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OXIDATION OF ACETATE, CLUCOSE, OR GLYCINE TO CO2 IN MICE EXHIBITING THE HEREDITARY OBESITY SYNDROME

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ABSTRACT

The oxidation of acetate-2-C<sup>14</sup>, glucose-C<sup>14</sup><sub>6</sub>, and glycine-2-C<sup>14</sup> to C<sup>14</sup>O<sub>2</sub> was studied in the hereditary obesity syndrome of mice. Both the rate of excretion and cumulative excretion of C<sup>14</sup>O<sub>2</sub> from acetate was depressed in the obese mice as compared to the controls. Glucose metabolism was not changed appreciably except for the obese males that showed a mild-diabetic-type pattern. Glycine oxidative metabolism was not particularly affected in the obese mice.

## OXIDATION OF ACETATE, GLUCOSE OR GLYCINE TO CO<sub>2</sub> IN MICE EXHIBITING THE HEREDITARY OBESITY SYNDROME

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A strain of mice, developed at the Jackson Memorial Laboratory, Bar Harbor, Maine, which exhibits a hereditary obesity-diabetes syndrome has been rather extensively studied. A concise review of this work is presented by Guggenheim and Mayer.<sup>1</sup> In the same paper, they also report studies on acetate metabolism in this strain of mice, with a decreased production of C<sup>14</sup>O<sub>2</sub> from labeled acetate over a 3-hr period, and suggest a partial block of the C<sub>2</sub>-fragment oxidation as the characteristic biochemical lesion in these animals. Parson and Crispell<sup>2</sup> present data that do not confirm the results of Guggenheim and Mayer.

Mayer, Bates, and Dickie<sup>3</sup> report a high blood-sugar level in these obese mice: 200 mg% compared to 110 mg% for the nonobese controls. The blood-sugar level of the obese mice was lowered 50 to 60% by fasting for four hours, while that of the controls remained unchanged. The obese mice also proved to be very insulin-resistant.

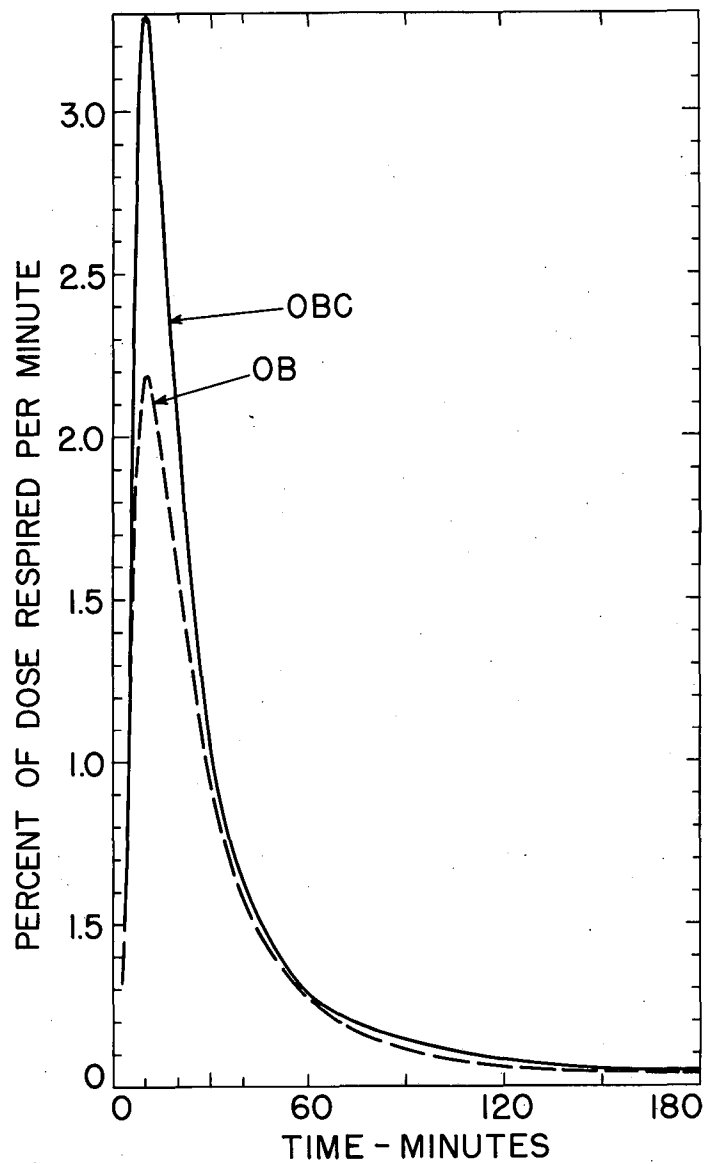
In order to obtain additional data on these animals and their metabolic abnormality, we have determined the patterns for carbon-14 dioxide excretion following the injection of labeled glucose, acetate, or glycine. The C<sup>14</sup> respiratory patterns following glucose-C<sup>14</sup> administration were determined for one animal with and without insulin. These data have been used to define further abnormalities of intermediary metabolism rates or of metabolic-pool sizes.

