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EXPERIMENTS WITH NORMAL AND DIABETIC EATS USING CARBON-14 RESPIRATION PATTERNS

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DIABETIC RATS USING CARBON-14
RESPIRATION PATTERNS

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USING CARBON-14 RESPIRATION PATTERNS

Bert M. Tolbert and Martha R. Kirk

September 6, 1956

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ABSTRACT

Normal and alloxan-diabetic rats with and without insulin, Carbutamide, or Orinase have been studied by use of carbon-14 respiration patterns. Substrates have included glucose-C¹⁴₆, fructose-C¹⁴₆, sodium acetate-2-C¹⁴, and lactic-1-C¹⁴ acid. It has been shown that such patterns are a good index of diabetes, that the three drugs increase glucose oxidation in normal animals, that insulin can return the abnormal diabetic pattern to normal in all cases, and that Carbutamide and Orinase can do so only in certain cases. The data and time studies indicate that these last two drugs do not act by an anti-insulinase action, but seem to operate as insulin-production stimulators combined with some other action.

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INTRODUCTION

In a series of papers^{1, 2, 3} we have described a method for studying the metabolism of specific compounds *in vivo*. In these experiments, animals or humans are given a carbon-14-labeled compound, such as acetate, glucose, or glycine, and the rate of respiration of the C^{14} as CO_2 is measured and recorded automatically by an instrument consisting of a flow system, an ionization chamber for the C^{14} analysis, an infrared gas analyzer for the CO_2 measurement, and a multichannel ratio recorder to record the total C^{14} , percent CO_2 , and C^{14}/CO_2 (specific activity) values. The nature of the respiration curves obtained from animals can be modified by drugs that affect specific biochemical systems or by physiological conditions that affect the body chemistry.

The metabolism of glucose in normal and diabetic rats represents a problem that is of current interest and has many incompletely understood facets. The roles of insulin, Orinase,⁴ and Carbutamide⁴ as hypoglycemic agents are not known. Such a problem can be easily studied by use of the carbon-14 respiration-pattern method outlined above.

In the following paper we present carbon-14 respiration patterns for normal and alloxan-diabetic rats, with and without insulin, Orinase, or Carbutamide, and using labeled acetate, glucose, and fructose as substrates. We have found that such patterns serve as excellent measures of the presence or absence of diabetes and the extent of the disease. Glucose- C^{14}_6 metabolism to $C^{14}O_2$ is greatly modified by diabetes, while acetate and fructose metabolism are only slightly changed. Insulin is capable of returning the abnormal diabetic glucose-respiration curves to near normal in all cases, but this is not true for Orinase and Carbutamide.

EXPERIMENTAL

Animals

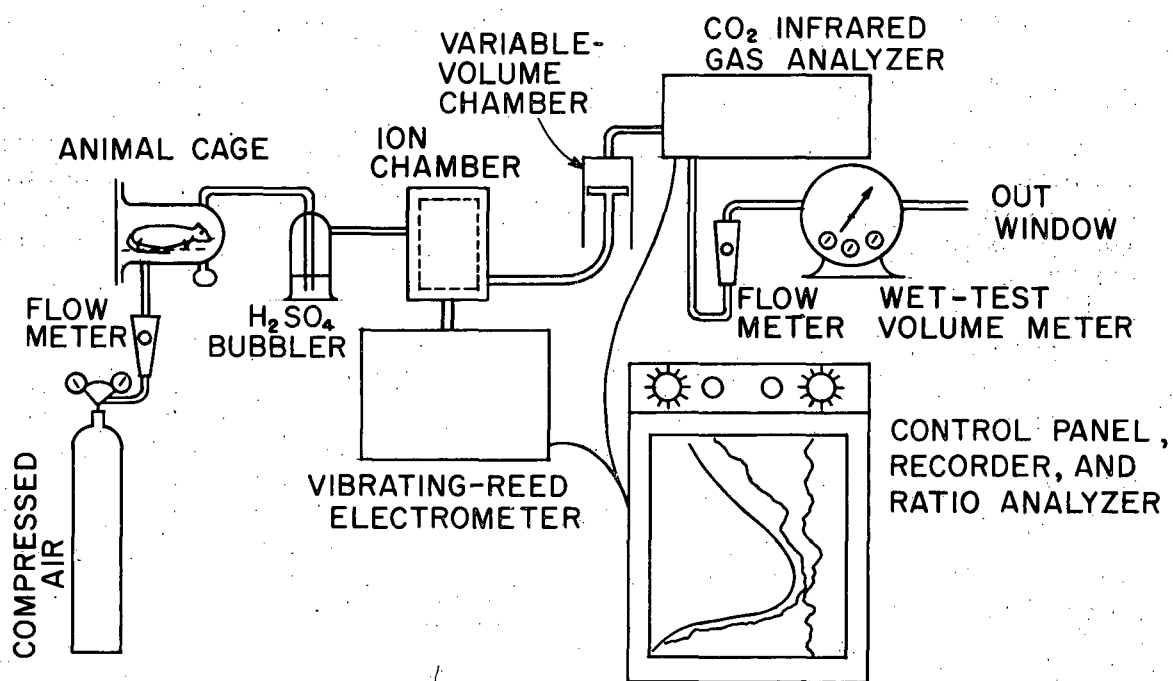
Animals used for these experiments were Long-Evans males. Normal rats weighed approximately 250 grams. For the diabetic studies, rats weighing 100 to 150 grams were chosen. After a 3-day fast, each was given a single intravenous injection of alloxan (50 mg/kg) dissolved in physiologic saline.^{5,6} They were then maintained on protamine zinc insulin (6 units per day) for one week. Tests were begun about the fourteenth day after the alloxan treatment. Rats were considered diabetic if they showed more than 2% sugar in the urine and oxidized not more than 10% of injected glucose to CO_2 in 2 hours. Unless otherwise noted, urine sugar percentages reported are for tests made in the morning before any drugs were given.

Compounds

Radioactive glucose- C^{14}_6 and fructose- C^{14}_6 were obtained from canna leaves by biosynthesis.^{7,8} These two compounds were purified by paper chromatography and contained less than 1% radioactive impurity. Sodium acetate-2- C^{14} was synthesized from $\text{C}^{14}\text{H}_3\text{I}$ and tested for purity by paper chromatography.⁹ The lactic acid was prepared as previously described.¹⁰ Radioactive compounds were administered by intraperitoneal (i. p.) injection of 1 mg of the compound dissolved in 0.1 ml water containing 10 μC of radioactivity per injection.

To test the effect of insulin on respiration patterns, crystalline insulin (Eli Lilly and Co.) was given by intraperitoneal injection at the rate of 0.5 unit/kg for normal rats and 8 units per injection for diabetic rats. The oral hypoglycemic agents, Carbutamide and Orinase, were dissolved in 1 N NaOH, brought to pH 8 to 9 with 1 N NaCl, and diluted with water to bring the final concentration to 100 mg/ml. It was given to the rats by stomach tube in the amount of 1 g/kg. Unless otherwise stated, hypoglycemic agents, including insulin, were administered one hour before the radioactive substrate was given.

