Title
EFFECT OF FEEDING ON C1402 RESPIRATION OF LABELED METABOLITES IN RATS

Authors
Kirk, Martha R.
Lepkovsky, Samuel
Tolbert, Bert M.

Publication Date
1958-02-19

UCRL 3854

UNIVERSITY OF CALIFORNIA

Radiation Laboratory

BERKELEY, CALIFORNIA

DISCLAIMER

UCRL-3854

UNIVERSITY OF CALIFORNIA

Radiation Laboratory

EFFECT OF FEEDING ON $C^{14}O_2$ RESPIRATION OF LABELED METABOLITES IN RATS

Martha R. Kirk, Samuel Lepkovsky and Bert M. Tolbert

February 19, 1958

Berkeley, California

EFFECT OF FEEDING ON $C^{14}O_2$ RESPIRATION OF LABELED METABOLITES IN RATS*

Martha R. Kirk, Samuel Lepkovsky and Bert M. Tolbert/

Radiation Laboratory and Department of Poultry Husbandry,
University of California, Berkeley, California

ABSTRACT

The effect of feeding on carbon-14 respiration patterns following injection of acetate-2-C^{14}, glucose-C_6^{14}, fructose-C_6^{14} or glycine-2-C^{14} was studied in rats. Feeding increases total CO_2 production. Respiration patterns from acetate are not greatly changed by feeding. Feeding increases glucose and fructose oxidation to CO_2 and greatly increases glycine oxidation. The data are interpreted in terms of the specific dynamic action of food.

---

* The work described in this paper was sponsored in part by the United States Atomic Energy Commission and in part by the Department of Poultry Husbandry, University of California, Berkeley, California.

/ Present address: Dept. of Chemistry, University of Colorado, Boulder, Colorado.

EFFECT OF FEEDING ON $C^{14}O_2$ RESPIRATION OF LABELED METABOLITES IN RATS*

Martha R. Kirk, Samuel Lepkovsky and Bert M. Tolbert/

Radiation Laboratory and Department of Poultry Husbandry,
University of California, Berkeley, California

It has long been known that metabolic levels in animals change as a function of the time after food intake. After a big meal an animal will use more oxygen and will give off more CO_2.[1] However, the rate of oxidation to CO_2 of specific compounds was not known. One objective of this study was to determine differences in respiration rate patterns which might occur among fatty acids, sugars, and amino acids. A second objective was to determine whether food intake merely increased total oxidation by means of a mass action effect, or whether it increased or decreased the fraction of a given metabolic pool that was oxidized to CO_2 per unit time.

---

* The work described in this paper was sponsored in part by the United States Atomic Energy Commission and in part by the Department of Poultry Husbandry, University of California, Berkeley, California.

/ Present address: Dept. of Chemistry, University of Colorado, Boulder, Colorado.

Rats were trained to eat their daily food in a couple of hours. Normal animals with dietary habits of regular but intermittent feeding alternate between periods of surplus and storage and periods of living off the stores. The extreme instance of single-meal training represents exaggerations of the phases of metabolism of normal animals.[2] Thus, they are particularly well suited for this type of study. According to the report of Wilhelmi[3] the ability of animals to make the necessary adjustments in metabolism depends mainly on a balance between the secretions of the pancreatic islets, the pituitary, and the adrenal cortex.

At selected time intervals after feeding, these trained rats were injected with a tracer dose of carbon-14 labeled acetate, glycine, glucose or fructose. The cumulative breath excretion as well as the specific radioactivity of the $C^{14}O_2$ was measured and recorded. These data are presented and interpreted in terms of metabolic rates in the animal as a function of time after feeding.

EXPERIMENTAL

By a training period of three months, Long-Evans rats were taught to consume their total food intake for 24 hours in a two-hour period. On such a schedule they develop and gain weight normally. The training was begun when the females were three months of age and the males four months so that they were six and seven months old at the time of the experiments. The average weight of the females at this time was about 250 grams, while the males weighed from 425 to 475 grams. They were fed on a diet that consisted of

whole wheat ground, 62.0; whole milk powder, 10.0; commercial casein, 15.0; NaCl, 1.0; $CaCO_3$, 2.0; beef fat, 10.0; vit. A 250 mg/kg (20,000 U.S.P. Units/gm).[4]

The labeled compounds used in this experiment were sodium acetate-2-C^{14};[5] DL-glycine-2-C^{14};[6] and the sugars, glucose-C_6^{14} and fructose-C_6^{14}, which were prepared by biosynthesis[7,8] from Canna leaves by Dr. E. W. Putman. These sugars were then repurified by paper chromatography so that the final products contained not more than 1% radioactive impurities. All solutions for injection were made so that an injection of 0.2 ml contained 2 mg substrate with 20 μc C^{14}.