### EXPERIMENTAL

Obese mice, both male and female, and their litter-mate controls were obtained from the Jackson Memorial Laboratory. Acetate-2-C<sup>14</sup>, glucose-C<sup>14</sup>, and glycine-2-C<sup>14</sup> were the compounds tested. In each case the dose, injected either intraperitoneally (i. p.) or intravenously (i. v.), was 1 mg and contained about 5 μc of C<sup>14</sup>. Respiratory excretion of C<sup>14</sup>O<sub>2</sub> was followed thereafter for 7 hr. In this method of studying respiratory metabolism, the animal is injected with a labeled compound and immediately placed in a glass cage. Air is drawn through the animal cage, then through an ionization-chamber, vibrating-reed-electrometer combination which continuously measures the radioactivity in the air passing through the chamber and records the value on a recording potentiometer.<sup>4</sup> The data obtained in this manner can be expressed as a rate curve (percent per minute of injected dose respired as C<sup>14</sup>O<sub>2</sub> plotted vs. time after injection) and as a cumulative breath-excretion curve (cumulative percent of injected dose excreted as C<sup>14</sup>O<sub>2</sub> plotted vs. time after injection). Each experimental curve given in this paper represents the average of data obtained from 12 animals.

### RESULTS

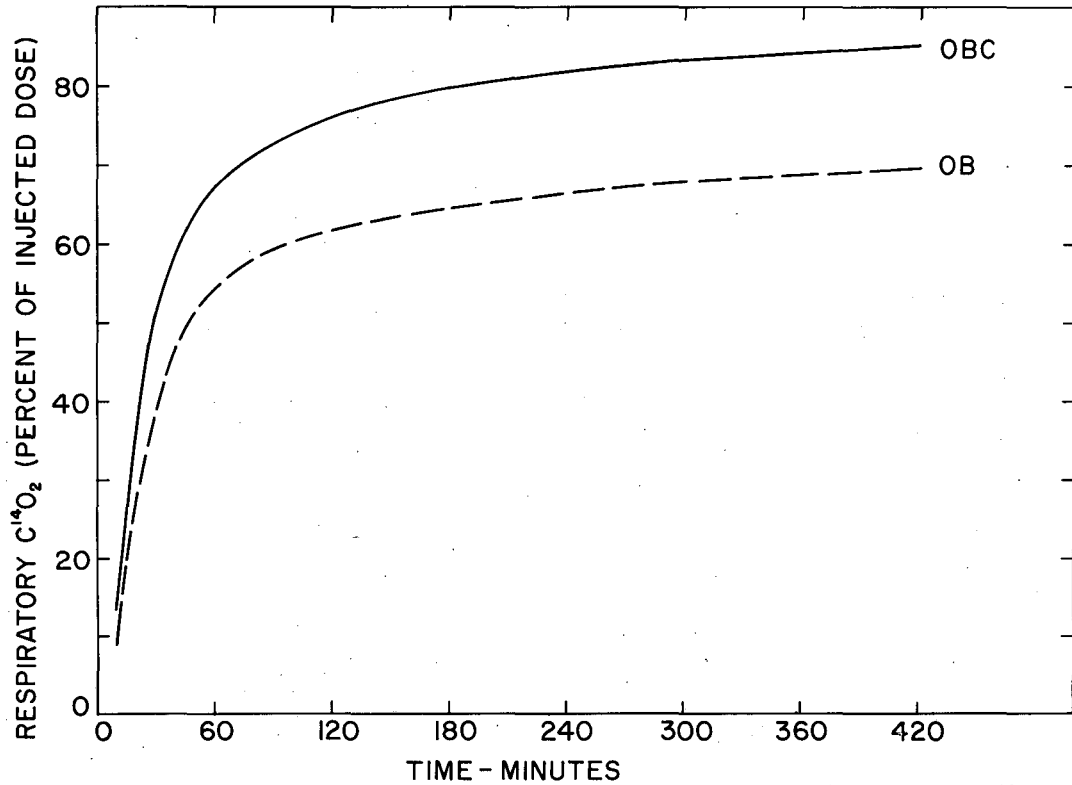
The first experiments, in which acetate was injected i. p., showed a striking difference between the obese and control animals (see Figs. 1 and 2). It was felt that owing to the great quantity of fatty tissue in the abdominal cavity of the obese mice, the considerable differences seen in respiration could be mostly caused by a



