained from the specific activity of its barium carbonate precipitate and the total urinary glucose from its reducing value.

The validity of the fermentation procedure was tested on a sample of 3,4-C^{14}-labeled glucose. This glucose was prepared by a modification of the method of Solomon *et al.* (10) as follows. A 200 gm. rat was fasted for 24 hours. 340 mg. of Na lactate[3] were administered by stomach tube. 1 hour later the animal was injected intraperitoneally with 1 millicurie of $NaHC^{14}O_3$ contained in isotonic $NaHCO_3$. 2 hours after the administration of the labeled $NaHCO_3$ the animal was sacrificed and glycogen isolated from its liver (13). Glucose was obtained by hydrolyzing the isolated glycogen for 3 hours with 0.6 N HCl.

According to Wood *et al.* (14) all the C^{14} atoms in this glucose are in the 3 and 4 positions. Our finding that the specific activity of the $C^{14}O_2$ produced by fermentation was 3 times as high as that of the CO_2 when the whole glucose molecule was oxidized seems adequate evidence that the fermentation with *Torula monosa* is a reliable method for the determination

[3] The amount of lactate administered was based on earlier observations of Cori and Cori dealing with liver glycogen formation from lactic acid (11). It was found in the laboratory that during the first 6 hours glycogen is deposited at a uniform rate in the liver of the 24 hour fasted rat fed lactate. Liver glycogen was determined by the method of van Wagtendonk (12).

of the specific activity of both carbon atoms 3 and 4 of the glucose molecule.

Isolation of Osazone—The glucose content of a sample of urine was determined, and for each gm. of glucose present 10 gm. of phenylhydrazine hydrochloride and 15 gm. of sodium acetate trihydrate were added. The mixture was heated on a steam bath for 1.5 hours and then kept at 4° for approximately 12 hours. The mixture was centrifuged, and the osazone crystals obtained were washed several times with distilled water. The crystals were next dissolved in hot 50 per cent alcohol, and insoluble materials present removed by filtration through a steam jacket Büchner funnel. The glucosazones were allowed to crystallize at 4° and then dried in a vacuum desiccator over $CaCl_2$.

The glucosazones were oxidized by heating them with a chromic-sulfuric acid mixture, according to Van Slyke and Folch (9), and the carbon dioxide evolved was trapped in a mixture of 0.25 N barium hydroxide and 2 per cent barium chloride and treated as described above. 92 to 93 per cent of the osazone carbon was recovered as barium carbonate. The barium carbonate was mounted on an aluminum disk as described above, and its radioactivity measured. The measurement of the radioactivity of the glucose is much more tedious by this method than by the fermentation procedure. Furthermore, the counts per mg. of $BaCO_3$ obtained by the osazone procedure suffer a 3-fold dilution by the phenylhydrazine carbon.

Tables III and IV show that the values obtained for radioglucose by the two methods (osazone and fermentation) are in fairly good agreement. This agreement implies that the specific activity of the 3rd and 4th carbons of the glucose molecule is approximately equal to that of the other carbons. This is important since it excludes the possibility that the expired $C^{14}O_2$ is derived only from one or two specific carbons of the glucose chain.

Results

Conversion of Radioglucose to CO_2 by Normal and Diabetic Rats

Normal Rats—Rats N1 and N2 received intraperitoneally 100 mg. of radioglucose (Table II), whereas Rats 3 to 5 were injected intraperitoneally with 1 gm. of labeled glucose per kilo of body weight. The amounts of radioactive CO_2 recovered in the expired air of each rat are recorded in Table II. During the first 4 hours, the administered labeled glucose was rapidly oxidized by the normal rat. By the time 6 hours had elapsed, 40 to 60 per cent of the administered C^{14} was recovered in the expired CO_2. Although in 6 hours Rat N2 eliminated as CO_2 as much as 55 per cent of the radioglucose that it received, only an additional 11 per cent was recovered as expired radioactive CO_2 during the next 18 hours. The more rapid conversion of the administered labeled glucose to CO_2 during the early intervals

means, of course, that at these times the animal's labeled glucose pool had a high specific activity and that at the later intervals this had been diluted by unlabeled glucose derived either from the diet or from endogenous sources.