The rate of oxidation of these compounds to $C^{14}O_2$ was determined at specific times after the rats had completed their normal two-hour daily feeding. At 1, 8.5 or 22 hours after the feeding period, the animal was injected intraperitoneally with the radioactive compound and placed in a metabolism cage (see Fig. 1). Air was passed through this cage at a constant 400 cc/min, through an ion chamber-vibrating reed electrometer unit to measure the C^{14} in the gas, and then through an infrared CO_2 gas analyzer. A modified three channel recording potentiometer recorded not only the C^{14} per unit time and the percent CO_2 in the breath, but also divided one by the other to give the specific activity of the $C^{14}O_2$ which was also recorded.[9,10]

The concentration of $C^{14}O_2$ and CO_2 in the respired air was measured continuously for two hours after the injection and at least four rats (two males and two females) were studied for each compound and for each interval after feeding. The data from each measurement was analyzed to give the

average rate of CO_2 excretion in milligrams per minute and to give the curves for the cumulative percent of the injected dose expired as CO_2 and the specific activity of the $C^{14}O_2$ both as a function of time.

RESULTS

The cumulative respiratory excretion of $C^{14}O_2$ from acetate-2-C^{14}, glucose-C_6^{14}, fructose-C_6^{14} and glycine-2-C^{14} is plotted in Figs. 2 and 3 as a function of time after injection of the labeled compound. For each compound three curves are given, representing 1, 8.5 or 22 hours after feeding. These data are summarized in Table I, which also gives the average production of CO_2 in milligrams per minute for each group of animals. In calculating these data the CO_2 production for each animal was corrected to an adjusted animal weight of 250 gms by multiplying the actual CO_2 output by the ratio $(250/W)^{0.75}$ where "W" is the animal's weight in grams.[11,12]

The specific activity of the $C^{14}O_2$ was expressed as millimicrocuries of carbon-14 per gram of carbon (in the breath CO_2) for a 20 μc injected dose of C^{14}. The data for each animal was again adjusted to an animal weight of 250 grams by multiplying the specific activity by the factor $(W/250)^{0.75}$. The use of Kleiber's factor in these calculations not only corrects for the change in surface area-to-mass ratio encountered with our widely varying animal weights, but at the same time it normalizes the radioactive dose so that we are comparing equivalent amounts of radioactivity injected into equivalent sized animals.

Figs. 4 through 7 show the $C^{14}O_2$ specific activity vs. time after

TABLE I

Cumulative Excretion of $C^{14}O_2$ from Rats at Various Times after Feeding*

Metabolite	Time of Injection Hours after Feeding	Cumulative $C^{14}O_2$ excretion (1% injected C^{14}) Minutes after injection					Average Production of CO_2, mg/min
		0-20	0-40	0-60	0-90	0-120	
Acetate-2-C^{14}	1	9.71	22.48	30.95	39.60	44.23	7.52
	8.5	9.37	22.87	33.14	42.76	48.46	8.42
	22	10.44	24.63	34.08	44.27	49.68	7.59
Glucose-C_6^{14}	1	2.95	9.36	16.71	25.69	30.65	9.07
	8.5	3.46	10.60	17.90	26.41	32.34	8.55
	22	1.31	5.35	9.91	17.60	23.30	6.36
Fructose-C_6^{14}	1	3.95	11.20	17.21	23.59	27.78	8.88
	8.5	2.61	8.63	14.57	21.84	27.85	8.39
	22	1.71	5.95	10.55	16.59	21.97	6.37
Glycine-2-C^{14}	1	2.27	5.35	7.79	9.87	11.23	10.40
	8.5	.83	2.59	3.91	5.85	7.10	8.22
	22	.33	1.15	1.90	3.10	4.12	7.73

* Each value is the average of data from four animals.

injection curves for labeled acetates, glucose, fructose and glycine metabolism as a function of time after feeding.

DISCUSSION

As indicated in the introduction of this paper, we expected a decrease in average CO_2 production as a function of time after eating. Except for one instance this was observed in all four series of experiments. As a similar result we found that the fraction of a given tracer dose of glycine-2-C^{14}, glucose-C_6^{14} or fructose-C_6^{14} that is oxidized to CO_2 also decreases with time after feeding. Of these three compounds glycine showed the greatest decrease (percent oxidized went from 11.2 to 4.1). Glucose and fructose both showed about the same relative percentage change. Acetate metabolism to CO_2, on the other hand, increases slightly with time after feeding going from 44.2% to 49.6% for the one hour and the 22 hour period, respectively.

Some of the changes in the oxidation of these labeled compounds can be easily interpreted on the basis of known intermediary metabolism. It is only necessary to assume a "specific dynamic action" of food. Black, Maddy and Swift[13] define the "specific dynamic action of foodstuffs" as the increase in heat production as a result of the ingestion of food. This is generally estimated as about 6% of the caloric value of food eaten and is highest when protein rich substances are fed. This assumption is substantiated by our average CO_2 production data, as well as by other research.[14,15]

Acetate metabolism is less disturbed by feeding than any of the four compounds. The acetate to CO_2 specific activity curves follow no consistent

pattern change, the 22 hour curve showing the highest peak of specific activity and the 8.5 hour curve, the lowest. Neither is there any change in the shape of the curves. We infer then, that the fraction of the acetate pool being oxidized to CO_2 in a given time (via the Krebs cycle) remains about constant, and the specific activity will also not sharply change. The acetate figures then would not be changed by the specific dynamic action of food, although the pool size may change appreciably. Also the evidence indicates that fat can be mobilized from the depots to the liver. Levin and Farber[16] have shown that in the mouse this process is under the control of the growth hormone, ACTH, and perhaps some enzymatic regulation. In the rat control is regulated by the growth hormone, the adrenal cortex and the adrenal medulla.[17] This continuing process of fat storage and remobilization would also tend to stabilize acetate oxidation.

