UCRL 3851

UNIVERSITY OF CALIFORNIA

Radiation Laboratory

TWO-WEEK LOAN COPY

This is a Library Circulating Copy which may be borrowed for two weeks. For a personal retention copy, call Tech. Info. Division, Ext. 5545

BERKELEY, CALIFORNIA

DISCLAIMER

This document was prepared as an account of work sponsored by the United States Government. While this document is believed to contain correct information, neither the United States Government nor any agency thereof, nor the Regents of the University of California, nor any of their employees, makes any warranty, express or implied, or assumes any legal responsibility for the accuracy, completeness, or usefulness of any information, apparatus, product, or process disclosed, or represents that its use would not infringe privately owned rights. Reference herein to any specific commercial product, process, or service by its trade name, trademark, manufacturer, or otherwise, does not necessarily constitute or imply its endorsement, recommendation, or favoring by the United States Government or any agency thereof, or the Regents of the University of California. The views and opinions of authors expressed herein do not necessarily state or reflect those of the United States Government or any agency thereof or the Regents of the University of California.

UCRL-3851

UNIVERSITY OF CALIFORNIA
Radiation Laboratory
Berkeley, California

Contract No. W-7405-eng-48

EFFECT OF CANCER AND STARVATION ON THE OXIDATION OF LABELED ACETATE, GLUCOSE, AND GLYCINE TO $C^{14}O_2$

Denham H. Harmon, Martha R. Kirk, and B. M. Tolbert

October 7, 1957

Printed for the U. S. Atomic Energy Commission

EFFECT OF CANCER AND STARVATION ON THE OXIDATION OF LABELED ACETATE, GLUCOSE, AND GLYCINE TO $C^{14}O_2$

Denham H. Harmon, Martha R. Kirk, and B. M. Tolbert

Donner Laboratory of Medical Physics and Radiation Laboratory
University of California, Berkeley, California

October 7, 1957

ABSTRACT

The metabolism of acetate-2-C^{14}, glucose-C^{14}_6, and glycine-2-C^{14} was studied in rats as a function of starvation and tumor growth. So measured, acetate metabolism was not affected, glucose utilization was at first depressed and later showed a moderate increase. Glycine was conserved in the tumor-bearing rat during the period of weight gain, but oxidation to CO_2 increased markedly during weight loss in both the starved and tumorous animals. The glycine and glucose rate curves all showed a delay of the time of peak specific activity with starvation, presumably a result of depressed intermediary metabolic rates.

EFFECT OF CANCER AND STARVATION ON THE OXIDATION OF LABELED ACETATE, GLUCOSE, AND GLYCINE TO $C^{14}O_2$*

Denham H. Harmon,† Martha R. Kirk, and B. M. Tolbert§

Donner Laboratory of Medical Physics and Radiation Laboratory
University of California, Berkeley, California

October 7, 1957

Cancer cells are believed to liberate a "toxohormone" into the blood stream.[1, 2, 3] To this compound (or mixture) is attributed the progressive depression (probably through inactivation) of the iron porphyrins--most marked in liver catalase. Inasmuch as the mechanism of inactivation of other oxidative enzymes may be similar to that of the iron porphyrins, the presence of cancer may result in a progressive change in the rate of metabolism of common foodstuffs by the host.

As a prelude to possible work with human cancer patients, the ability of Curtis-Dunning rats to metabolize acetate-2-C^{14}, glucose-C^{14}_6, and glycine-2-C^{14} to $C^{14}O_2$ has been studied as a function of time before and after inoculation with cancer and then at intervals until death. Since cachexia is such a prominent feature of late cancer, the effect of starvation was investigated also.

In this procedure, the animal is injected with the labeled metabolite and placed in a cage. Automatic equipment then measures and records the breath excretion rate for CO_2 and C^{14} and calculates and records the C^{14}/CO_2 ratio, which is the specific activity of the excreted $C^{14}O_2$. These data are presented in this paper as a $C^{14}O_2$ specific activity rate curve and a cumulative C^{14} breath-excretion curve.

EXPERIMENTAL PROCEDURE

The apparatus used in this work has been described previously,[4] and consists of a flow-control system, an ionization chamber-vibrating-reed electrometer for C^{14} measurements, an infrared CO_2 gas analyzer, and a ratio-analyzer-recorder. The arrangement of the equipment is as shown in Fig. 1.

The carbon-14 respiration rate patterns were measured as follows: The rat was injected intraperitoneally (i. p.) with a fraction of a milliliter of solution containing about 5 μC in 1 mg, or less, of the compound. The rat was placed immediately in the metabolism cage and the excretion pattern measured for a period of 7 hours.

---

*The work described in this paper was sponsored by the U. S. Atomic Energy Commission.

§Present address: Department of Chemistry, University of Colorado, Boulder, Colorado.

†Present address: United States Veterans Administration Hospital, San Francisco, California

A strain of Curtis-Dunning rats was used in which an undifferentiated carcinoma had been carried for some years. A given animal was used with a given compound for the entire series of measurements. Two patterns were obtained for each animal in the period before starvation began or before tumor inoculation, and thereafter patterns were measured every 3 to 5 days during the time the animals remained alive. The rats were measured in rotation. The starvation experiments (no food, water ad lib.) were conducted in the same manner as the tumor experiments, using tumor-free animals of the same strain.

The time elapsed between measurements was sufficient for background radioactivity in the breath to become relatively constant over the 8-hour period. This background figure could then be subtracted by proper adjustment of the controls before a given run was started. In general, these background radioactivity values were less than 1% of the average radioactivity for a given run.