MU-10564

Fig. 1. Rate of respiration of  $C^{14}O_2$  following i.p. injection of acetate-2- $C^{14}$ . Percent per minute of the injected dose respired as  $C^{14}O_2$  is plotted vs. time after injection.

OB = obese mice  
OBC=litter-mate control mice



MU-10563

Fig. 2. Cumulative respiratory excretion of  $C^{14}O_2$  following i. p. injection of acetate-2- $C^{14}$ . Cumulative percent of injected dose respired is plotted vs. time after injection.

OB = obese mice  
OBC = litter-mate control mice.



slower absorption of the injected acetate. Therefore, the experiments were repeated, injecting the acetate i.v. instead of i.p. The same type of respiratory pattern differences were observed (see Figs. 3 and 4).

In the first experiments involving the metabolism by obese mice of i.p. - injected glucose, we found a rather wide variation among animals, depending to a large extent on their physical activity. In an attempt to reduce this variation, and also to eliminate the possibility of poor absorption of the i.p. glucose by the obese mice, we repeated the experiments, injecting the glucose i.v. The results from these later experiments showed a considerably improved reproducibility, but there was such a difference between the male and female mice that the results on glucose-to- $\text{CO}_2$  oxidation are presented separately for each sex (see Figs. 5 and 6).

As can be seen from Figs. 7 and 8, there was no striking difference between obese and control mice in the  $\text{C}^{14}$  respiration patterns from glycine-2- $\text{C}^{14}$ .

## DISCUSSION

The rate at which a tracer carbon-14-labeled metabolite is excreted as  $\text{C}^{14}\text{O}_2$  in the breath is a function of many factors, including the rates of oxidation for the several steps required to convert the compound to  $\text{CO}_2$ , the sizes of the several metabolic pools on this pathway(s) and, finally, the size and turnover rate of the body bicarbonate pool itself. It is not the purpose of this paper to attempt to analyze this complex of reaction kinetics. Rather, we will attempt to interpret the data presented above in a qualitative way.

In the first hour following injection of labeled acetate, there is a significant decrease in the fraction of the activity excreted as  $\text{C}^{14}\text{O}_2$  for the obese mice as compared to the controls. In addition, the rate of excretion of the  $\text{C}^{14}\text{O}_2$  from the obese mice is significantly depressed as compared to that of the controls. These data confirm the conclusion of Guggenheim and Mayer<sup>1</sup> who suggest that a partial block of the  $\text{C}_2$  fragment oxidation is the characteristic biochemical lesion in these animals. However, a direct comparison of results cannot be made because we used methyl-labeled acetate whereas the other workers with obese mice have used carboxyl-labeled acetate.

The turnover and equilibration of the acetate pool is sufficiently rapid that in one hour most of the injected acetate-2- $\text{C}^{14}$  will either have been excreted or equilibrated with many other compounds. We can infer, therefore, that the reduced oxidation rate for this  $\text{C}_2$  fragment must reflect either an increased synthesis of fat or sugars, or a vastly increased body pool of intermediates leading to these substances.