Glucose and fructose are presumed oxidized via the pentose shunt and the Emden-Myerhoff reactions. The specific dynamic action of food will here increase the amount of glucose appearing as CO_2, both directly and via acetate thus increasing the $C^{14}O_2$; both that appearing directly and that going through acetate.

The drop in blood sugar which normally occurs with time after eating indicates a lowered glucose and fructose pool size. A lowered pool size would result in less dilution of the C^{14} label from the injected hexoses and a higher specific activity of the $C^{14}O_2$. Thus the difference in oxidation rate (Figs. 2, 3, 5 and 6, Table I) must reflect a much greater drop in the absolute amount of the carbohydrate converted to CO_2 than is indicated solely

by a comparison of the percentage of $C^{14}O_2$ excreted at 1 hour and 22 hours.

The change in the shape of the specific activity curves for these two sugars is also probably a reflection of the specific dynamic action of food. The time of peak specific activity is increased as a function of time after feeding. Concomitantly, the curve changes shape, becoming broad and flat topped. We think this represents a slowing down of most of the metabolic steps involved in the handling of these sugars, probably due to changes in co-enzyme concentrations and enzyme activity. It is the same modification of specific activity curve shape that the authors have observed for starved or very sick (cancer) rats with glycine, glucose and acetate.[18]

When the body has a surfeit of amino acids, as following a large meal in a healthy individual, it may use some of these compounds as an energy source. At other times, it will conserve its amino acid supply using it principally to maintain body proteins. Both the specific activity and cumulative excretion curves for glycine-2-C^{14} seem to reflect this conservation of glycine carbon. The peak specific activity of the CO_2 goes down from 166 to 45 mμc C^{14}/gm C in going from 1 hour after feeding to 22 hours after feeding. Although the dynamic action of food seems to be greatest when protein rich substances are eaten, Forbes and Swift[17] report that the dynamic effects of diets are not the additive effects of their components and are not in accord with their protein content. Also, inasmuch as there is no scientific means of apportioning energy effects of values among different dietary constituents, the dynamic effects of individual foods or nutrients are without significance as constants. In view of these facts it is not certain that the changes in glycine metabolism as a function of time after feeding are due entirely to the specific dynamic action of feeding or to amino acid conservation. Perhaps both contribute

their share in view of the considerable drop in rate of glycine oxidation to $C^{14}O_2$ between 8.5 and 22 hours after the meal even though any effects of the specific dynamic action of feeding would have long since disappeared.

The results, therefore, substantiate the concept of the "specific dynamic action" of food, but the effect observed depends upon the class of food involved. Acetate metabolism was little changed by eating or short-term fasting, whereas the $C^{14}O_2$ production from fructose and glucose decreased by about 22 percent between one and 22 hours after feeding and that from glycine decreased by 65 percent.

LIST OF REFERENCES

1. Wright, Sampson, "Applied Physiology," Oxford Univ. Press, VI, 600 (1947).
2. Dickerson, V. C., Tepperman, J., and Long, C. H. N., Yale J. Biol. Med., 15, 875 (1943).
3. Wilhelmi, A. E., Ciba Foundation Colloquia on Endocrinology, "Hormonal Factors in Carbohydrate Metabolism," Little, Brown and Co., Boston, VI, 70 (1953).
4. Lepkovsky, S., Lyman, R., Fleming, D., Nagumo, M., Dimick, M., Am. J. Physiol., 188, 327 (1957).
5. Calvin, M., Heidelberg, C., Reid, J. C., Tolbert, B. M., and Yankwich, P.E., "Isotopic Carbon," John Wiley and Son, 193 (1949).
6. Tolbert, B. M., and Hughes, D. M., University of California Radiation Laboratory Report, UCRL 705 (1950).
7. Putman, E. W., Hassid, W. Z., Krotkov, G. and Barker, H. A., J. Biol. Chem., 173, 785 (1948).
8. Putman, E. W., Hassid, W. Z., J. Biol. Chem., 196, 749 (1952).
9. Tolbert, B. M., Kirk, M. and Baker, E. M., Am. J. Physiol., 185, 269 (1956).
10. Tolbert, B. M., Lawrence, J. H. and Calvin, M., Proc. International Conference on Peaceful Uses of Atomic Energy, 12, 281 (1956). U.N. Publication.
11. Kleiber, M., Hilgardia, 6, 315 (1932).
12. Kleiber, M., Physiol. Rev., 27, 511 (1947).
13. Black, A., Maddy, K. H., Swift, R. W., J. Nutrition, 42, 415 (1950).
14. Bell, G. H., Davidson, J. N., Scarborough, H., "Textbook of Physiology and Biochemistry," The Williams and Wilkins Co., X, 132 (1950).
15. Forbes, E. B., Swift, R. W., J. Nutrition, 27, 453 (1944).