Instrumentation for determining the C^{14}O_2 excretion patterns has been described previously.^{1,2,3} The illustration (Fig. 1) shows the flow system by which air is introduced into the cage and carried through the ionization chamber that measures the C^{14} and the infrared gas analyzer for measuring total CO_2 . The multichannel potentiometer records not only the total radioactivity and the CO_2 , but also by a special switching arrangement, records the ratio of $\text{C}^{14}/\text{CO}_2$, i. e., the specific activity of C^{14}O_2 respired. The data from each run were analyzed to give a cumulative C^{14}O_2 excretion curve, C^{14}O_2 specific-activity curve, and the rate of CO_2 production per minute.



MU-9185

Fig. 1. Schematic Diagram of Respiratory $C^{14}O_2$ Analyzer.

RESULTS

Glucose Oxidation in Normal Rats

Orinase and Carbutamide, as well as insulin, are capable of increasing the oxidation of a trace of glucose- C^{14}_6 to $C^{14}O_2$ in rats. To show this effect, normal fasted animals were treated with one of the drugs and one hour later a respiration measurement was made on the animals with a tracer dose of labeled glucose. The summarized data are presented in Table I. We see here that a normal fasted rat oxidized about 5% of a given dose of glucose to CO_2 in 40 minutes, and about 28% in two hours. After an injection of 0.5 units/kg of crystalline insulin, the rat oxidized 10% of the glucose to CO_2 in 40 minutes but the 2-hour total was about the same as for a normal animal. When Orinase was given 1 hour before the injection, 11% was oxidized to CO_2 in 40 minutes and 37% in 2 hours. This enhanced oxidation was clear-cut and was observed in all cases for Orinase. When Carbutamide was given 1 hour before the injection, the results sometimes showed an enhanced oxidation effect and sometimes showed no effect. We made measurements on eleven animals to make sure that these variable results were real. We do not know the explanation for the observed variability, but it might be due to the size of the drug dose administered or in the basic effect of the drug itself.

Fructose Oxidation in Rats

Neither insulin, Orinase, nor Carbutamide drastically affects the oxidation of uniformly labeled fructose to $C^{14}O_2$. Table II shows the data for a series of rate measurements exemplifying this point. Some measurements on a diabetic rat, B-3, with and without insulin, are included to show whether or not the fructose metabolism is greatly changed. The diabetic animal showed a depressed $C^{14}O_2$ production, and this effect is reversed by the insulin. There seems to be a real difference here, but it is only about a factor of two instead of ten (as observed for labeled glucose--see below).

Acetate Oxidation in Normal and Diabetic Rats

There is no very large difference observed in the oxidation of sodium acetate-2- C^{14} to $C^{14}O_2$ in normal and alloxan-diabetic rats with and without insulin, Orinase, or Carbutamide (see Table III). It seems that the drug-treated animals oxidized less of the tracer acetate dose to $C^{14}O_2$ from lactate-1- C^{14} than did the untreated animals. There was no significant difference in the two conditions and the experiments were not repeated.

Glucose Oxidation in Nondiabetic Rats

After the series of rats were treated with alloxan and allowed a recuperation period sustained by insulin, they were deprived of insulin and tested for urine sugar, and their glucose respiration patterns were determined. Figure 2 shows a series of curves from fasted and fed rats, both normal and alloxan-treated, that failed to become diabetic, i.e., to show a positive urine sugar. The 2-hour cumulative excretion of $C^{14}O_2$ is from 25% of 40% of the carbon-14 given in tracer glucose injection. The spread of these values represents a normal variation in animals or groups of animals. However, there is a significant difference between fasted and non-fasted rats.¹¹ The fasted animals show a smaller production of $C^{14}O_2$

Oxidation of glucose-C¹⁴₆ to C¹⁴O₂ in normal fasted rats.

Cumulative excretions as percent of injected glucose-C¹⁴ dose.

Drug	% C ¹⁴ O ₂ respired				CO ₂ respiration ^a			wt (grams)
	0 to 20 (min)	0 to 40 (min)	0 to 60 (min)	0 to 120 (min)	5 to 20 (min)	20 to 60 (min)	60 to 120 (min)	
None	0.89	5.21	10.7	25.1	9.5	7.7	6.4	228
None	0.93	4.99	11.4	29.5	11.4	8.2	9.0	197
None	0.76	5.32	11.2	28.7	10.7	8.1	7.1	205
None	1.19	5.04	11.3	28.1	8.7	8.2	9.5	287
Insulin	2.60	10.2	16.9	28.2	9.2	7.7	6.8	259
Insulin	2.72	8.3	13.7	24.5	9.6	6.6	5.5	290
Insulin	3.30	11.0	17.0	27.8	8.4	6.0	5.4	244
Orinase	1.62	8.7	17.0	32.2	10.5	9.4	8.3	206
Orinase	4.10	14.0	23.1	40.3	7.6	9.7	9.8	247
Orinase	1.98	9.2	17.5	35.3	8.7	7.7	7.5	270
Orinase	2.45	12.4	21.8	39.4	10.3	9.8	9.3	234
Carbutamide	1.11	4.9	10.2	24.2	8.9	7.0	6.5	219
Carbutamide	1.50	5.5	10.6	24.1	7.7	6.4	6.5	268
Carbutamide	1.74	9.6	19.4	34.9	9.9	10.4	8.3	192
Carbutamide	3.58	11.3	18.6	34.2	7.9	7.5	6.9	209
Carbutamide	1.08	6.2	13.6	30.3	7.8	7.8	7.9	202
Carbutamide	1.25	6.8	13.6	31.6	8.1	7.8	6.6	216
Carbutamide	0.80	4.7	10.2	24.4	6.9	7.2	6.5	226
Carbutamide	1.38	5.5	12.3	29.9	8.3	6.2	6.1	272
Carbutamide	0.91	4.5	9.9	23.7	7.7	6.8	6.9	264
Carbutamide	2.11	7.9	14.4	30.2	9.8	6.8	6.4	212
Carbutamide	1.42	6.9	13.6	28.7	7.0	6.9	6.9	220

^a Mg CO₂/min / $\left(\frac{250}{\text{wt rat}}\right)^{0.75}$; i.e., amount of CO₂/min normalized to a 250-gram rat.

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Table I

Oxidation of Fructose-C¹⁴₆ to C¹⁴O₂ in normal fasted rats.
Cumulative excretion is given as percent of injected fructose-C¹⁴₆ dose.