RESULTS

The C^{14} respiration pattern obtained from the injection of a labeled compound in an animal is the reflection of a great number of biochemical reactions and equilibrations, as well as physiological processes, such as the body-bicarbonate clearance time. Therefore, the authors have chosen to present their experimental results as a series of curves, rather than numerical data on peak specific activity and time--which, incidentally, can be quite variable for a given labeled substrate.

The complete $C^{14}O_2$ respiration pattern may be expressed as two curves: one is the cumulative excretion of $C^{14}O_2$ plotted as a function of time after injection, and the other is the specific activity of the $C^{14}O_2$ plotted as a function of time after injection. The first curve is not an integration of the second, since the total $C^{14}O_2$ per unit of time includes not only the specific activity of the CO_2 but also the weight of CO_2 that is produced per unit of time. In order to simplify the presentation of the data, cumulative $C^{14}O_2$ excretion has been presented in tabular form wherever possible.

In order to correct the data for changes in animal weight, all $C^{14}O_2$ values have been adjusted to a given dose of radioactivity for a given weight of animal using the ratio $(\text{average weight (250 g)}/\text{animal weight})^{0.75}$.

The carcionoma used in this work usually killed the animal in about 30 to 40 days. During this time about ten respiration patterns were measured. Since it is impossible to present all these data, four representative curves were chosen for each animal condition (cancer and starvation) and compared. The data for the oxidation of acetate-2-C^{14} to $C^{14}O_2$ are given in Fig. 2 and Table I. As can be seen, the acetate-to-CO_2 specific activity curves do not change much with tumor growth. The glucose-C^{14}_6 and glycine-2-C^{14} curves are given in Figs. 3 and 4, and also in Table I.

In general, fewer respiration patterns were made for the starved rats than for the tumor-bearing rats because of the shorter duration of the experiments. Figs. 5 through 7 present the specific activity curves for the

Table I

Cumulative excretion of $C^{14}O_2$ following injection of labeled compounds in tumor-bearing animals. Data are expressed as percent of injected dose so excreted, for the 1-, 2-, and 7- hour time intervals.

Animal condition	Tumor growth (days*)	Acetate-2-C^{14}			Glucose-C_6^{14}			Glycine-2-C^{14}		
		Σ1 hr	Σ2 hr	Σ7 hr	Σ1 hr	Σ2 hr	Σ7 hr	Σ1 hr	Σ2 hr	Σ7 hr
Normal	0	39.80	54.26	64.57						
	0				25.57	44.36	58.82			
	0							5.84	9.45	15.89
Incubation phase	9	31.00	45.42	57.30						
	12				31.23	47.97	60.49			
	7							5.13	8.30	14.48
Growth phase	21	33.30	46.27	55.68						
	19				23.30	45.30	59.75			
	12							3.47	6.06	11.25
Peak cancer size	27	32.08	47.00	59.53						
	28				9.20	30.08	55.93			
	19							2.46	5.17	11.18
Weight-loss period	34	28.01	43.11	57.33						
	33				5.27	18.22	48.04			
	25							5.13	12.52	25.16
Moribund	37	26.32	42.23	57.92						(died 39)
	36				10.13	27.30	58.71			(died 39)
	28							2.65	12.40	29.96 (died 28 days)

*Time of cancer inoculation was taken to be 0.

$C^{14}O_2$ respired for acetate-2-C^{14}, glucose-$C^{14}{}_6$, and glycine-2-C^{14}, respectively. In Table II are presented the cumulative excretion data for the $C^{14}O_2$ in terms of percent of injected dose.

In an additional longer-time experiment, metabolism of glycine-2-C^{14} to $C^{14}O_2$ was measured for two moribund rats, one from the tumor experiment and one from the starvation experiment, for about 20 hours. The cumulative curves of respiration of $C^{14}O_2$ are shown in Fig. 8 and therein compared with the curves for the same rats in a normal state before the experiment began.

In addition to the respiration-pattern data just presented, weight and CO_2-production data were kept for each animal. Representative records for a tumor-bearing animal and a starved animal are given in Figs. 9 and 10. The weight of the tumor animals was relatively constant for about 10 days, then increased rapidly to a peak at about 25 days, after which the animals did not eat much more but went into a rapid decline, losing weight quickly until death ensued. Starved animals lost weight continuously throughout the experiment and died in 12 to 15 days.

DISCUSSION

Acetate-to-CO_2 metabolism is apparently one of the most unchangeable processes of the animal biological system. Neither very severe starvation nor the final stages of cancer could appreciably change the acetate respiration patterns. As long as the animal was alive the fraction of the pool oxidized to CO_2 and the rate of oxidation of this fragment remained relatively constant. This observation is consistent with feeding experiments in this laboratory in which sudden large food intake in rats did not appreciably change the acetate respiration patterns.[6]

Glucose-$C^{14}{}_6$ is oxidized to CO_2 more slowly as the tumorous rat begins to lose weight, and this characteristic was also observed in the starved rat. Both the tumor-bearing and the starved rats displayed a moderate increase in glucose oxidation as the animal neared death.