16. Levin, L. and Farber, R. K., Proceedings of the Laurentian Hormone Conference, Recent Progr. Hormone Research, Academic Press, New York, N. Y., VII, 399 (1955).

17. Wool, I. G., Goldstein, M. S., Ramez, E. R. and Leven, R., Am. J. Physiol., 178, 427 (1954).

18. Kirk, M. R., Harmon, D. and Tolbert, B. M., University of California Radiation Laboratory Report, UCRL 2932, 12 (March, 1955).

LEGENDS

Fig. 1. Schematic diagram of the respiratory $C^{14}O_2$ analyzer used.

Fig. 2. Cumulative excretion of $C^{14}O_2$ from rats injected with acetate-2-C^{14} of fructose-C^{14}. Each curve is the average of four animals. Cumulative percent of the injected dose respired as Co_2 is plotted vs. time after injection.

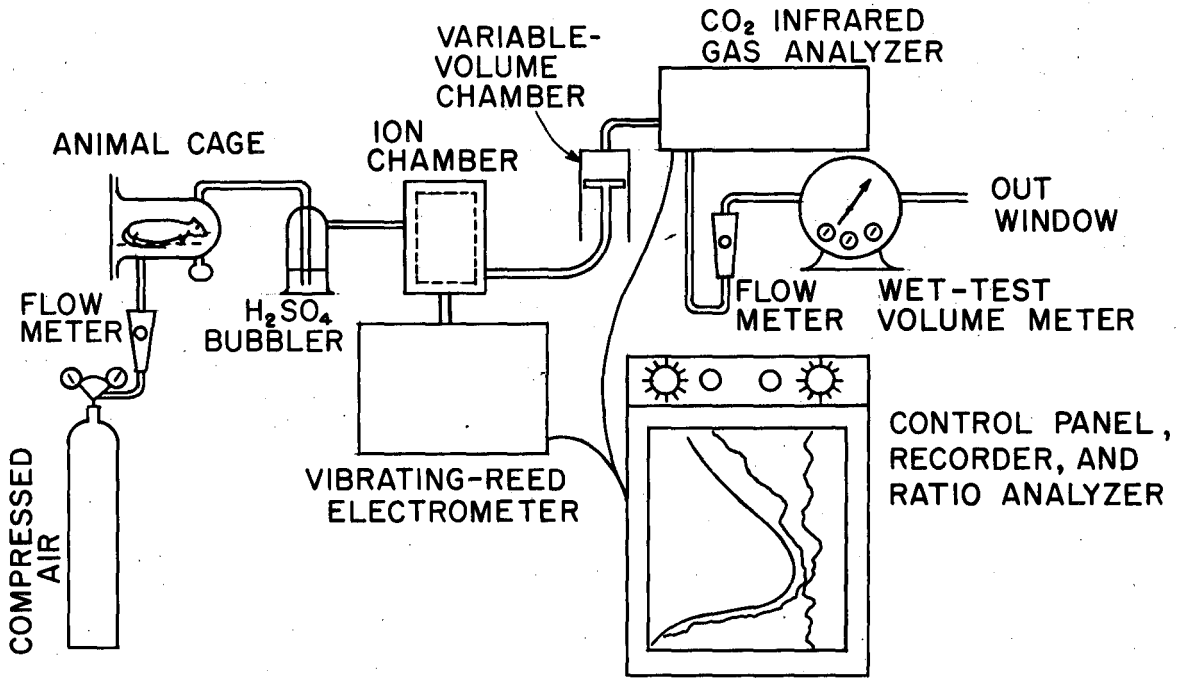
Fig. 3. Cumulative excretion of $C^{14}O_2$ from rats injected with glucose-C^{14} and glycine-2-C^{14}. Each curve is the average of four animals. Cumulative percent of the injected dose respired as Co_2 is plotted vs. time after injection.

Fig. 4. Specific activity of $C^{14}O_2$ from rats injected with acetate-2-C^{14}. Millimicrocuries per gm carbon per 20μc C^{14} injected normalized to a 250 gm rat is plotted vs. time after injection.

Fig. 5. Specific activity of $C^{14}O_2$ from rats injected with glucose-$C_6{}^{14}$. Millimicrocuries per gm carbon per 20 μc C^{14} injected normalized to a 250 gm rat is plotted vs. time after injection.

Fig. 6. Specific activity of $C^{14}O_2$ from rats injected with fructose-$C_6{}^{14}$. Millimicrocuries per gm carbon per 20 μc C^{14} injected normalized to a 250 gm rat is plotted vs. time after injection.