The discrepancy between Guggenheim and Mayer's work and that of Parson and Crispell<sup>2</sup> may well be a matter of dose size. Guggenheim and Mayer's value was about 0.8 mg acetate/gm body weight which is a very high dose. Whether this is critical for carboxyl-labeled acetate is difficult to say. For methyl-labeled acetate, it is the maximum that one may use and still get anything like a normal respiration pattern. Parson and Crispell do not define a gravimetric dose, although we assume it was less than that of Guggenheim and Mayer judging from their radiochemical source. The carboxyl carbon of acetate is much more rapidly and completely oxidized to  $\text{CO}_2$  than is the methyl carbon. It is, therefore, more difficult to measure changes in the rate of acetate metabolism using this labeled isomer.

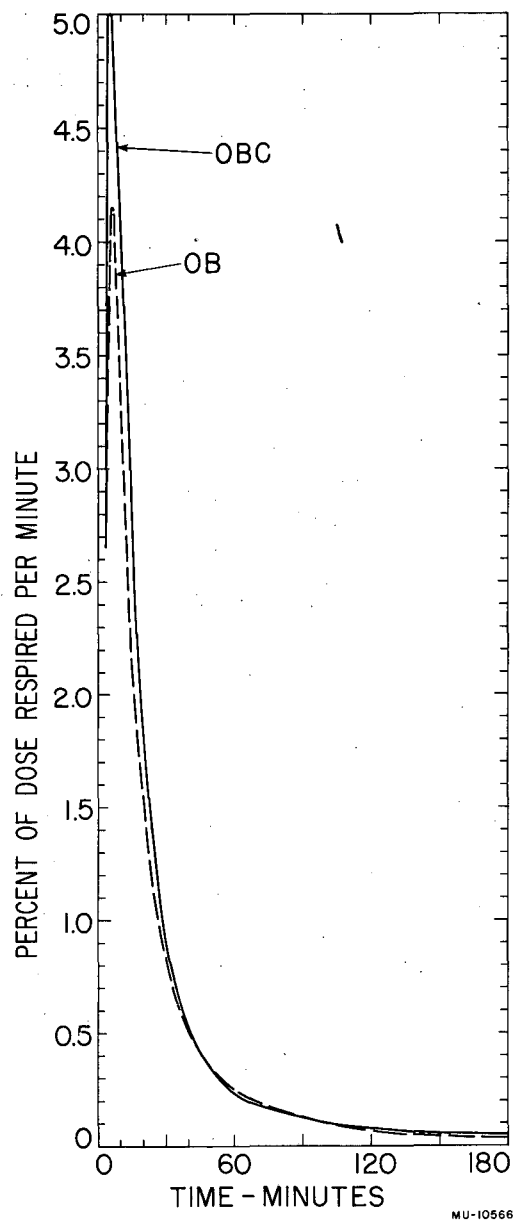


Fig. 3. Rate of respiration of  $C^{14}O_2$  following i. v. injection of acetate-2- $C^{14}$ . Percent per minute of the injected dose respired as  $C^{14}O_2$  is plotted vs. time after injection

OB = obese mice  
OBC = litter-mate control mice.