Glycine-2-C^{14} oxidation to $C^{14}O_2$ followed a distinct progressive course in the tumor-bearing animals. First, glycine was conserved as the tumor grew, i.e., during the period of weight gain. Then glycine-to-CO_2 oxidation increased progressively as the animal lost weight and refused to eat and the tumor necrosed. The starved animal displayed a marked progressive increase in rate of conversion of glycine to CO_2 as a function of starvation time. This is consistent with the work of Bansi, who found that in starvation, although fat is still present in the depots, and the majority of caloric needs are met by burning up the fat reserves, nevertheless more protein is used up (as indicated by increased excretion of nitrogen) in this state of affairs for the purpose of energy than corresponds to the wear-and-tear quota--that is, to the endogenous nitrogen minimum.[7]

All the glucose and glycine rate curves show an interesting phenomenan in the later stages of the experiments, i.e., as the tumor growth and (or) starvation progress, the peak-specific-activitiy time comes later and later.

Table II

Cumulative excretion of $C^{14}O_2$ following injection of labeled compounds in starving animals. Data are expressed as percent of injected dose respired for the 1-, 2-, and 7- hour time intervals.

Animal conditions	Day	Acetate-2-C^{14}			Glucose-C^{14}_6			Clycine-2-C^{14}		
		Σ1 hr	Σ2 hr	Σ7 hr	Σ1 hr	Σ2 hr	Σ7 hr	Σ1 hr	Σ2 hr	Σ7 h
Normal	0	31.28	46.88	60.21						
	0				20.06	35.88	57.26			
	0							3.24	7.73	13.61
Mild starvation	3	22.47	39.11	57.09						
	4				4.69	15.12	31.95			
	5							3.11	6.21	13.65
Severe starvation	9	25.77	40.67	59.19						
	11				6.53	17.47	45.94			
	8							6.03	11.35	19.26
Moribund	14	(Not determined)								
	15				18.68	35.30	62.1			
	11							3.26	9.79	28.85

Furthermore, the specific-activity curves become more rounded and the peak is flatter. This change of rate pattern probably reflects a slowing down of all the metabolic processes in the body--a result, we presume, of smaller enzyme and cofactor concentrations. However, in no case can we attribute any specific effect of this type to the cancer, exclusive of the generalized debilitation and starvation.

In conclusion, we observe that if these particular cancer cells do liberate some substance such as toxohormone into the circulatory system, it appears to have little effect on the ability of the host to handle simple foodstuffs.

CAPTIONS

Fig. 1. Schematic diagram of C^{14} respiration-pattern analyzer for animal work. The ionization chamber has a volume of 1000 cc or 250 cc, depending on the sensitivity of analysis required.

Fig. 2. Specific activity of $C^{14}O_2$ from injected acetate-2-C^{14} as a function of tumor growth. The amount of C^{14} per gram of expired carbon per 10 microcuries of injected C^{14} is plotted against time after injection.

Fig. 3. Specific activity of $C^{14}O_2$ from injected glucose-$C^{14}{}_6$ as a function of tumor growth. The amount of C^{14} in millimicrocuries per gram of expired carbon per 10 microcuries of injected C^{14} is plotted against time after injection.

Fig. 4. Specific activity of $C^{14}O_2$ from injected glycine-2-C^{14} as a function of tumor growth. The amount of C^{14} in millimicrocuries per gram of expired carbon per 10 microcuries of injected C^{14} is plotted against time after injection.

Fig. 5. Specific activity of $C^{14}O_2$ from injected acetate-2-C^{14} as a function of starvation. The amount of C^{14} in millimicrocuries per gram of expired carbon per 10 microcuries of injected C^{14} is plotted against time after injection.

Fig. 6. Specific activity of $C^{14}O_2$ from injected glucose-$C^{14}{}_6$ as a function of starvation. The amount of C^{14} in millimicrocuries per gram of expired carbon per 10 microcuries of injected C^{14} is plotted against time after injection.

Fig. 7. Specific activity of $C^{14}O_2$ from injected glycine-2-C^{14} as a function of starvation. The amount of C^{14} in millimicrocuries per gram of expired carbon per 10 microcuries of injected C^{14} is plotted vs time after injection.

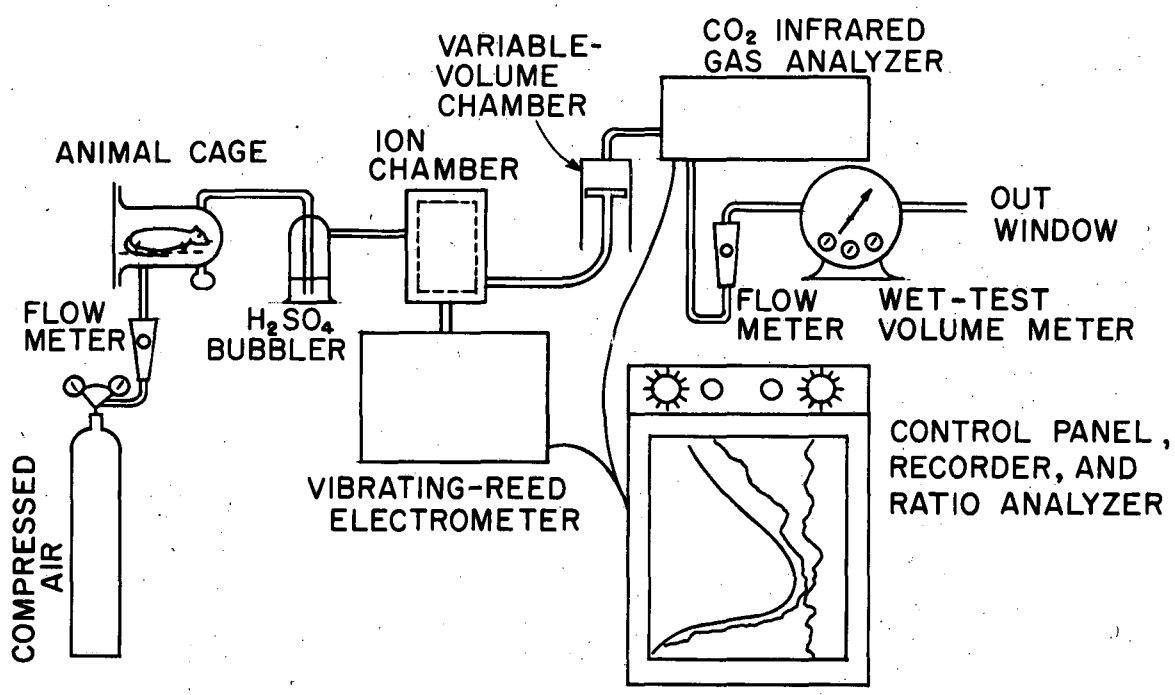
Fig. 8. Cumulative excretion of $C^{14}O_2$ from glycine-2-C^{14} as a function of animal condition. Cumulative percent of the injected dose respired is plotted against time after injection.