Fig. 7. Specific activity of $C^{14}O_2$ from rats injected with glycine-$C_6{}^{14}$. Millimicrocuries per gm carbon per 20 μc C^{14} injected normalized to a 250 gm rat is plotted vs. time after injection.



SCHEMATIC DIAGRAM OF RESPIRATORY $C^{14}O_2$ ANALYZER

MU-9185

Fig. 1.

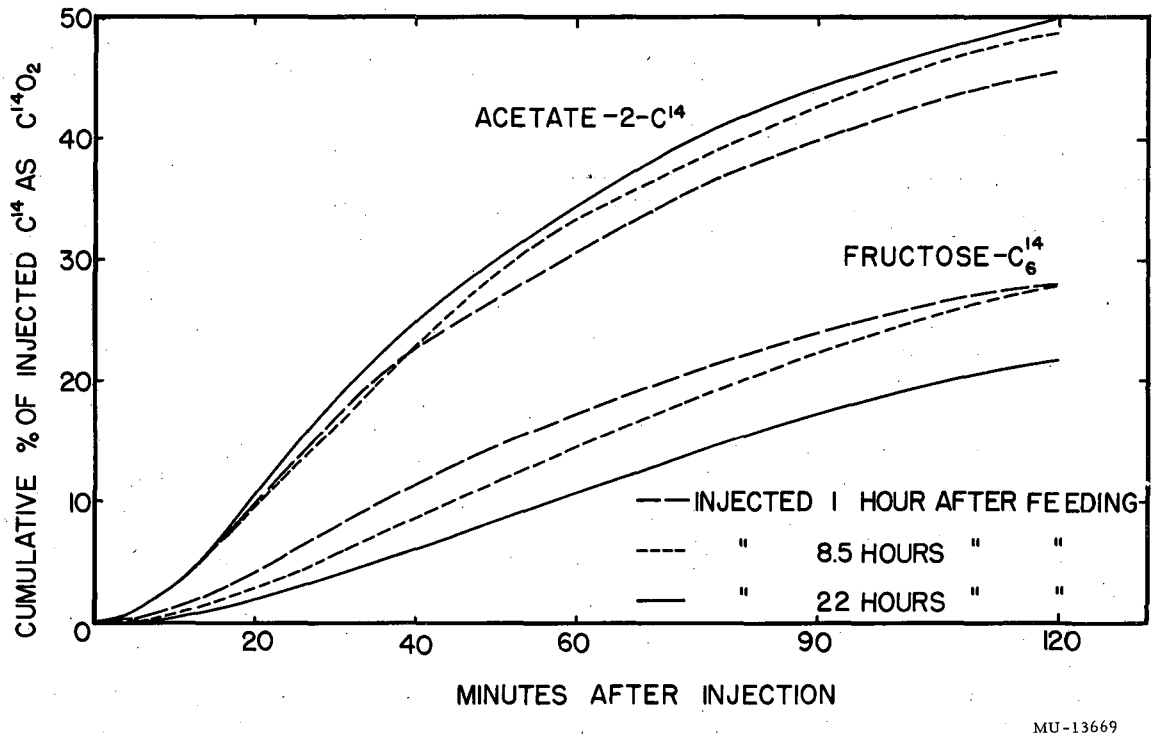

CUMULATIVE % OF INJECTED C14 AS C14O2
50
40
30
20
10
0
20
40
60
90
120
MINUTES AFTER INJECTION
ACETATE-2-C14
FRUCTOSE-C6 14
——INJECTED 1 HOUR AFTER FEEDING
---- " 8.5 HOURS " "
—— " 22 HOURS " "
MU-13669