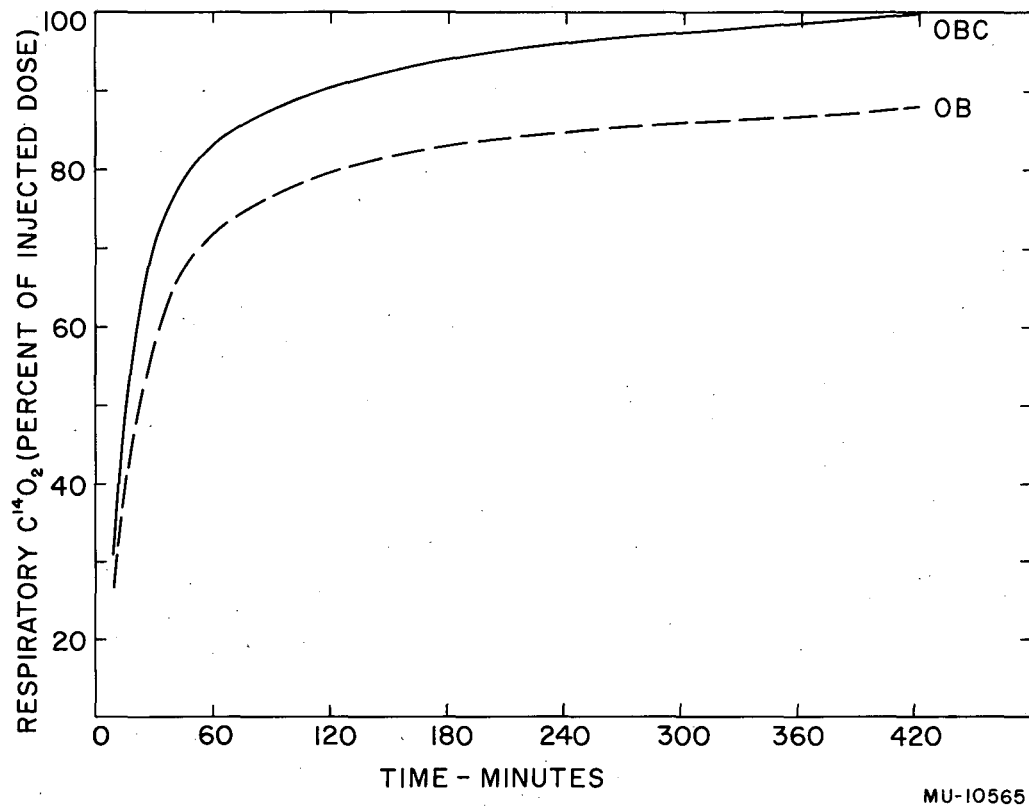


Fig. 4. Cumulative respiratory excretion of  $C^{14}O_2$  following i. v. injection of acetate-2- $C^{14}$ . Cumulative percent of injected dose respired is plotted vs. time after injection.

OB = obese mice  
OBC = litter-mate control mice.

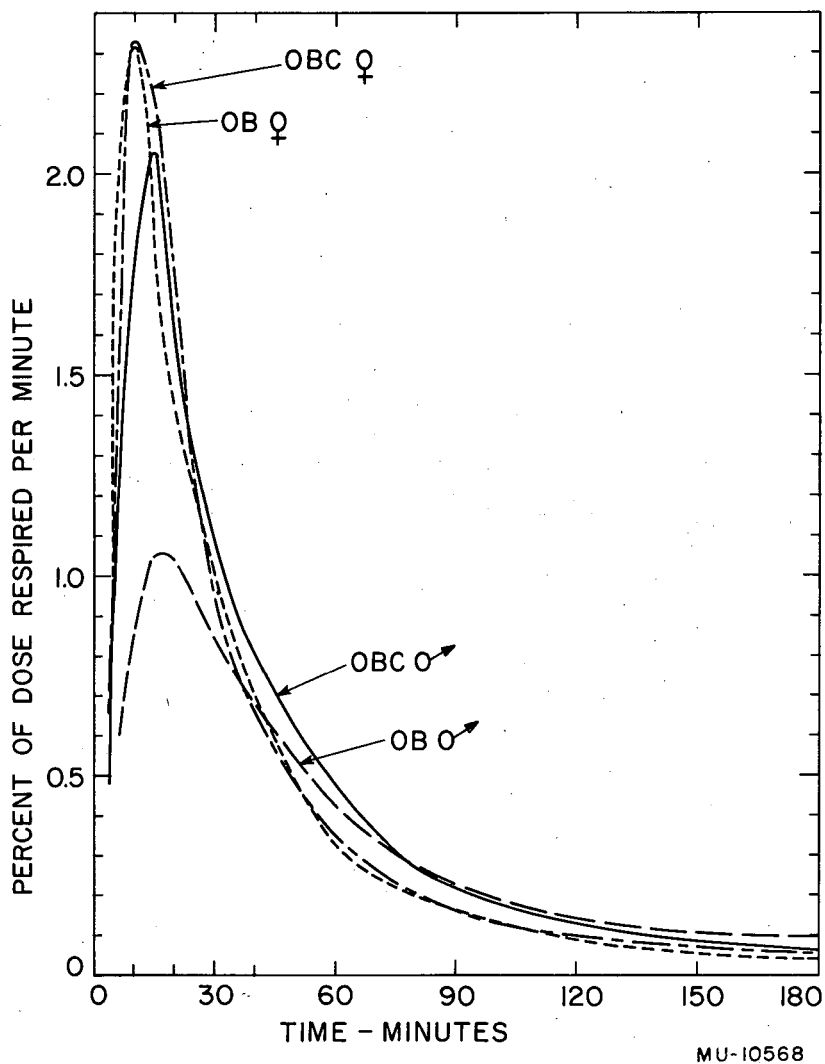


Fig. 5. Rate of respiration of  $C^{14}O_2$  following i.v. injection of glucose- $C^{14}_6$ . Percent per minute of the injected dose respired as  $C^{14}O_2$  is plotted vs. time after injection.