Diabetic Rats—At the time radioactive glucose was injected, diabetes had existed in the animals used for periods varying from 10 to 48 days (Table I). The rats displayed such manifestations of diabetes as polyuria, glycosuria, polyphagia, and weight loss. Each rat excreted from 50 to 120 cc. of urine per day. The amount of glucose excreted was, of course, not constant from day to day. The maximum found before the administration of radioglucose amounted to 11 gm. per 24 hours.

TABLE II

*Oxidation of Intraperitoneally Injected Radioglucose to CO_2 by Normal Rats**

Rat No.	Weight	Radioglucose injected intraperitoneally	Per cent of administered glucose† converted to CO_2 at end of				
			2 hrs.	4 hrs.	6 hrs.	12 hrs.	24 hrs.
	gm.	*mg.*					
N1	172	100			56		
N2	250	100	27.6	48.4	55	61.6	65.7
N3	230	230‡			56.7	63.3	
N4	270	270‡			45.9	53.6	
N5	280	280‡			39.4	49.6	

* The rats had access to food throughout the period of observation.

† The administered glucose contained 1 to 2 million counts per minute.

‡ Equivalent to 1 gm. per kilo of body weight.

In contrast to the normal rat, which had converted approximately 40 to 60 per cent of the administered glucose to CO_2 in 6 hours, the five diabetic rats used in this study (Tables III and IV) oxidized only 11 to 24 per cent of the injected labeled glucose in 6 hours. An examination of the urine revealed, however, that most of the administered radioglucose was not available for oxidation but had been excreted. Thus Rat D20 excreted more than 60 per cent of the administered glucose in the first 12 hours after its injection (Table III). Little more radioglucose was excreted in the next 36 hours. This does not mean that the rat did not continue excreting glucose, but rather that after 12 hours the specific activity of the body glucose had dropped to low levels. In 6 hours the other four rats (Table IV) excreted from 30 to 45 per cent of the labeled glucose they had received intraperitoneally.

Calculation of Rate of Glucose Oxidation

From the amount of $C^{14}O_2$ expired during a given interval after the injection of the radioglucose, it is possible to obtain an approximate value for the amount of plasma glucose that had been oxidized during that interval.

TABLE III

Oxidation of 100 Mg. of Intraperitoneally Injected Radioglucose by Diabetic Rat D20

Interval	Total glucose	Radioglucose, per cent of injected dose determined by		Specific activity* of urinary glucose × 10^3	Expired $C^{14}O_2$, per cent of administered glucose†	Glucose oxidized
		Fermentation	Osazone			
hrs.	*mg.*					*mg. per hr.*
0- 2	775	49.3	43.1	60	6.6	55
2- 4	513	11.0	9.7	20	3.3	82
4- 6					1.5	
4-12	2,260	3.3	5.0	1.9		
6-12					2.1	
12-24	5,350	0.7‡	‡	0.13	1.0	
24-48	9,300	‡	‡		0.7‡	
Total.....	18,200	64.3			15.2	

* The specific activity is expressed as the per cent of the injected C^{14} per mg. of glucose. The values are based on averages between fermentation and osazone.

† The injected dose contained 1,800,000 counts per minute.

‡ The sample counted less than 1.2 × background.

TABLE IV

Oxidation of Intraperitoneally Injected Glucose by Diabetic Rats D3, D6, D23, and D40

Rat No.	Weight	Radio-glucose injected	Urinary glucose								Expired $C^{14}O_2$, per cent of administered glucose*		Glucose oxidized‡
			Total		Radioglucose, per cent of injected dose* determined by				Specific activity† of urinary glucose × 10^3				
					Fermentation		Osazone						
			1st 6 hrs.	2nd 6 hrs.	1st 6 hrs.	2nd 6 hrs.	1st 6 hrs.	2nd 6 hrs.	1st 6 hrs.	2nd 6 hrs.	1st 6 hrs.	2nd 6 hrs.	
	gm.	*mg.*	*mg.*	*mg.*									*mg. per hr.*
D3	158	100	1130		34		36.8		31		23		122
D6	150	100	360		46		44.5		125		24		32
D23	163	163§	379	2070	30	13.5	30.5	10.9	79	6.0	15	3.7	32
D40	174	174§	1360	2840			45.7	3.0	34	1.1	17.5	1.8	87

* The administered glucose contained 1 to 2 million counts per minute.