Drug	% C ¹⁴ O ₂ respired				CO ₂ respired ^a		
	0 to 20 (min)	0 to 40 (min)	0 to 60 (min)	0 to 120 (min)	5 to 20 (min)	20 to 60 (min)	60 to 120 (min)
None	1.86	6.5	11.7	25.7	11.7	7.5	7.3
None	1.63	6.2	10.5	22.8	11.0	7.1	6.3
None ^b	0.94	3.9	7.1	15.9	-	-	-
None ^b	0.88	3.6	6.3	12.3	9.7	8.6	8.3
Insulin	2.16	10.1	17.6	31.5	11.1	9.4	8.3
Insulin	1.58	6.4	11.9	24.3	11.0	7.7	6.9
Insulin	2.36	7.9	14.1	29.7	10.6	7.6	9.1
Insulin ^b	1.88	8.0	14.8	27.4	-	-	-
Insulin ^b	1.63	6.0	10.0	17.2	-	-	-
Orinase	0.86	5.2	11.4	25.1	7.7	6.1	6.1
Orinase	1.58	6.4	12.5	27.0	7.9	5.9	5.6
Carbutamide	1.38	5.4	10.5	23.8	10.4	7.0	6.4
Carbutamide	0.62	3.3	7.5	20.8	4.9	5.9	6.6

^a Mg CO₂/min / $\left(\frac{250}{\text{wt rat}}\right)^{0.75}$; i.e., amount of CO₂/min normalized to a 250-gram rat.

^b Alloxan-diabetic rat No. B-3.

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Table II

Oxidation of acetate-2-C¹⁴ to C¹⁴O₂ in normal fasted rats.
 Cumulative excretion is given as percent of injected acetate-2-C¹⁴ dose.

Drug	% C ¹⁴ O ₂ respired				CO ₂ respiration ^a		
	0 to 20 (min)	0 to 40 (min)	0 to 60 (min)	0 to 120 (min)	5 to 20 (min)	20 to 60 (min)	60 to 120 (min)
None	9.78	26.6	38.9	53.7	14.5	10.6	8.0
None	8.99	25.4	36.2	-	9.9	7.5	6.8
None ^b	7.33	23.1	35.1	50.4	13.4	11.6	9.6
None ^b	7.43	24.9	35.3	53.8	11.5	10.3	9.7
None ^c	6.26	19.3	29.3	43.3	10.7	8.3	7.8
Insulin	5.11	17.3	29.8	50.4	7.4	5.8	5.8
Insulin	6.27	19.7	32.2	51.3	10.1	7.6	7.0
Orinase	6.20	19.6	32.2	52.2	9.3	7.3	7.0
Orinase	4.56	18.8	30.8	50.9	7.2	6.8	7.6
Carbutamide	3.12	11.7	20.1	39.3	5.7	5.0	5.1
Carbutamide	5.32	18.2	28.7	48.5	6.6	6.4	6.2

^a Mg CO₂/min / $\left(\frac{250}{\text{wt rat}}\right)^{0.75}$; i.e., amount of CO₂/min normalized to a 250-gram rat.

^b These were alloxan-treated rats that did not become diabetic.

^c This was an alloxan-treated rat, severely diabetic.

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Table III

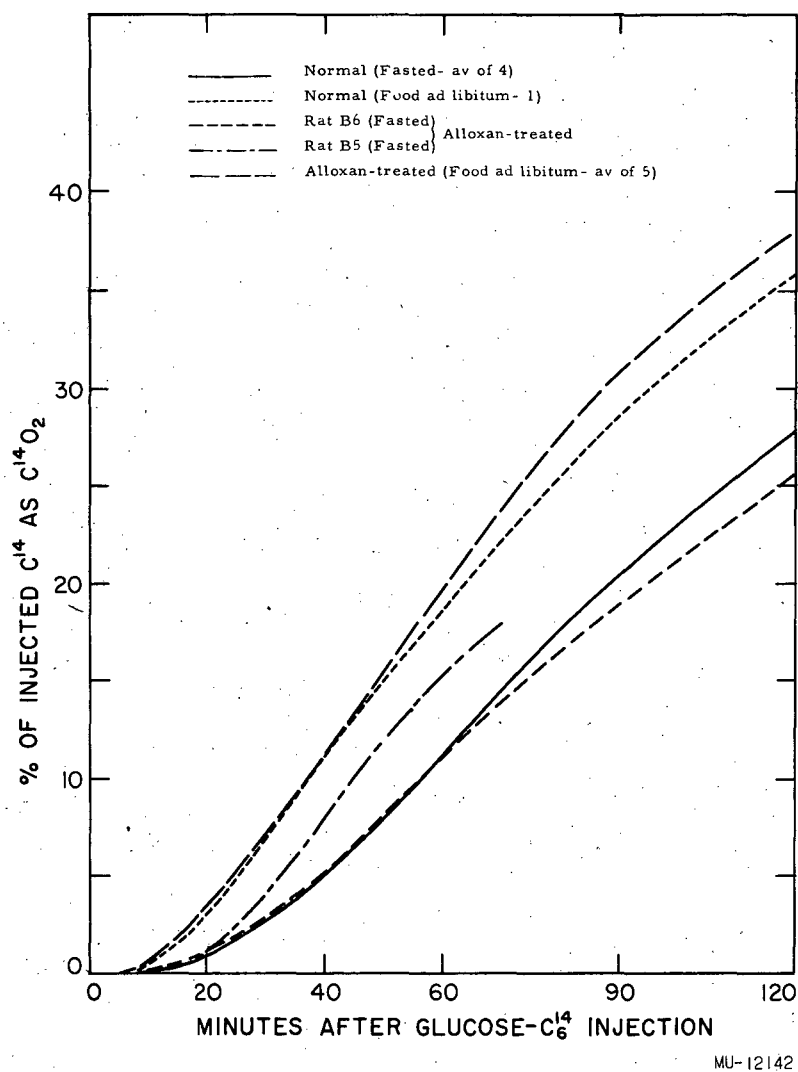


Fig. 2. $Glucose-C^{14}_6$ Respiration Patterns: Normal and Alloxan-Treated Rats which Failed to Become Diabetic.

from the labeled glucose; the absolute difference is about 5 to 10%, which corresponds to about 30% relative change.

Glucose Metabolism in Diabetic Rats

The alloxan-diabetic rat shows a greatly depressed rate of oxidation of glucose- C^{14}_6 to $C^{14}O_2$. The depressed oxidation rate can be quickly returned to normal with insulin. In Fig. 3 are shown the cumulative $C^{14}O_2$ excretion curves from the glucose- C^{14}_6 substrate for one rat (B-1). The curves on the tenth and twenty-first day after the alloxan treatment are typical curves observed in diabetic animals, i. e., less than 10% of the glucose is oxidized to $C^{14}O_2$ in 2 hours. The curves made on the fifteenth, sixteenth, and seventeenth days are normal-type curves and may be compared with those in Fig. 2.

Following this series of measurements this same rat, B-1, was given a course of Orinase treatments. Figure 4 presents the data for this experiment using the same units as before, i. e., cumulative $C^{14}O_2$ excretion from glucose- C^{14} . As before, control curves without drugs show less than 10% of the glucose oxidized to $C^{14}O_2$ in 2 hours, typical of the diabetic animal. One hour after the first oral administration of Orinase, a glucose- CO_2 respiration measurement was started, but the slowness of the action of Orinase is such that the 2% increase in total C^{14} is hardly significant. Note, however, that what increase there is occurs mostly in the second hour of the measurement, after the Orinase has had two full hours to be absorbed and begin acting.