OB = obese mice  
OBC = litter-mate control mice.

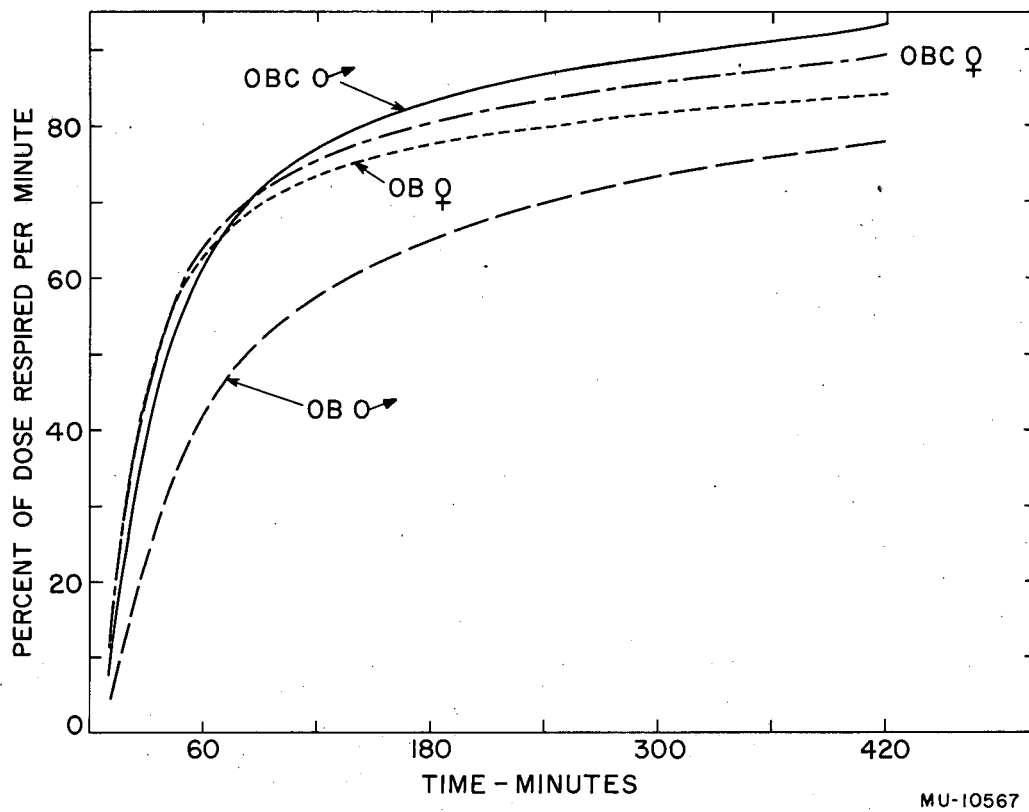
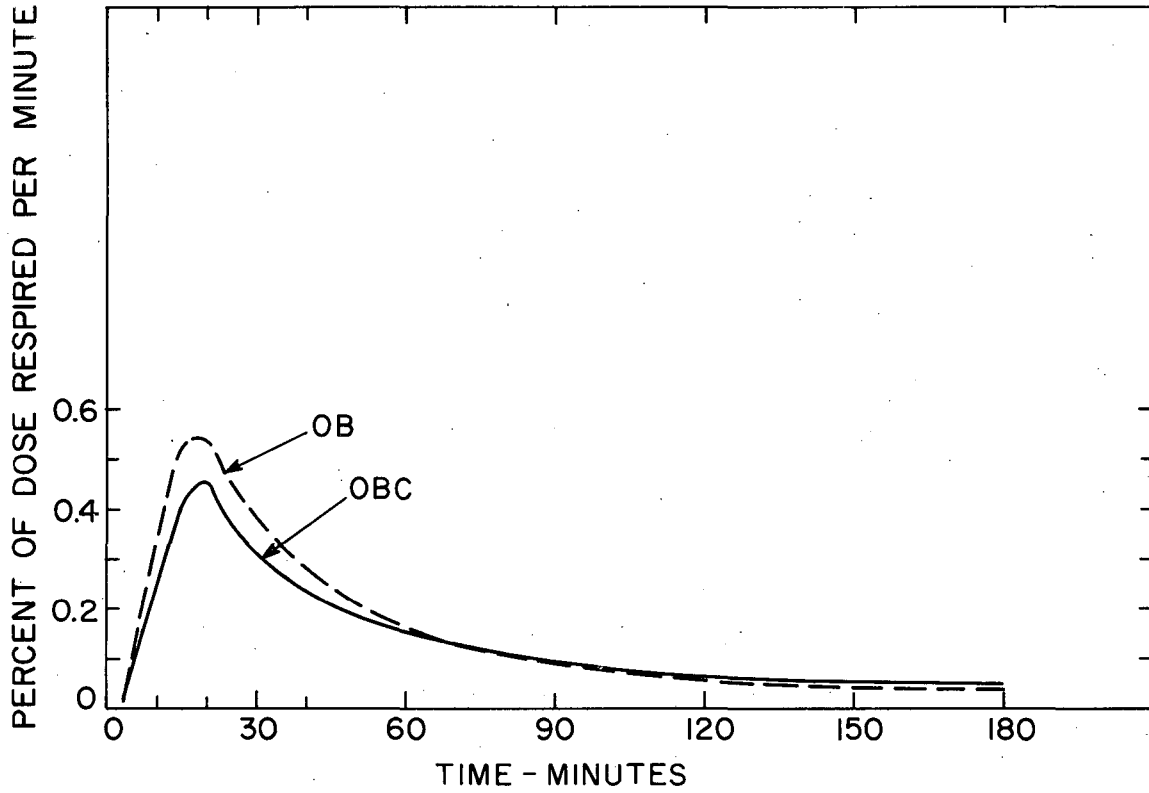