Fig. 2

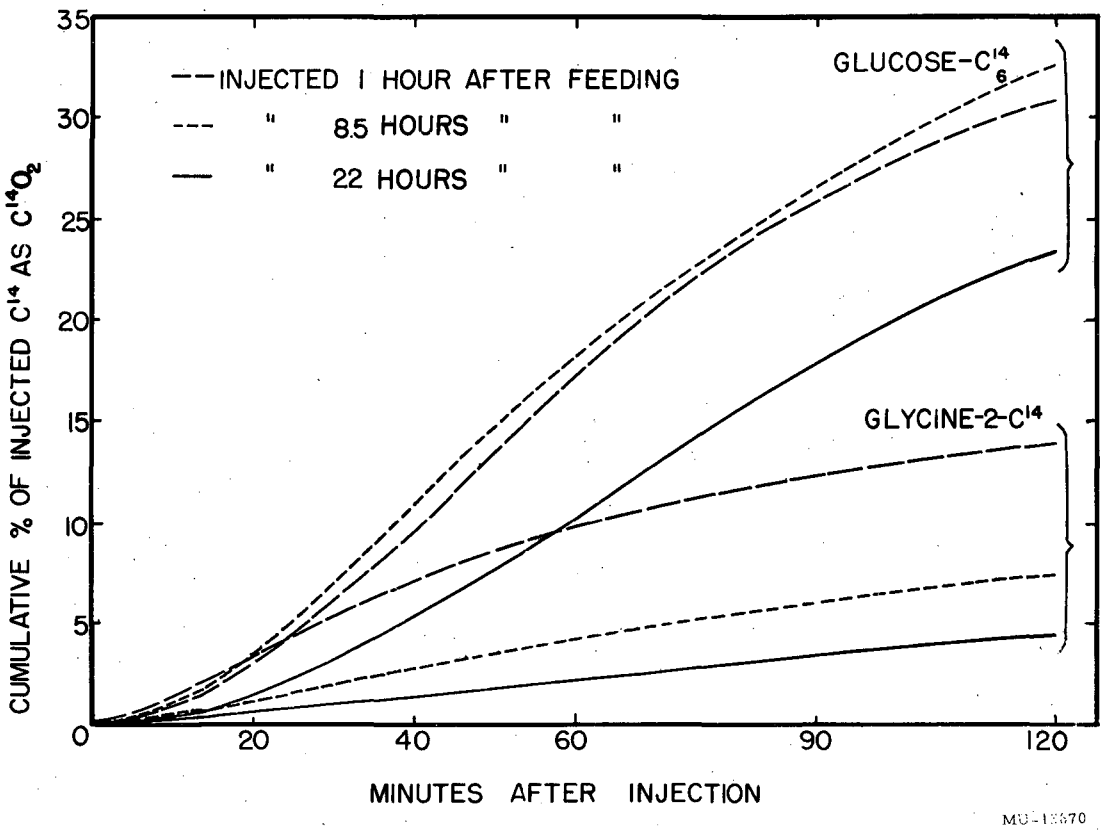

INJECTED 1 HOUR AFTER FEEDING
" 8.5 HOURS " "
" 22 HOURS " "
GLUCOSE-C14 6
GLYCINE-2-C14
CUMULATIVE % OF INJECTED C14 AS C14O2
MINUTES AFTER INJECTION
0
5
10
15
20
25
30
35
20
40
60
90
120
MU-15870

Fig. 3

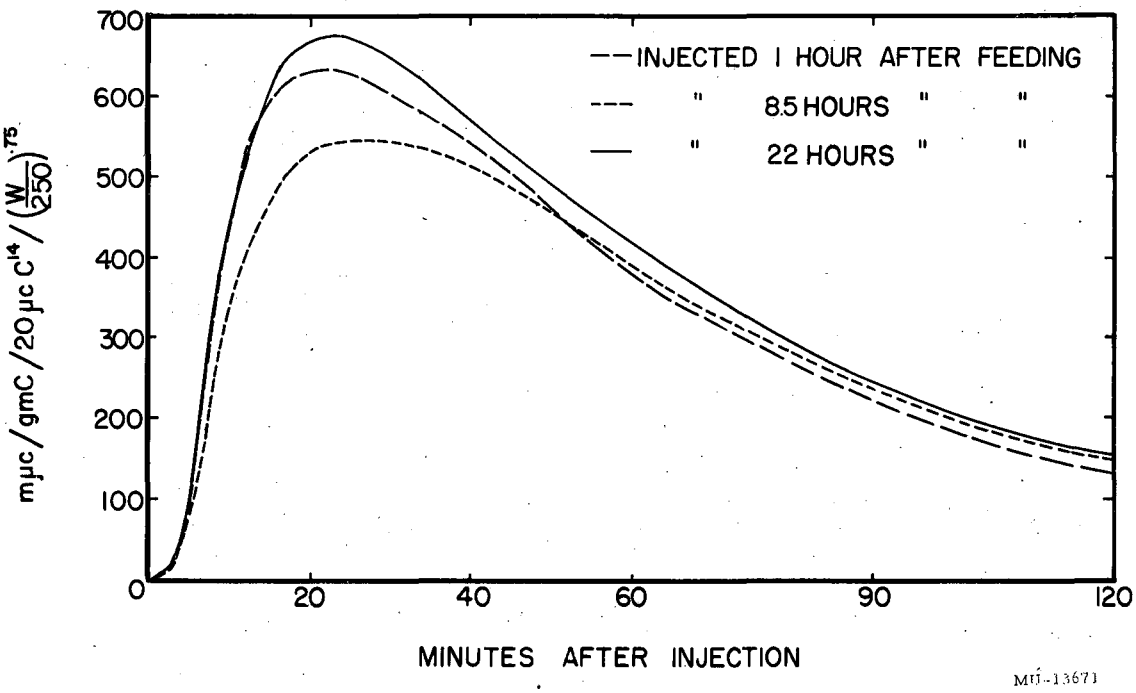

700
600
500
400
300
200
100
0
20
40
60
90
120
mμc/gmC/20μc C14/(W/250).75
MINUTES AFTER INJECTION
--INJECTED 1 HOUR AFTER FEEDING
--- " 8.5 HOURS " "
— " 22 HOURS " "
MU-13671