Fig. 9. Weight and CO_2 production as a function of tumor growth for a rat inoculated with carcinoma. These data are from the glycine-2-C^{14} experiment.

Fig. 10. Weight and CO_2 production for a rat as a function of starvation. These data are from the glycine-2-C^{14} experiment.

REFERENCES

1. Endo, Sugimura, Ono, and Konno, Gann 46, 51-57 (1955).
2. Nakagawa, Kosuge, and Tokunaka, Gann 46, 585-96 (1955).
3. Jesse P. Greenstein, Biochemistry of Cancer (Academic Press, New York; 1954), p. 534-36.
4. Tolbert, Kirk, and Baker, Am. J. Physiol. 185, 269 (1956).
5. M. Kleiber, Physiol. Rev. 27, 511 (1947).
6. Kirk, Lepkovsky, and Tolbert, Effect of Feeding on $C_{14}O^2$ Respiration in Rats from Labeled Metabolities, UCRL-3854, 1957.
7. H. W. Bansi, "Metabolism in Obesity" from Ciba Foundation on Endocrinology: Hormonal Factors in Carbohydrate Metabolism VI (Little, Brown, Boston, 1953), p. 187.



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

MU-9185

Fig. 1.

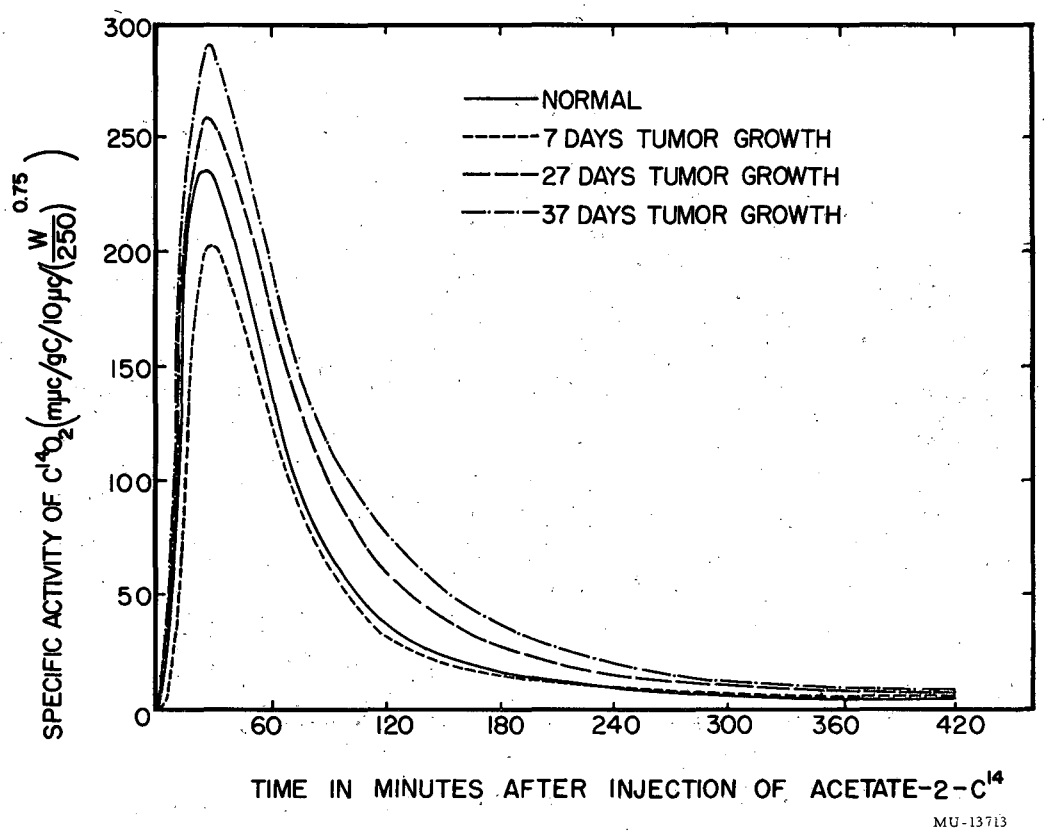

300
250
200
150
100
50
0
60
120
180
240
300
360
420
NORMAL
7 DAYS TUMOR GROWTH
27 DAYS TUMOR GROWTH
37 DAYS TUMOR GROWTH
SPECIFIC ACTIVITY OF $C^{14}O_2$ (mμc/gC/10μg/(W/250)^0.75)
TIME IN MINUTES AFTER INJECTION OF ACETATE-2-C^{14}
MU-13713

Fig. 2.