Fig. 6. Cumulative respiratory excretion of  $C^{14}O_2$  following i.v. injection of glucose- $C^{14}_6$ . Cumulative percent of injected dose respired is plotted vs. time after injection.

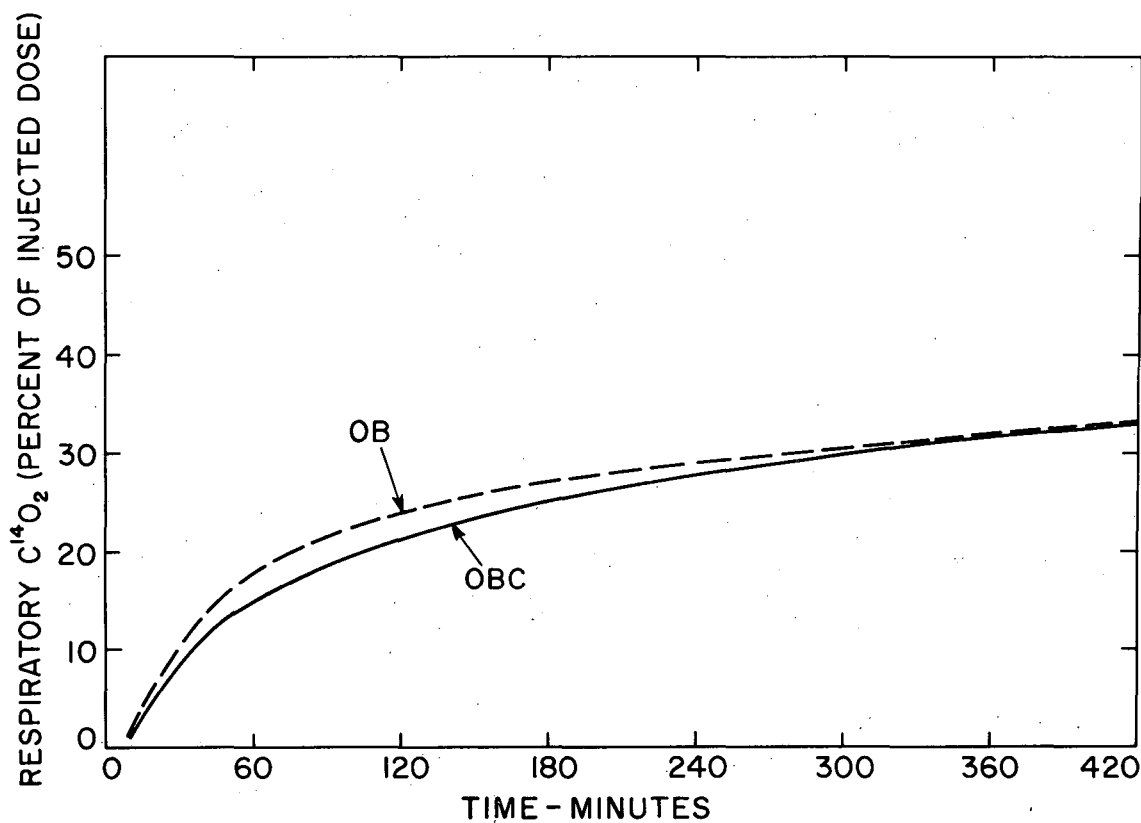
OB = obese mice  
OBC = litter-mate control mice