† The specific activity is expressed as the per cent of the administered C^{14} per mg. of glucose.

‡ These values are determined from the specific activity of the urinary glucose and the expired $C^{14}O_2$ during the first 6 hours of the experiment.

§ The glucose injected equals 1 gm. per kilo of body weight.

To make such a calculation, we used the *average* specific activity of plasma glucose during the period under consideration. But we have to be

reasonably certain that the expired $C^{14}O_2$ is derived only from labeled glucose; as a rule, this will be true only during the early intervals after the injection of radioglucose when little or none of it has been converted to other compounds.

To determine the specific activity of plasma glucose, an aliquot of the plasma sample was oxidized and its total radioactivity determined as $BaCO_3$, whereas on a different aliquot of this same plasma the glucose content was determined from its reducing value. In the diabetic animals we were able to check this procedure by demonstrating that the specific activity so obtained was equal to the specific activity of the urinary glucose. This is shown in Fig. 1.

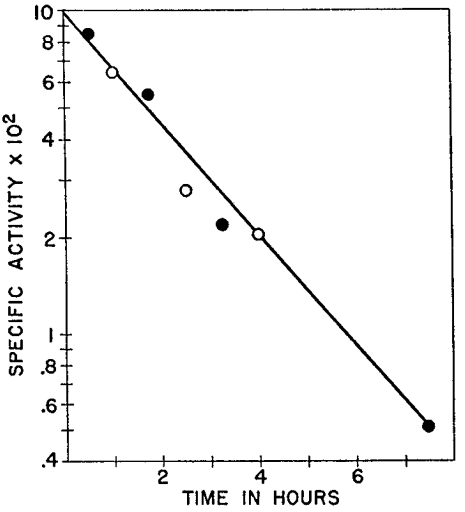

FIG. 1. Changes with time in specific activity of urinary and plasma glucose of Rat DJ2. The ordinate scale is logarithmic. ○ plasma glucose; ● urine glucose.

Values for specific activities of plasma and urinary glucose of two diabetic rats (Rats DJ2 and D20) are presented in Figs. 1 and 2; here the values are plotted against time on semilog paper. This plot was found to be a straight line during the first 6 hours (15). During the interval from 0 to 2.5 hours the average specific activity of plasma glucose, expressed as a percentage of the injected dose per mg. of glucose, is approximately 6×10^{-2} (see Fig. 1). The amount of $C^{14}O_2$ exhaled during that interval was approximately 6 per cent of the administered radioactivity. Therefore, the amount of glucose oxidized in this period is equal to $6/(6 \times 10^{-2}) = 100$ mg. or 40 mg. per hour. The same calculation for the interval from 2.5 to 4 hours, in which 2 per cent of the injected dose was exhaled, gives an oxidation of 50 mg. of glucose per hour.

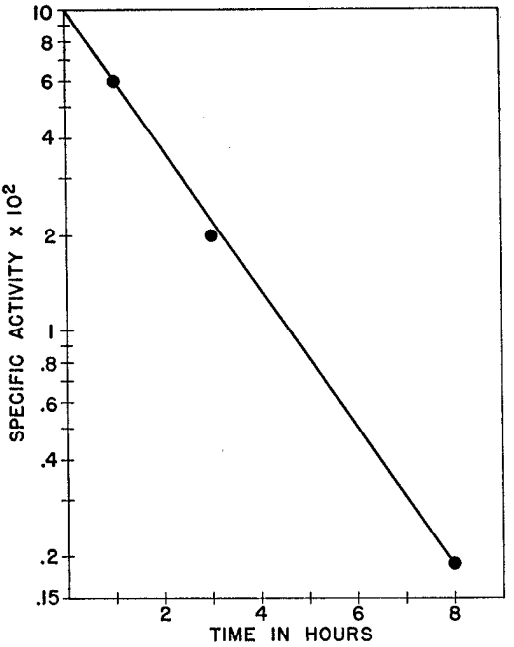

FIG. 2. Changes with time in specific activity of urinary glucose of Rat D20 (see Table III).