Fig. 4

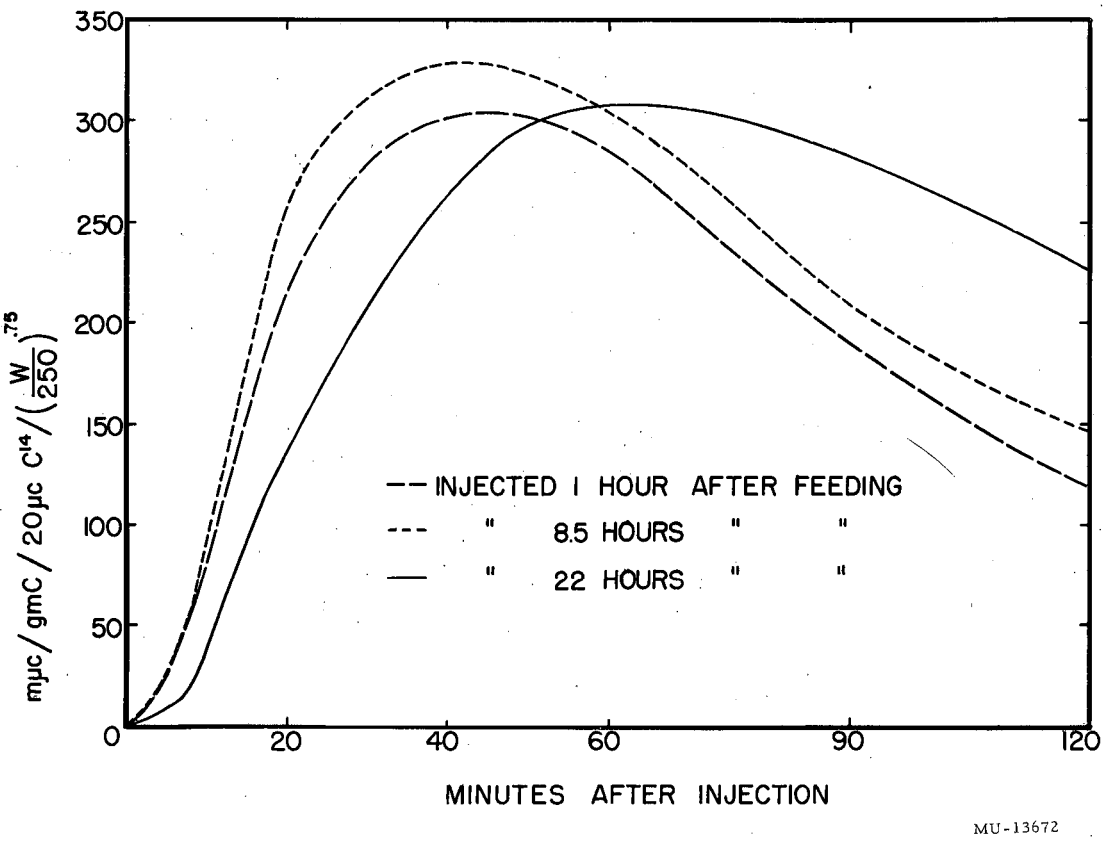

350
300
250
200
150
100
50
0
20
40
60
90
120
mμc / gmC / 20μc C14 / (W/250).75
MINUTES AFTER INJECTION
-- INJECTED 1 HOUR AFTER FEEDING
--- " 8.5 HOURS " "
—— " 22 HOURS " "
MU-13672