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Fig. 7. Rate of respiration of  $C^{14}O_2$  following i. p. injection of glycine-2- $C^{14}$ . Percent per minute of the injected dose respired as  $C^{14}O_2$  is plotted vs. time after injection.

OB = obese mice  
OBC = litter-mate control mice.



MU-10569

Fig. 8. Cumulative respiratory excretion of  $C^{14}O_2$  following i. p. injection of glycine-2- $C^{14}$ . Cumulative percent of injected dose respired is plotted vs. time after injection.

OB = obese mice  
OBC = litter-mate control mice.

In our work the difference observed in the rate of respiration of  $C^{14}O_2$  from labeled acetate derived from i. p. or i. v. injection is probably due to the slower absorption following i. p. injection. The fact that the cumulative respiration of  $C^{14}O_2$  is also decreased, even over seven hours, is more difficult to explain. <sup>2</sup> Because most of the peritoneally absorbed acetate must go through the liver before it can enter general circulation and metabolism, we feel that this difference could represent the metabolic difference between a first pass in the liver vs. a first pass in the over-all body tissues. This picture is consistent with the present knowledge of the liver as a very active site of metabolic synthesis, and with some unpublished results from this laboratory on respiration of  $C^{14}O_2$  from the several labeled lactates and succinates as a function of the site of injection.

The metabolism of glucose to  $CO_2$ , measured as a rate study (Fig. 5) or as a cumulative oxidation of the glucose to  $CO_2$  (Fig. 6), is very comparable for the female obese or control mice and for the male controls. The obese male mice present a rather different picture. This abnormal glucose-metabolism pattern is probably derived from the high blood-sugar levels found in these obese mice by Mayer, Bates, and Dickie. <sup>3</sup> The depressed activity and cumulative  $C^{14}O_2$  respiration is in accord with the data of Tolbert and others <sup>5-8</sup> who have studied the oxidation of glucose  $C^{14}_6$  to  $C^{14}O_2$  in diabetic rats and mice.

It is interesting to note that the obese male mice show this glucose-oxidation abnormality clearly, whereas it is just barely inferred from the data for the obese female mice. Clinical tests for diabetes in these mice were negative except for one male mouse that was not included in the above data. This mouse showed a positive urine-sugar test and the glucose metabolism was so modified that only 10% of the injected glucose was recovered as  $CO_2$  in the 7-hr period. The administration of insulin with glucose tripled the amount of glucose- $C^{14}_6$  oxidized to  $C^{14}O_2$ .

The rate of oxidation of glycine-2- $C^{14}$  to  $C^{14}O_2$  is slightly faster for obese mice during the first hour after injection of the glycine than for the controls. Cumulative excretion of the  $C^{14}O_2$  for the obese vs. control mice becomes nearly identical after the first few hours, although the peak-rate difference is about 20%. The direction of change is consistent with the picture that the obese mice are producing active acetate from any available food sources for the synthesis of fat. In the control mice, less of the glycine would be degraded to this fat precursor, so less activity would appear in metabolic pools where it could be quickly oxidized to  $CO_2$ .

#### ACKNOWLEDGMENT

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FOOTNOTES

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