A more dramatic way to show such effects is by use of the specific-activity curves of the animal's respired $C^{14}O_2$. Figure 5 shows the marked change in the specific activity between the alloxan-diabetic and relieved-diabetic animal. At 30 minutes after glucose injection the normal animal's specific $C^{14}O_2$ activity is about ten times that of the diabetic animal. Here, also, the gradual relief after the first Orinase dose can be seen clearly.

Carbutamide and Diabetes

The drug Carbutamide is also capable of relieving the inability of the alloxan-diabetic rat to oxidize glucose- C^{14}_6 to $C^{14}O_2$. Figure 6 shows the respiration curves for rat B-3 in the early phase after alloxan treatment. During a 4-day course of Carbutamide treatment this rat showed one normal and one nearly normal respiration pattern, although in between these patterns it exhibited two distinctly diabetic curves.

It is not always possible to relieve a diabetic animal with Orinase or Carbutamide. The above data for rat B-3 shows such relief, but 1 month later this same rat produced only standard diabetic curves during a course of Carbutamide therapy, i. e., its diabetes could no longer be relieved by this drug.

Figure 7 shows a series of measurements on rat B-1 after it had become quite diabetic. After four days of Carbutamide treatments this rat failed to show any change in its glucose- CO_2 respiration pattern. On the fifth day, 8 units of insulin returned the respiration pattern of this rat to a normal type. A sixth-day treatment was made with Orinase. The respiration pattern was of the diabetic type.

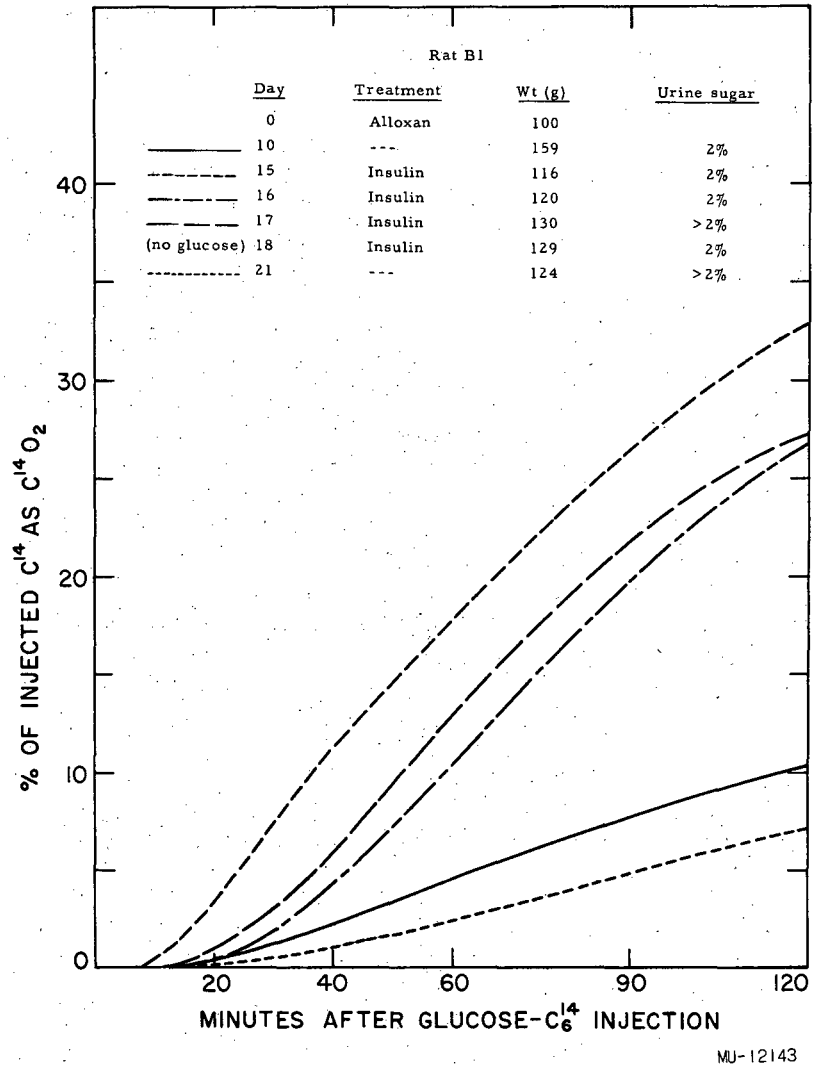


Fig. 3. Glucose- C_6^{14} Respiration Patterns: Effect of Insulin on Diabetes.

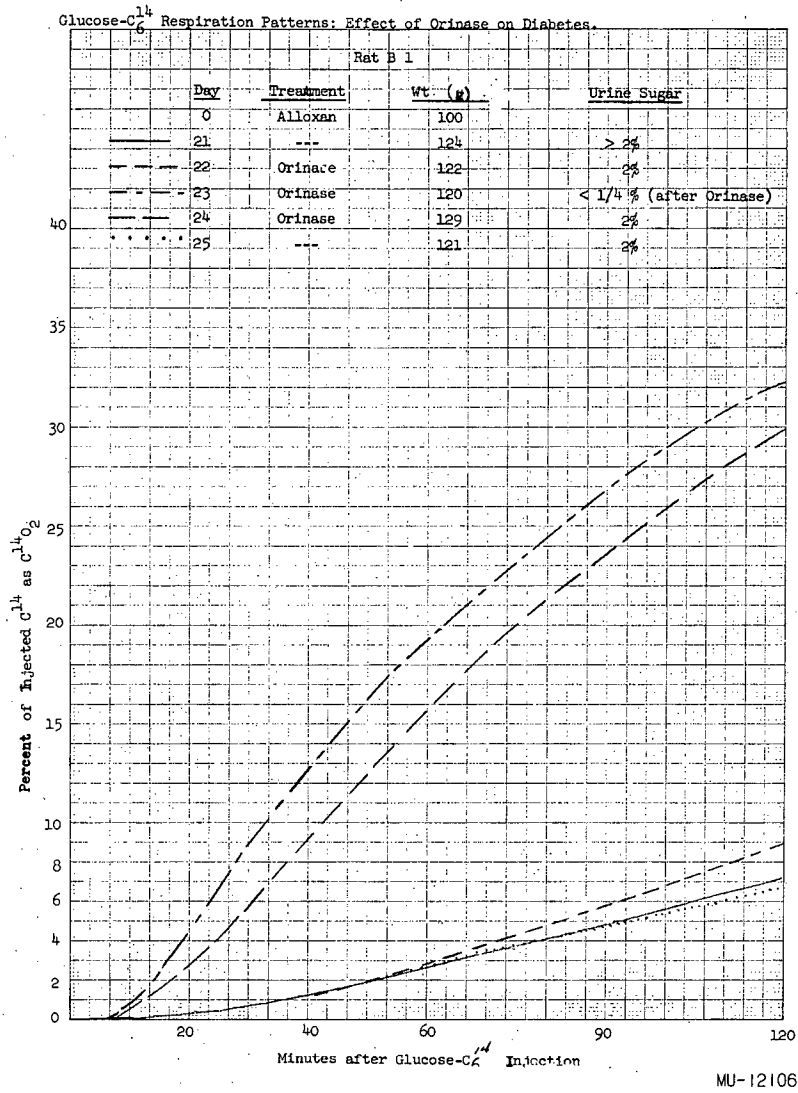


Fig. 4. Glucose-C¹⁴₆ Respiration Patterns: Effect of Orinase on Diabetes.