Fig. 5

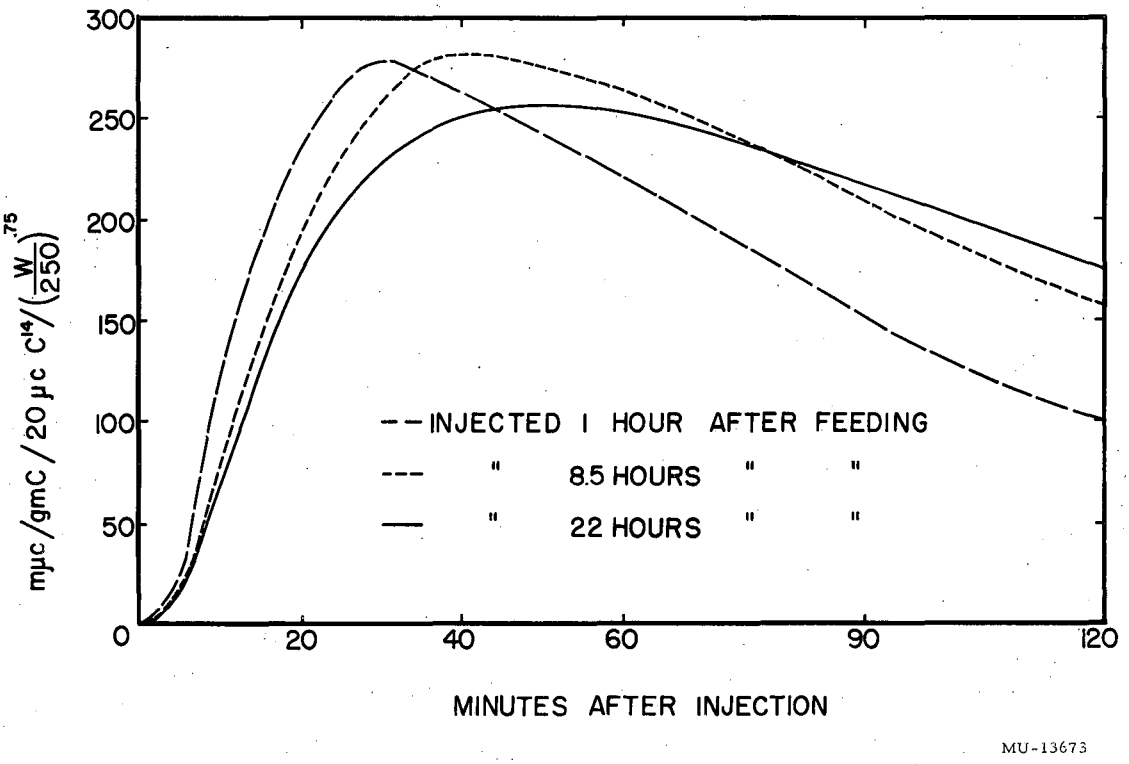

300
250
200
150
100
50
0
20
40
60
90
120
mμc/gmC/20 μc C14/(W/250).75
--INJECTED 1 HOUR AFTER FEEDING
--- " 8.5 HOURS " "
—— " 22 HOURS " "
MINUTES AFTER INJECTION
MU-13673

Fig. 6

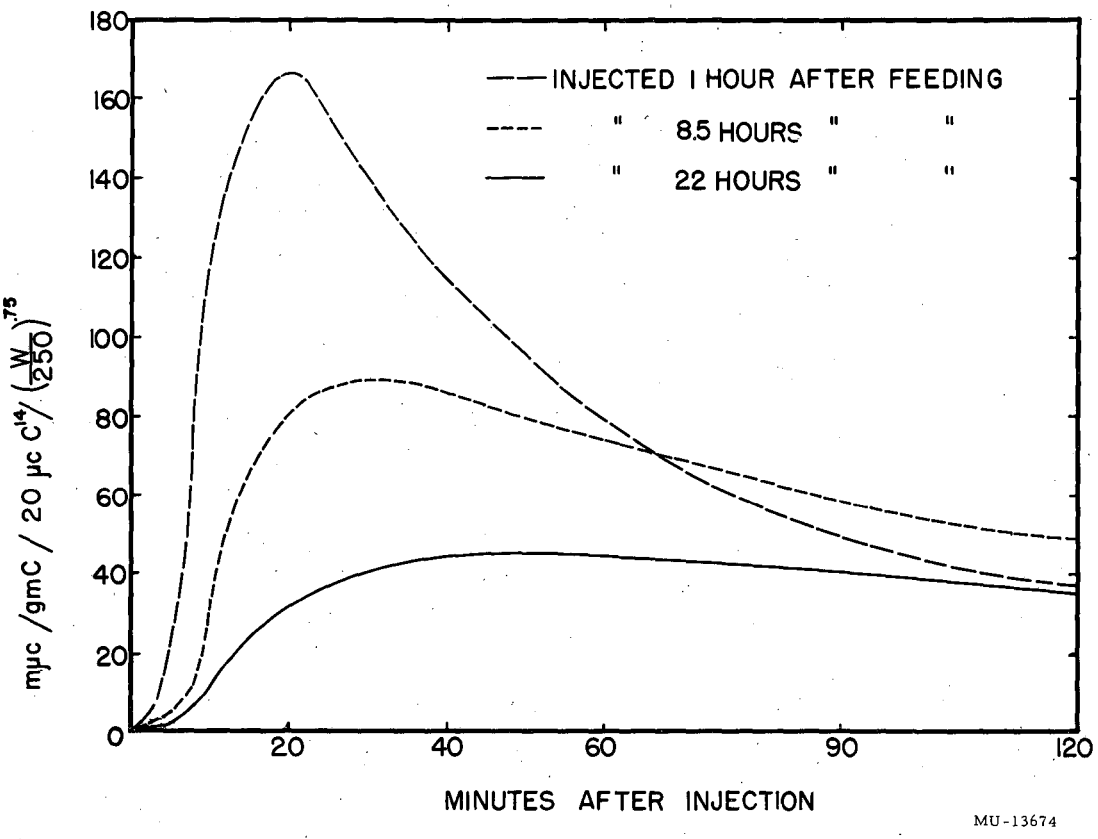

180
160
140
120
100
80
60
40
20
0
20
40
60
90
120
INJECTED 1 HOUR AFTER FEEDING
" 8.5 HOURS " "
" 22 HOURS " "
mμc /gmC / 20 μc C14/ (W/250)^.75
MINUTES AFTER INJECTION
MU-13674

Fig. 7