TABLE V

Rate of Oxidation of Glucose by Normal Rats

Each rat was injected intraperitoneally with 15 mg. of labeled glucose.

Rat No.	Weight	$C^{14}O_2$ collected		Plasma glucose		Glucose oxidized
		Interval	Per cent of injected dose*	Time after injection	Specific activity† × 10^2	
	gm.	*hrs.*		*hrs.*		*mg. per hr.*
NJ1	220	0–1	13.6		‡	50
		1–2	10.6	1	20.8	66
		2–4	5.5	3	6.9	40
		4–6	1.84	6	4.6	
NJ2	242	0–1	8.75		‡	32
		1–2	9.0	1	19	69
		2–4	5.25	3	4.1	64
		4–6	2.73	6	4.1	
NJ3	250	0–1	7.93		‡	28
		1–2	14.1	1	21.8	85
		2–3	4.63	3	7.6	48

* The injected glucose contained 1,600,000 counts per minute.

† The specific activity is expressed as the percentage of the injected C^{14} per mg. of glucose.

‡ The average specific activity of plasma glucose was obtained by extrapolation (see the text).

The values obtained by this type of calculation for the amounts of glucose oxidized per hour by normal rats are recorded in Table V and by diabetic rats in Tables III and IV. The range of values calculated for the normal rat was similar to that found for the diabetic.

Comparison of Ability of Nephrectomized Normal and Nephrectomized Alloxan-Diabetic Rats to Convert Radioglucose to CO_2

Since the diabetic rat excreted a good portion of the administered glucose in the urine, the amount of $C^{14}O_2$ exhaled, as noted above, provided no basis for comparing the capacities of normal and diabetic rats to oxidize administered glucose. To prevent loss of glucose by this route, radio-

TABLE VI

Conversion of Intravenously Injected Radioglucose to CO_2 by Nephrectomized Normal and Nephrectomized Alloxan-Diabetic Rats

Each rat received 1 gm. of labeled glucose per kilo of body weight.

Rat No.	Weight	Per cent of administered glucose* recovered as CO_2 for each interval						Blood sugar at end of experiment
		0–0.5 hr.	0.5–1 hr.	1–2 hrs.	2–4 hrs.	4–6 hrs.	Entire 6 hrs.	
	gm.							*mg. per cent*
Normal. N6	180	5.7	5.3	8.0	14.0	3.9	36.9	131
" N7	172	2.7	4.7	6.0	12.3	3.9	29.6	157
" N8	202	3.9	4.4	8.7	15.6	3.3	35.9	125
" N9	174	5.6	4.8	8.5	16.7	3.6	39.2	138
Diabetic. D28	212	0.42	1.4	4.5	10.5	9.4	26.2	700
" D48	160	2.2	5.9	10.0	13.7	7.8	39.6	870
" D53	186	0.57	2.1	4.3	11.4	7.5	25.9	1060

* The administered glucose contained 1 to 2 million counts per minute.

glucose was injected intravenously into rats 15 minutes after they had been subjected to bilateral nephrectomy. Each rat received 1 gm. of glucose per kilo of body weight. This dose was selected because it was large enough to yield similar *initial* values for the specific activities of circulating glucose in both normal and diabetic rats. In other words, this dose minimized the differences in plasma glucose between the normal and the diabetic rats at the very beginning of the experiment. Data on the conversion of injected radioglucose to $C^{14}O_2$ by the two types of rats under these conditions are recorded in Table VI.

Judging from the $C^{14}O_2$ exhaled during the entire 6 hours, there appears to be no significant difference between nephrectomized normal and nephrectomized alloxan-diabetic rats in their ability to oxidize the administered glucose. It is interesting to note, however, that in the early intervals

two of the three diabetic rats exhaled less $C^{14}O_2$ than the normal ones, whereas at the later intervals (4 to 6 hours) the reverse was the case.