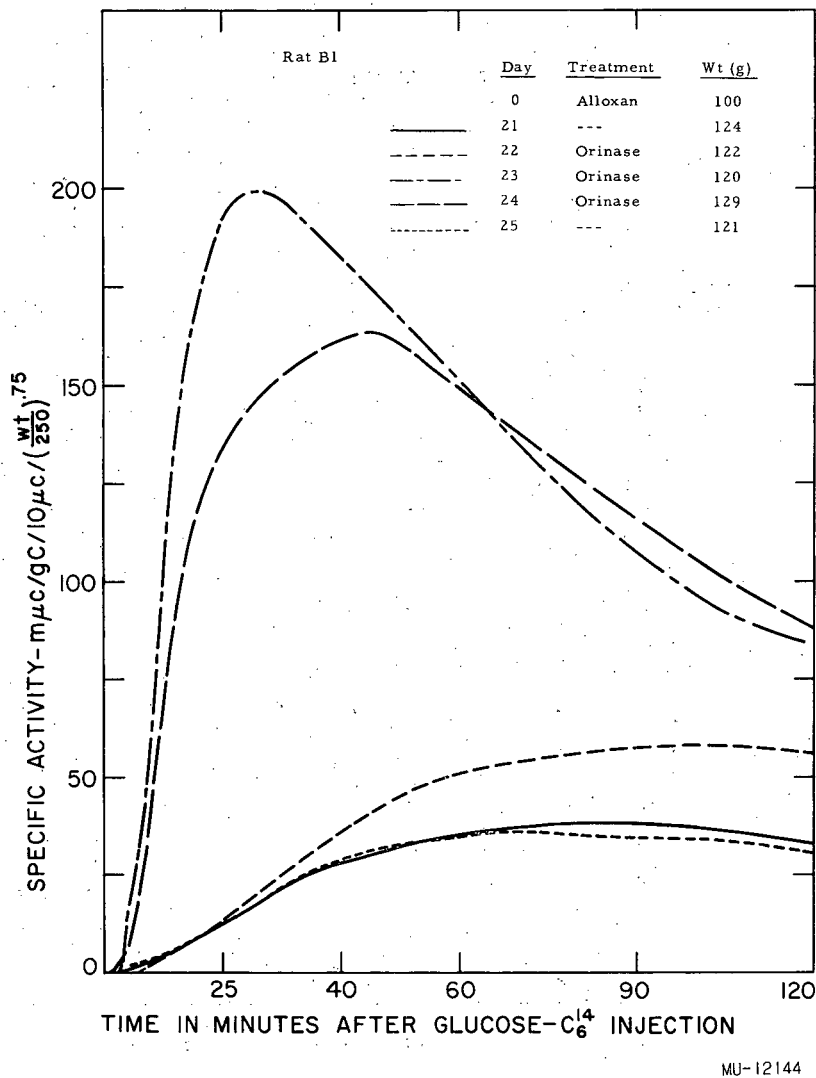
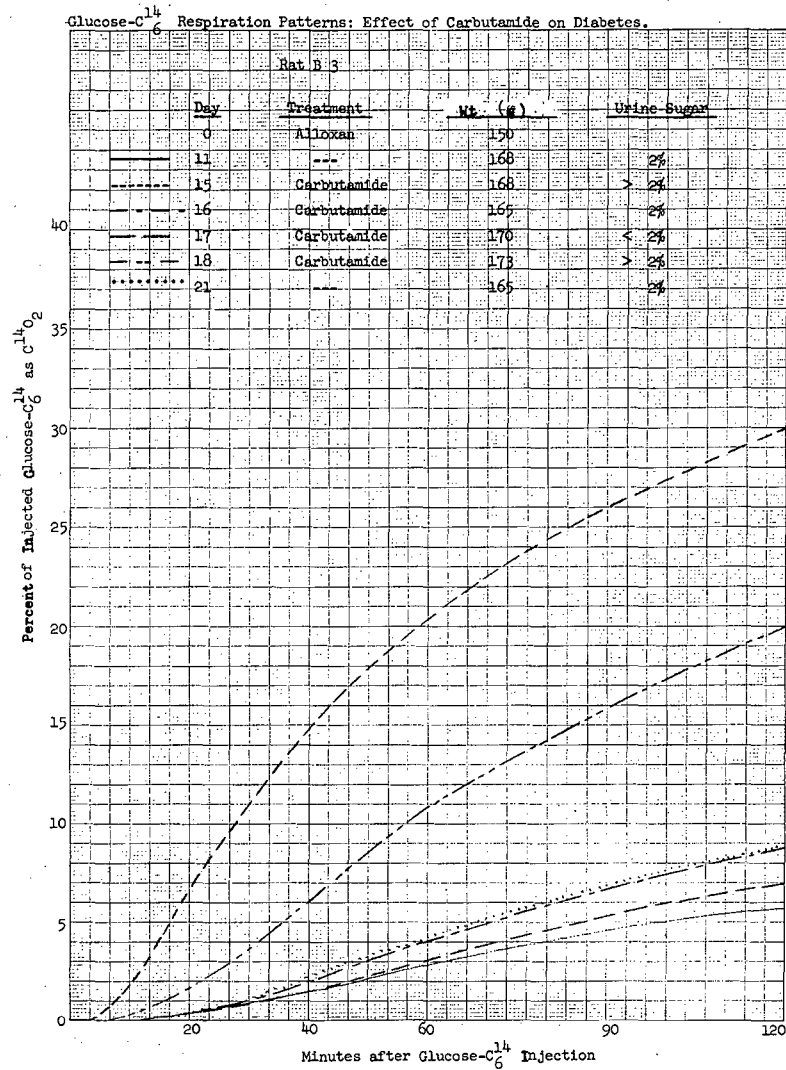


Fig. 5. Glucose-C¹⁴₆ Respiration Patterns:
Effect of Orinase on Diabetes.



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Fig. 6. Glucose-C¹⁴₆ Respiration Patterns: Effect of Carbutamide on Diabetes.