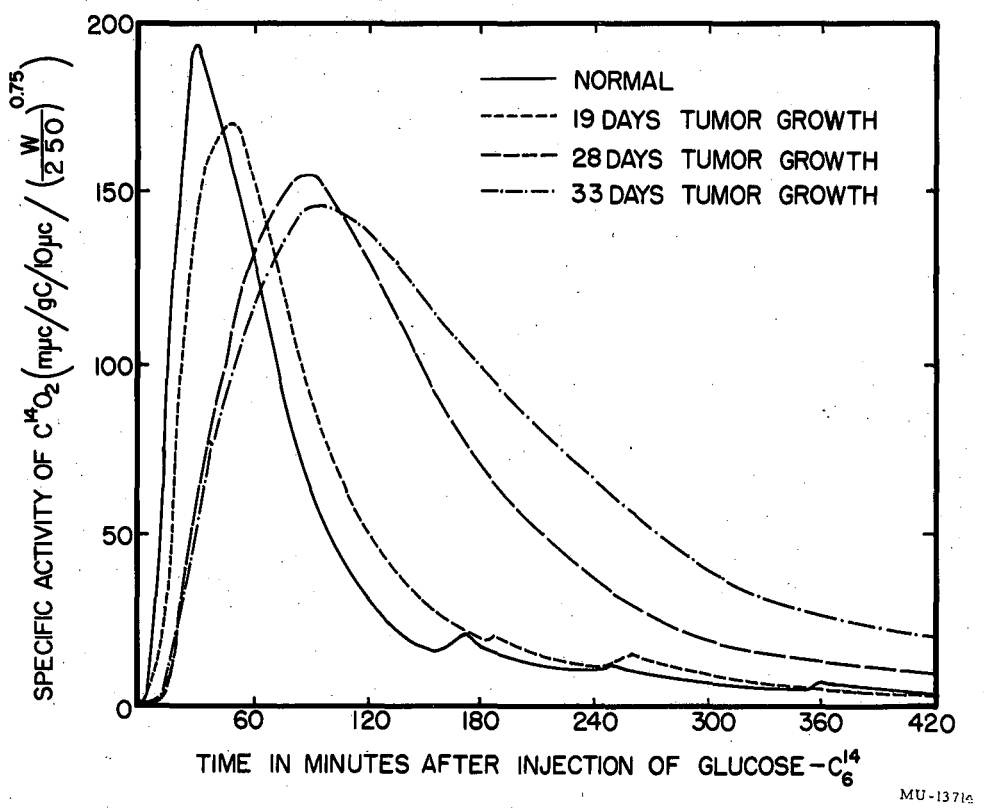

200
150
100
50
0
60
120
180
240
300
360
420
NORMAL
19 DAYS TUMOR GROWTH
28 DAYS TUMOR GROWTH
33 DAYS TUMOR GROWTH
SPECIFIC ACTIVITY OF $C^{14}O_2$ (mμc/gC/10μc / $(\frac{W}{250})^{0.75}$)
TIME IN MINUTES AFTER INJECTION OF GLUCOSE-C_6^{14}
MU-13716

Fig. 3.

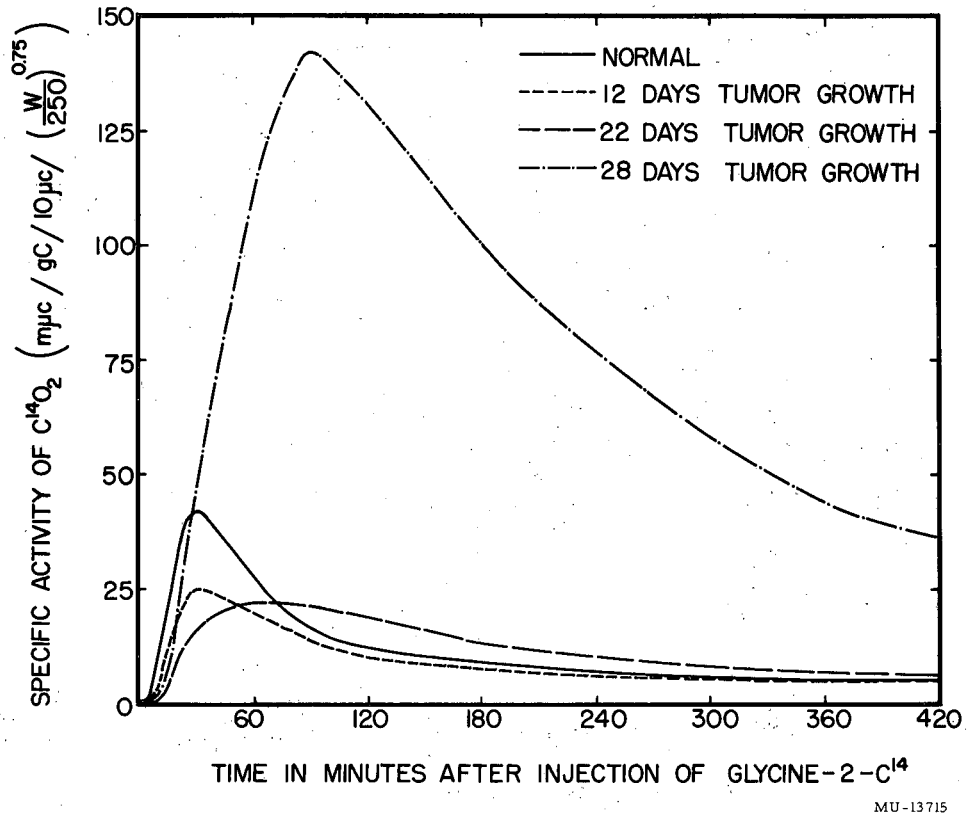

150
125
100
75
50
25
0
60
120
180
240
300
360
420
NORMAL
12 DAYS TUMOR GROWTH
22 DAYS TUMOR GROWTH
28 DAYS TUMOR GROWTH
SPECIFIC ACTIVITY OF $C^{14}O_2$ (mμc / gC / 10μc / $(\frac{W}{250})^{0.75}$)
TIME IN MINUTES AFTER INJECTION OF GLYCINE-2-C^{14}
MU-13715

Fig. 4.