6 hours after the administration of the large dose of labeled glucose, namely 1 gm. per kilo of body weight, the plasma glucose in the nephrectomized normal rats had returned to normal levels or levels close to normal; the highest value observed at the 6 hour interval was 160 mg. per cent. In the diabetic rats, on the other hand, plasma glucose of 700 to 1000 mg. per cent was found when they were sacrificed.

DISCUSSION

Normal rats injected intraperitoneally or intravenously with amounts of glucose varying from 100 to 280 mg. converted 40 to 50 per cent of it to CO_2 in 6 hours. In the nephrectomized but otherwise normal rat, $C^{14}O_2$ appeared in the exhaled gas within the first half hour, and at the end of the 1st hour nearly 10 per cent of the intravenously injected glucose was converted to CO_2. These findings are in agreement with the classical view that administered glucose is available for immediate oxidation by the animal body.

In order to compare the rates of *conversion* of *plasma* glucose to CO_2 by normal and diabetic rats, two methods of study were employed: one was based on the $C^{14}O_2$ evolved when a relatively large amount of labeled glucose was introduced intravenously into nephrectomized preparation; in the second the amount of glucose oxidized in the intact rat was derived from measurements of the $C^{14}O_2$ evolved and of the specific activity of plasma glucose. That the alloxan-diabetic rat can oxidize appreciable amounts of glucose cannot be doubted from the results obtained by the first method. As judged by the $C^{14}O_2$ eliminated during an entire 6 hour period of observation, the amounts of administered glucose oxidized by the nephrectomized normal and nephrectomized diabetic rats are of the same order. *The values for glucose oxidation obtained by the second method refer specifically to the conversion of plasma glucose (and such glucose as exists in rapid equilibrium with plasma glucose) to CO_2.*

The interpretation of our findings is not without some difficulty. The values for specific activity of plasma glucose are based on two measurements; namely, (1) the total radioactivity of plasma, which we have assumed to be glucose at the early intervals, and (2) the total reducing value of plasma, which we have also assumed to be glucose. The reliability of the specific activity measurements for plasma glucose is shown by the finding that in the diabetic rat the values obtained agreed with those found for the osazones prepared from urinary glucose. In the normal rat it was not possible to make such a comparison. The assumptions made in connection with the specific activity would appear, however, to be well supported by our

observations in the nephrectomized rats; the finding that the over-all capacity of the normal nephrectomized rat to oxidize glucose is of the same order as that of the diabetic involves none of the assumptions referred to above.

An interference was observed in the oxidation of the injected carbohydrate by the nephrectomized diabetic rat during the 1st hour, whereas at the later intervals the amount of $C^{14}O_2$ produced by these rats was of the same order of magnitude as that produced by normal rats. The interpretation of the results of the early intervals presents some difficulties. This phase is being investigated further.

In order to interpret the $C^{14}O_2$ data presented here for the alloxan-treated rats, the degree of diabetes that existed in these rats must be evaluated. Postmortem examination of their pancreases revealed massive necrosis of the islets. It cannot be inferred, however, that the alloxan-diabetic rat is deprived of *all* insulin-secreting tissue. But even though the presence of some residual insulin cannot be excluded in the diabetic rats used in this study, they nevertheless excreted, when fed, from 5 to 11 gm. of glucose daily and exhibited such manifestations of diabetes as polyphagia, polyuria, polydipsia, and loss of weight. Another indication of the degree of diabetes in the rats used here is provided by the finding of values for plasma glucose of 700 to 1000 mg. per cent 6 hours after excision of their kidneys.

SUMMARY

1. The rate of conversion of administered radioglucose to CO_2 was measured in normal and alloxan-diabetic rats.

2. $C^{14}O_2$ appeared within the first 30 minutes in the expired air of both normal and alloxan-diabetic rats.

3. The amount of *plasma* glucose converted to CO_2 was measured by two methods. The first was based on the specific activities of plasma glucose and the amounts of $C^{14}O_2$ in the expired air, the second on the amounts of $C^{14}O_2$ exhaled by the nephrectomized rat. As judged by these procedures, the rate of conversion of *plasma glucose* to CO_2 by the alloxan-diabetic rat does not differ significantly from that found in the normal.

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