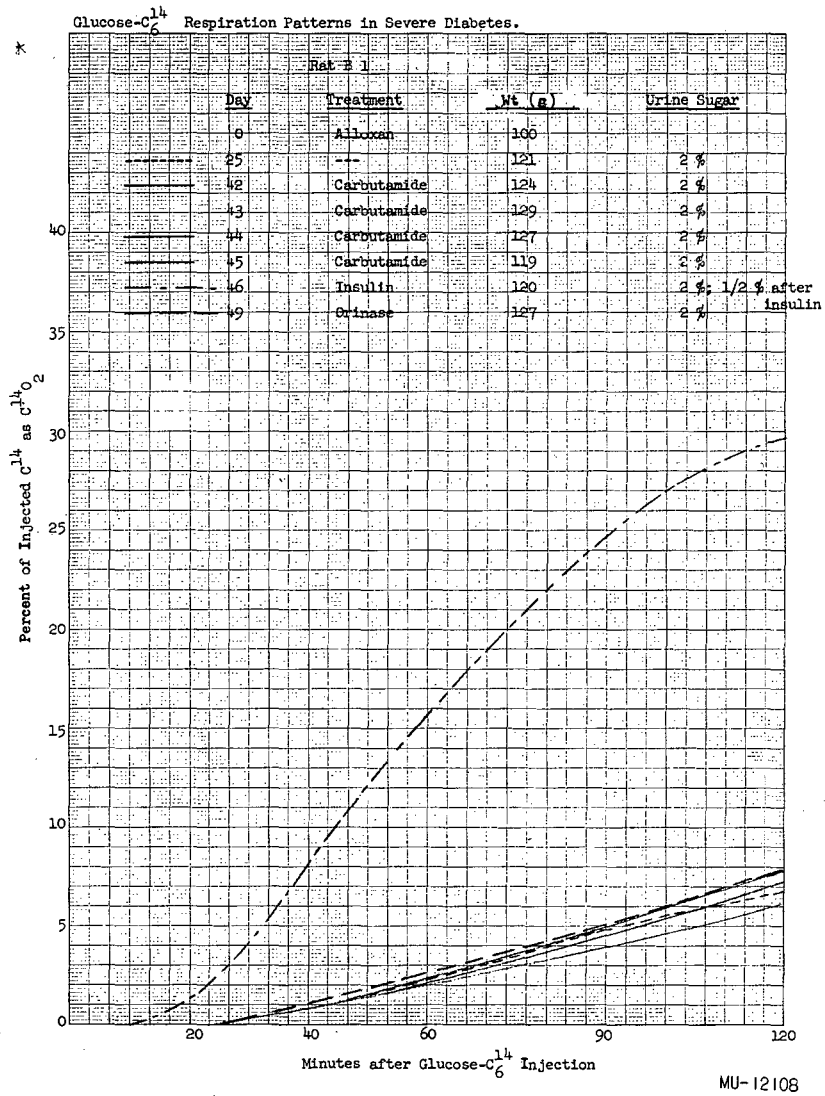


Fig. 7. Glucose-C¹⁴₆ Respiration Patterns in Severe Diabetes.

This same rat, B-1, was then series retested with Orinase to see if it could still respond to this drug. The results were negative, i. e., a 4-day course of Orinase treatments failed to change the typical diabetic-type glucose-to- CO_2 respiration curve. Thus the rat was by now unable to respond to either Carbutamide or Orinase but did respond to insulin.

Recovery of Diabetic Rats

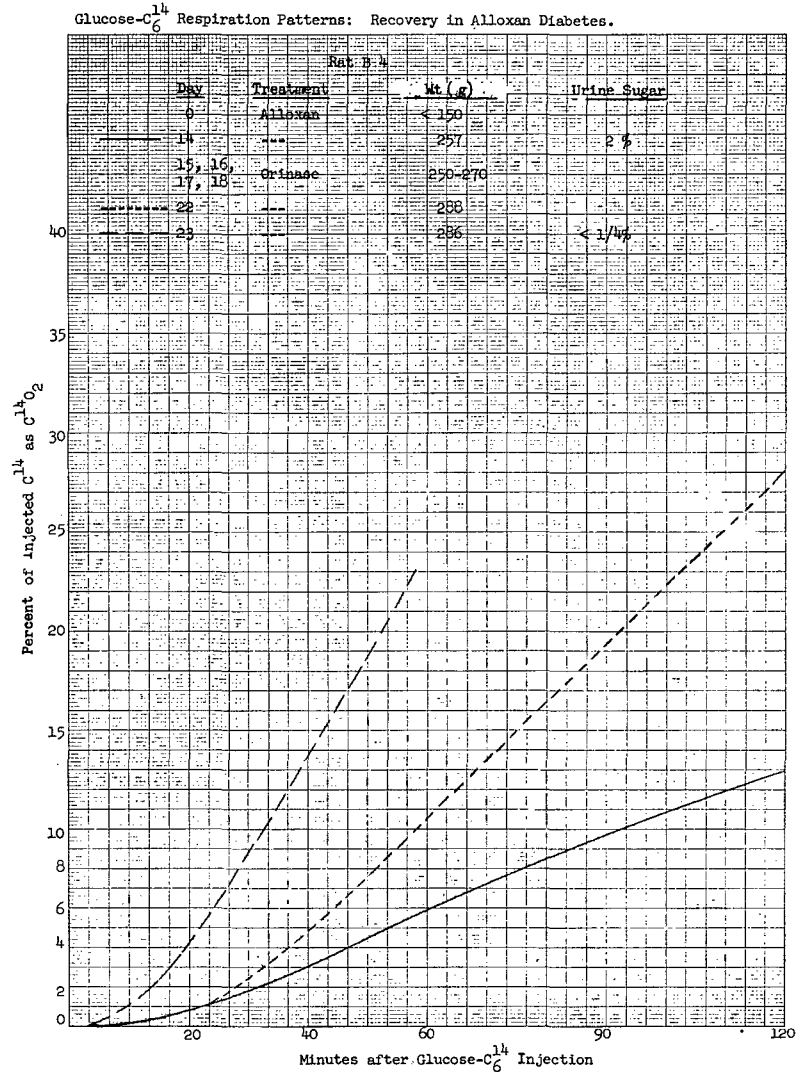
Some of the alloxan-diabetic rats show a recovery from their early inability to oxidize the labeled sugar rapidly, and the probability of such recovery seems enhanced if the glucose-to- CO_2 oxidation is not depressed too low, i. e., is less than the 25% minimum for normals but is more than the 10% maximum for diabetics. This recovery is not a new observation, but the experimental fact is clearly shown in Fig. 8, where the cumulative C^{14}O_2 excretion curves for rat B-4 are shown. At the end of the alloxan-treatment period the animal showed diabetes, although not as severe as in other rats. His curves on the twenty-second and twenty-third days were essentially normal, i. e., the rat had recovered. Note also that the rat gained weight in contrast to rats B-1 and B-3, which were severely diabetic and did not gain any appreciable weight.

Duration of Insulin Effect with and without Orinase

A series of studies was made on two diabetic rats that no longer responded to Carbutamide or Orinase treatment. The data were obtained in the following manner: The animal was given an ip injection of insulin with and without a two-dose course of Orinase treatment starting the day before. Immediately after the insulin injection, it was given a tracer glucose- C^{14}_6 injection and the 2-hour respiration curve was measured. At the end of 2 hours the glucose- C^{14}_6 injection was repeated, and this process was continued up to seven injections. The curves were analyzed by subtracting the extrapolated backgrounds from the previous injections; the condensed data are given in Table IV.