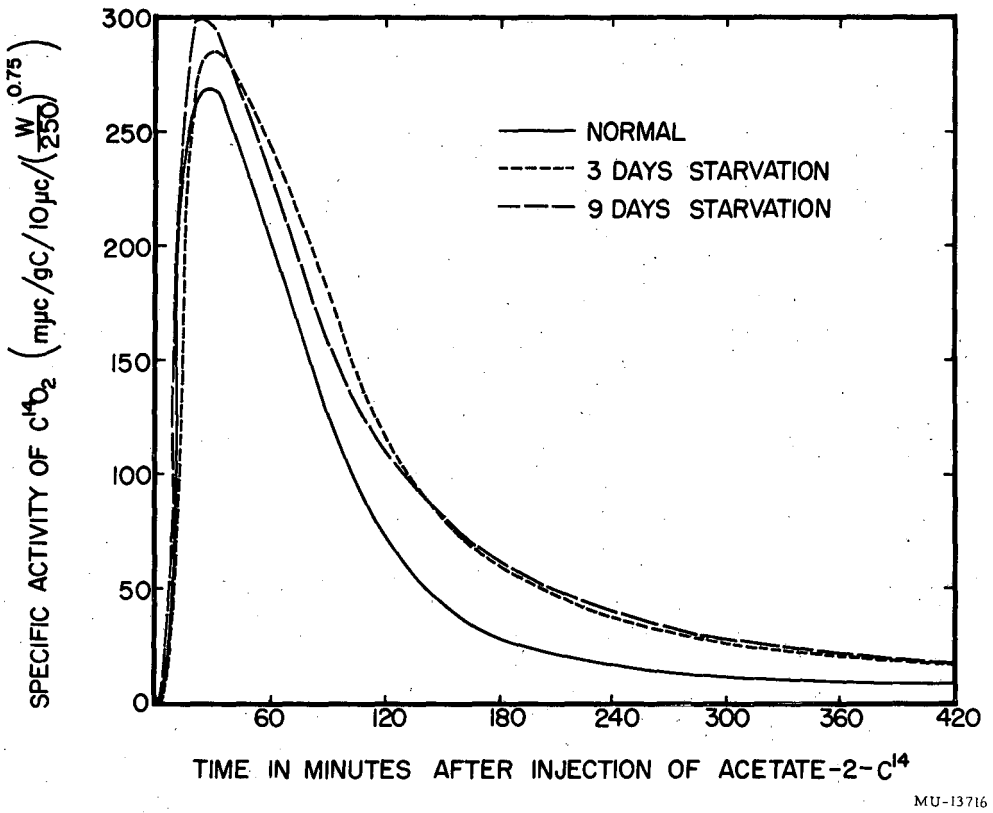

300
250
200
150
100
50
0
60
120
180
240
300
360
420
NORMAL
3 DAYS STARVATION
9 DAYS STARVATION
SPECIFIC ACTIVITY OF $C^{14}O_2$ $\left(m\mu c/gC/10\mu c/\left(\frac{W}{250}\right)^{0.75}\right)$
TIME IN MINUTES AFTER INJECTION OF ACETATE-2-C^{14}
MU-13716

Fig. 5.