This study shows that the effect of 8 units of insulin is to produce a distinctly enhanced glucose-to- CO_2 oxidation for about 10 hours; this amount of insulin had a maximum action between the fourth to eighth hour. The Orinase does not change the duration of the insulin effect within the limits of our measurement. It does, however, produce some odd effects: the insulin-Orinase combination slows down the glucose-to- CO_2 oxidation for the first 2 to 4 hours after insulin, and later (4 to 8 hours after insulin) accelerates the oxidation of the labeled glucose.

The above observations were confirmed not only by the cumulative percent glucose oxidized to CO_2 , but also by the peak specific activity of the C^{14}O_2 . The time of the peak specific activity is in agreement with these data, as also are the shapes of the curves.



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Fig. 8. Glucose-C¹⁴₆ Respiration Patterns: Recovery in Alloxan Diabetes.

Oxidation of glucose-C¹⁴₆ to C¹⁴O₂ in severely diabetic rats.

Duration of insulin effect with and without orinase.

Rat ^b	Treatment	Time after insulin (hr)	Cumulative % glucose-C ¹⁴ ₆ oxidized to C ¹⁴ O ₂				Time (min)	Max sp. act. C ¹⁴ O ₂ Value ^a
			0 to 20 (min)	0 to 40 (min)	0 to 60 (min)	0 to 120 (min)		
B-1	Insulin	0-2	2.93	9.9	17.7	35.7	50	155
	Insulin	2-4	0.59	3.9	10.6	27.4	60	187
	Insulin	4-6	1.27	7.9	18.4	28.8	40	242
	Insulin	6-8	1.29	7.8	20.2	35.2	46	278
	Insulin	8-10	0.64	5.1	13.5	25.6	50	168
	Insulin	10-12	0.28	2.1	5.5	14.8	60	92
	Insulin	12-14	0.73	3.0	7.0	12.0	40	67
B-1	Insulin + Orinase	0-2	0.31	2.0	5.2	18.4	90	104
	Insulin + Orinase	2-4	1.16	7.8	19.0	36.4	50	247
	Insulin + Orinase	4-6	2.72	16.6	25.8	37.3	30	270
	Insulin + Orinase	5-8	4.51	22.0	32.9	45.5	30	350
	Insulin + Orinase	8-10	1.73	12.8	25.4	37.1	35	205
	Insulin + Orinase	12-14	0.29	2.6	6.8	15.3	50	66
	B-3	Insulin	0-2	1.49	5.4	9.6	16.8	40
Insulin		2-4	1.64	10.0	17.6	27.8	34	100
B-3	Insulin + Orinase	0-2	0.37	2.0	4.1	9.3	60	35
	Insulin + Orinase	2-4	0.32	2.0	4.5	13.1	70	61
	Insulin + Orinase	4-6	4.07	9.1	14.0	25.8	15	139
	Insulin + Orinase	6-8	0.14	2.6	6.4	18.5	60	102
	Insulin + Orinase	8-10				approx. 5%	60	20

^a Millimicrocuries C¹⁴/g carbon/10 μc injected/(wt)^{0.75}

^b Measurements made 71 to 74 days after alloxan treatment.

MU-12114

Table IV

DISCUSSION

Detection of Diabetes

The foregoing data show clearly that the oxidation of glucose to CO_2 in the intact rat can be used as an excellent and reliable criterion of the diabetic state of the animal. Many of the alloxanized animals that showed positive urine sugars still showed a near-normal respiration pattern, indicating that insulin production was continuing in these animals. The diabetic animals that were helped by Carbutamide or Orinase would usually continue to show positive urine sugars, but the respiration pattern clearly indicated a relief of their inability to oxidize glucose rapidly. It would have been useful to correlate our data with blood-sugar levels, but these additional measurements were not within the scope of our research time. Furthermore, the repeated respiration measurements made on one animal would have suffered from too much bleeding.

Alloxan Diabetes in the Rat

The availability of a test that directly measures the biochemical abnormality of a diabetic animal permits an interesting confirmation of the nature of alloxan diabetes. Many previous investigators have commented on the difficulty in evaluating these animals. In a group of twelve animals treated with alloxan, we had three deaths within the first 2 weeks, five animals failed to show diabetic respiration patterns for the glucose- C^{14} substrate, and four animals were diabetic. These four animals showed the following histories. Rat B-1 first responded to Orinase. Later it became resistant to both Orinase and Carbutamide. Its weight remained constant. Rat B-3 at first responded to Carbutamide but later became resistant to this drug. It did not gain weight. Rat B-4 was at first diabetic and responded to Orinase. Sometime during these experiments it recovered partial insulin-producing ability and could oxidize glucose at near-normal rates. It became fat. Rat B-7 at first could be helped by Carbutamide treatment occasionally. Later it showed a partial ability to oxidize glucose to CO_2 , that is, its 2-hour C^{14}O_2 excretion was between 10% and 25%, which is neither in the normal range nor in the severe diabetic range. This animal also gained considerable weight.

This variation in the diabetic state of the four animals studied shows the utility of a good test for diabetes, and perhaps points out some of the reasons for previous discrepancies in literature results on diabetes experiments. It is very difficult to control the exact extent of the disease state.

Nature of the Diabetic Abnormality

The measurements on the glucose oxidation confirm the published evidence^{12, 13} that the severely diabetic rat cannot rapidly oxidize glucose to CO_2 . The 30-minute specific activity of the C^{14}O_2 for the sick animal is decreased to about 1/10 normal. The 2-hour cumulative excretion is reduced to about 1/4 normal.

Although fructose oxidation is decreased in diabetes, the effect is not nearly so large as for glucose, being at most a factor of 1/2 of the normal animals' respiration. A single acetate-2-C¹⁴ measurement on a diabetic animal indicated only a small reduction in C¹⁴O₂ output, confirming Chaikoff's data.^{14, 15}

Drug Effects in Normal Rats

The sulfonamide derivatives, Orinase and Carbutamide, have been shown to lower blood sugar in normal rats, dogs, rabbits, and humans.¹⁶ These compounds have also been shown to be effective in lowering blood sugar levels in certain types of diabetics.^{17, 18, 19} In our own experiments they proved capable of increasing the production of C¹⁴O₂ from glucose-C¹⁴₆ in normal rats. The effects are not always the same. Insulin stimulated the early production of C¹⁴O₂, but the 2-hour cumulative excretion was not appreciably changed. It may be that this was caused by the short-term effect of the small dose of crystalline insulin.

Orinase increased glucose oxidation appreciably over the entire 2-hour period. It is, of course, a long-acting drug. The compound Carbutamide sometimes produced a marked increase in the oxidation of glucose to CO₂ and sometimes did not. We have no explanation for this variability, except to ascribe it to differences in susceptibility of the several animals to Carbutamide.

Fructose-C¹⁴₆ and acetate-C¹⁴₂ oxidation to C¹⁴O₂ are not markedly affected by insulin, Orinase, or Carbutamide; neither is the oxidation of lactate-1-C¹⁴ affected by Orinase.