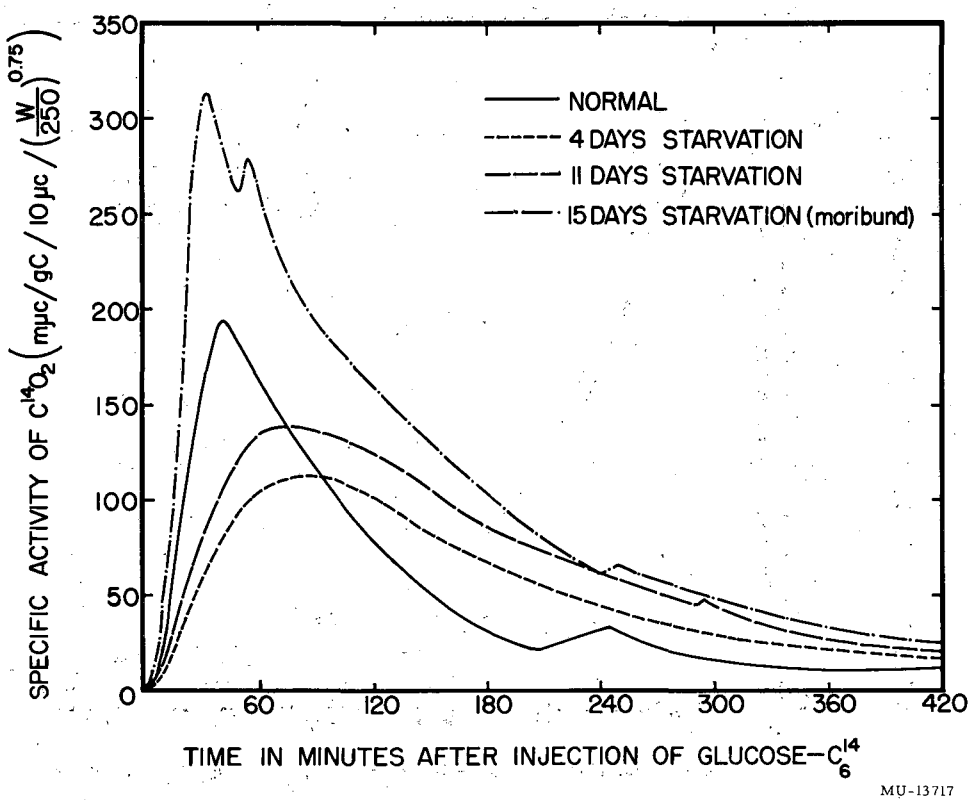

NORMAL
4 DAYS STARVATION
11 DAYS STARVATION
15 DAYS STARVATION (moribund)
SPECIFIC ACTIVITY OF $C^{14}O_2$ $\left(m\mu c/gC/10\mu c/\left(\frac{W}{250}\right)^{0.75}\right)$
TIME IN MINUTES AFTER INJECTION OF GLUCOSE-C_6^{14}
0
50
100
150
200
250
300
350
60
120
180
240
300
360
420
MU-13717

Fig. 6.

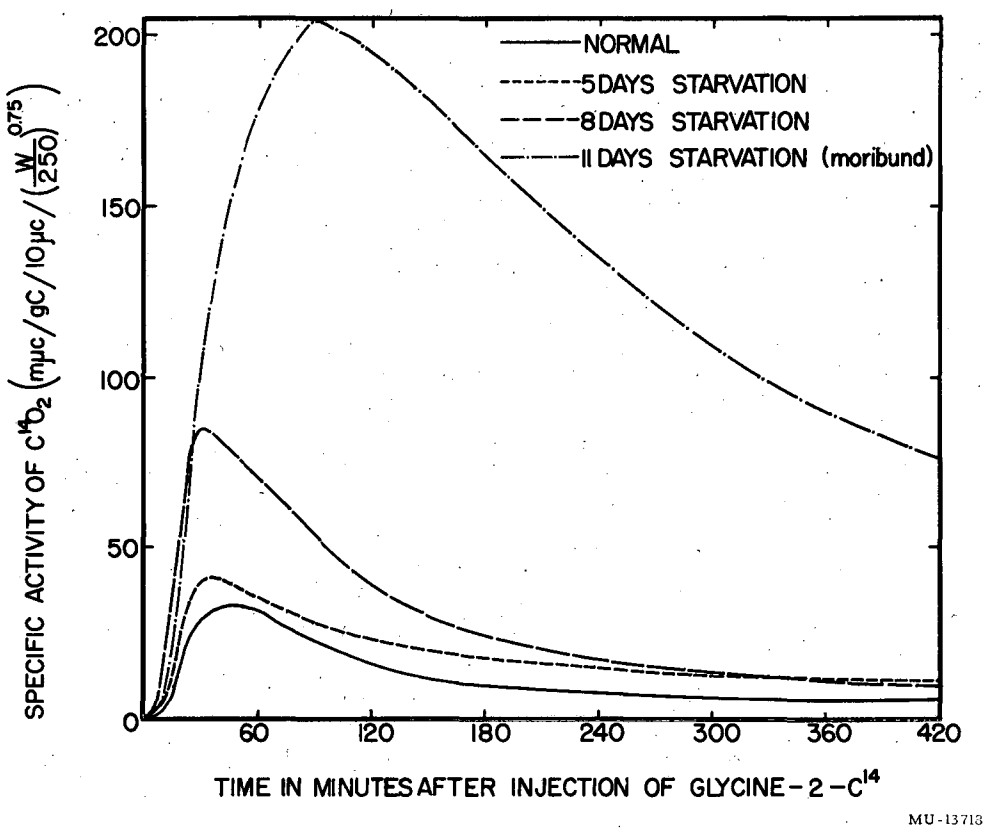

NORMAL
5 DAYS STARVATION
8 DAYS STARVATION
11 DAYS STARVATION (moribund)
SPECIFIC ACTIVITY OF $C^{14}O_2$ (mμc/gC/10μc/(W/250)^0.75)
200
150
100
50
0
60
120
180
240
300
360
420
TIME IN MINUTES AFTER INJECTION OF GLYCINE-2-C^{14}
MU-13713

Fig. 7.