Diabetic Relief by Drugs

The mechanism of the hypoglycemic action of the drugs, Orinase and Carbutamide, is not yet known. Mirsky et al.^{20, 21} suggest that it may be due to a noncompetitive inhibition of insulinase and a consequent decrease in the destruction of endogenous insulin. They also state that it cannot be due to the diminution in the availability or activity of glucagon, as these compounds are ineffective in lowering blood sugar in the severely diabetic, alloxanized animal. Moorhouse and Kark²² suggest that the action of Carbutamide is related to the release of hepatic glucose. Tyberghein^{23, 24} and co-workers found that treatment with Orinase reduced the spontaneous glucose output from rabbit liver slices and also reduced the formation of inorganic phosphate from liver homogenates in the presence of added glucose-6-phosphate. They conclude, therefore, that Orinase may produce hypoglycemia at least partially by decreasing glucose-6-phosphatase activity in the liver.

In a series of experiments with dogs, Anderson and co-workers²⁵ also suggest that the site of Orinase action may be proximal to the phosphorylase enzyme systems and take the form of suppression of glucagon production at the source, with resulting reduction in liver glucose output. Involvement of the phosphorylase enzyme systems is also mentioned by M. Vaughan.²⁶

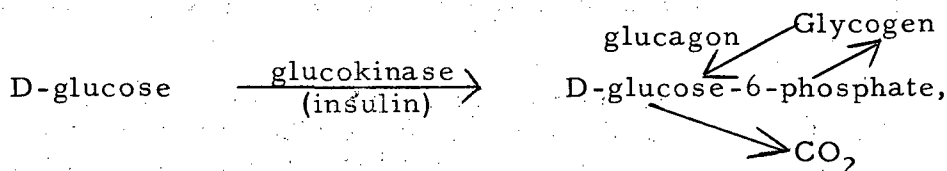
In our experiments all the cases tested showed that insulin is capable of returning the abnormal--i. e., diabetic--glucose-CO₂ respiration curve to near normalcy and that the ip crystalline insulin can produce this effect very quickly. In a limited number of cases, equivalent relief was provided diabetic animals by Carbutamide or Orinase. Figures 3 through 7 show that the control glucose-respiration patterns for the severely diabetic animal that could not be aided by Carbutamide or Orinase is the same flat curve as was observed earlier when the animal could be aided by these oral drugs.

If we assume that the severely diabetic rat that is drug-resistant is producing and releasing no insulin, then these experiments show that some diabetic rats can be helped by the oral drugs at a time when they are liberating virtually no insulin. Thus the Carbutamide and Orinase would seem to be acting by stimulating the release of insulin, and--perhaps indirectly--by stimulating the production of insulin.

The concept of these oral drugs as anti-insulinases requires that a certain production of insulin be taking place, which should be measurable in the animal. The similarity of the glucose diabetic-type oxidation curves, before and after complete resistance to the drug has set in, would indicate that no insulin release was occurring in the animal which was relieved by Carbutamide or Orinase, and would tend to discredit the anti-insulinase theory. This argument does not preclude the possibility of an intrapancrease production and destruction of insulin.

The data on the duration of the insulin action given in Table IV also would indicate that Carbutamide and Orinase do not work via an anti-insulinase mechanism. These data do indicate that the action of the oral drugs is not a simple stimulation of insulin production, for a definite decrease was observed in the glucose-to-CO₂ oxidation during the 4-hour period after insulin injection when the Orinase was also given. The 4-to-8-hour curves showed unusually fast oxidation rates for the labeled glucose, which cannot be explained by a simple insulin release theory. Our data would therefore indicate that the oral drugs such as Orinase and Carbutamide work not only by stimulation of insulin release and (or) production but also by changing the concentration of other factors. A likely possibility here is a modification of glycogen and glucose pool size, which could be accomplished via changes in glucagon concentration, according to the theory that the oral drugs have an antiglucagon action.^{23, 25}

If we consider the metabolic scheme for glucose,



we see that a diabetic animal that has little or no insulin has a depleted glycogen and glucose-6-P pool. If this animal is given one of the oral drugs, his glucagon activity will be decreased, but if the animal is severely diabetic and resistant to these drugs, no insulin will be produced. If the animal is now given insulin sometime after the oral drug, the first glucose-6-P

produced will be able to go rather irreversibly either to CO_2 or to glycogen, depending on many complex factors. The duration-effect experiments indicate that for the first 2 to 4 hours the glucose-6-P goes mostly to glycogen, and that later a large percentage goes to CO_2 . This very strong initial drive of the liver to form some glycogen pool is also observed in Tyberghein's experiments with starved rats under the influence of Orinase. The diabetic animal that receives only insulin has not had his glucagon activity suppressed, and so any glycogen formed can be readily remobilized to glucose-6-P.

The severely diabetic animal shows a glucose-to- CO_2 oxidation pattern that is surprisingly constant and virtually independent of urine sugar concentration and, therefore, more or less independent of blood sugar levels. This residual rate of glucose oxidation observed in all severely diabetic animals amounted to 5 to 10% of the injected radioactivity in 2 hours, and is interesting not only because of its constancy in individual cases and between cases in severe diabetes but also because of its appreciable magnitude. The oxidation may occur by alternate pathways by which glucose can be catabolized without going through the steps suggested by Mirsky in his review,²⁷ i. e., the glucose-to-glucose-6-phosphate step catalyzed by hexokinase and requiring the indirect action of insulin. A good possibility here is that glucose can be converted to glucose-1-phosphate at a slow but finite rate and that this substance can then be isomerized to glucose-6-phosphate and thence to CO_2 via normal channels.

If the oral drugs act solely as antiglucagon agents, we should expect the glucose-to- CO_2 rate to change under the effect of the drugs by not much more than the blood-sugar level changes. However, the peak specific activity of the C^{14}O_2 was increased 5 to 10 times when the diabetic animal responded to Carbutamide or Orinase. This change is several times as large as is observed in blood-sugar level changes with drug treatment. Thus, our data indicate that Carbutamide and Orinase must be affecting the glucose metabolism in the body more directly than by a mere concentration level control, as would be the case if these drugs were only antiglucagon agents.

We hope to repeat the measurements in humans in the near future as the equipment and techniques have been extended to such measurements.³ As seen from these data, alloxan diabetes in rats is a variable condition and the observations on the course of this disease cannot necessarily be extrapolated to humans. However, the mode of action of these drugs and the biochemical abnormality are probably the same, and certainly can be easily confirmed.

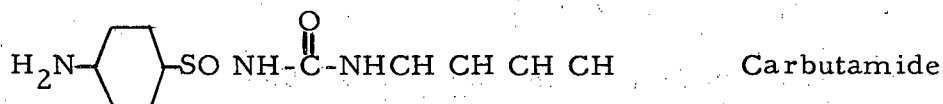
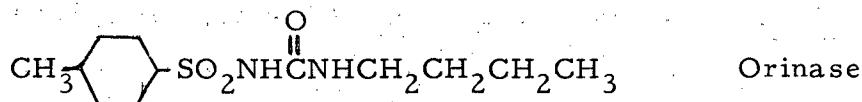
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