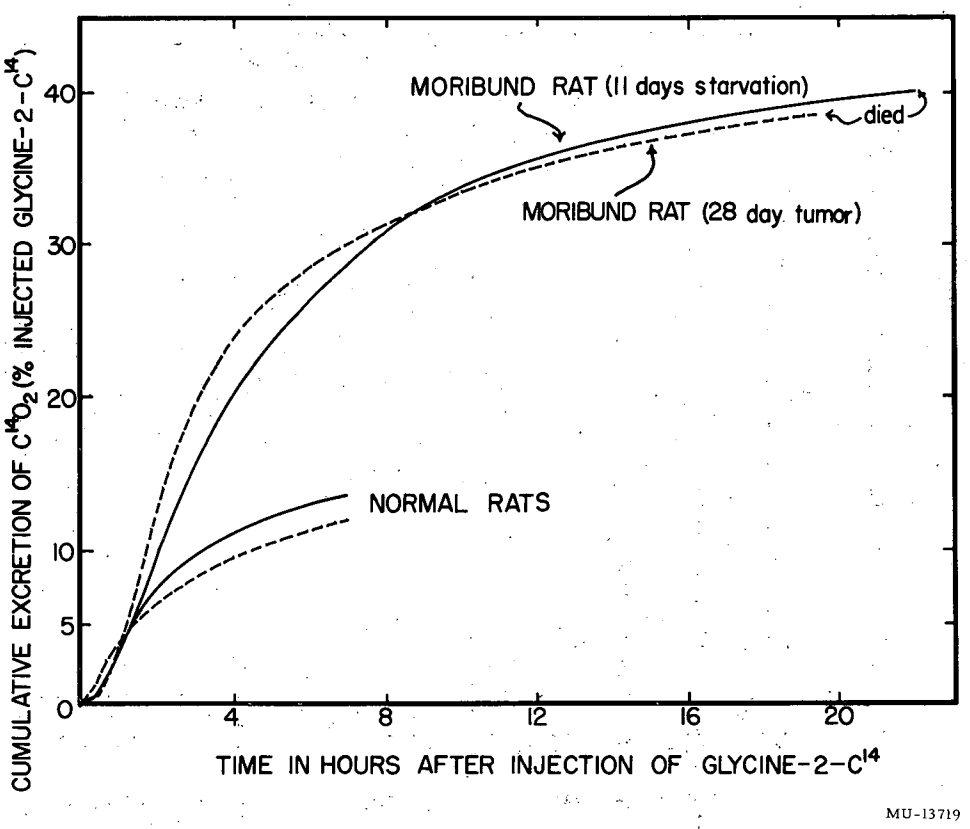

MORIBUND RAT (11 days starvation)
died
MORIBUND RAT (28 day tumor)
NORMAL RATS
CUMULATIVE EXCRETION OF $C^{14}O_2$ (% INJECTED GLYCINE-2-C^{14})
0
5
10
20
30
40
4
8
12
16
20
TIME IN HOURS AFTER INJECTION OF GLYCINE-2-C^{14}
MU-13719

Fig. 8.

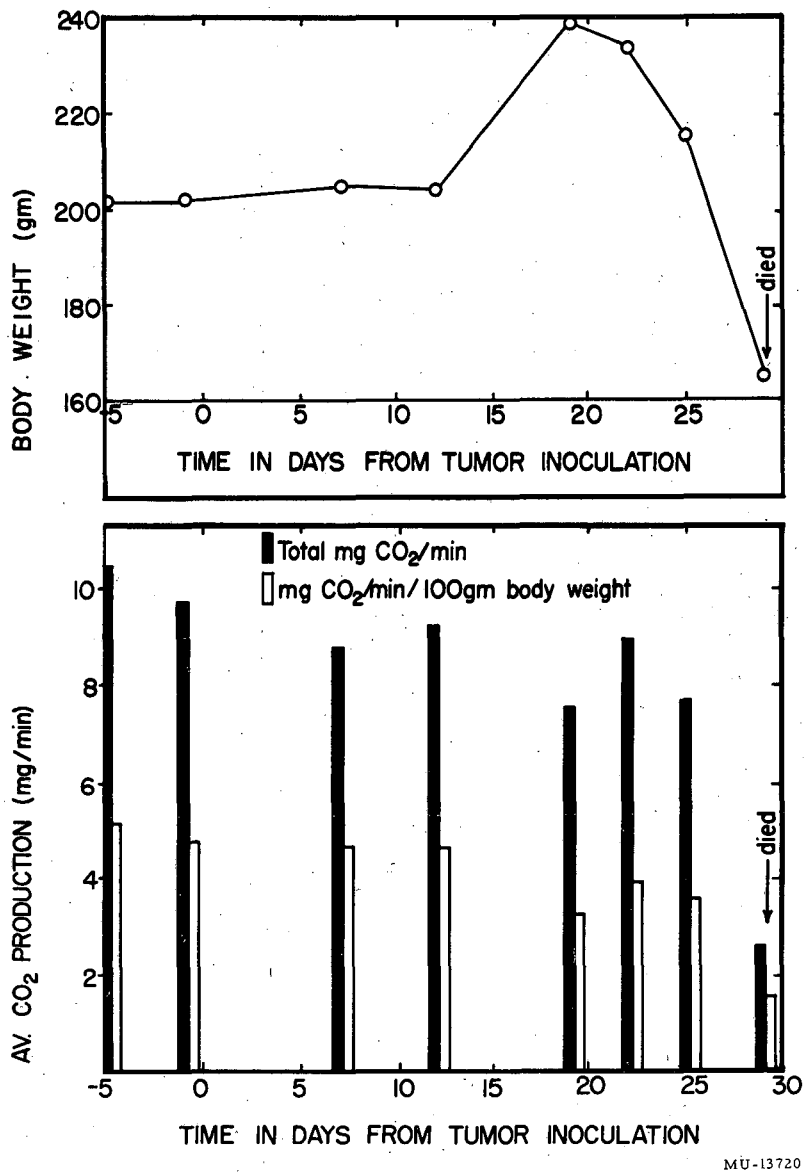

240
220
200
180
160
BODY WEIGHT (gm)
died
-5
0
5
10
15
20
25
TIME IN DAYS FROM TUMOR INOCULATION
Total mg CO_2/min
mg CO_2/min/ 100gm body weight
10
8
6
4
2
AV. CO_2 PRODUCTION (mg/min)
died
-5
0
5
10
15
20
25
30
TIME IN DAYS FROM TUMOR INOCULATION
MU-13720

Fig. 9.

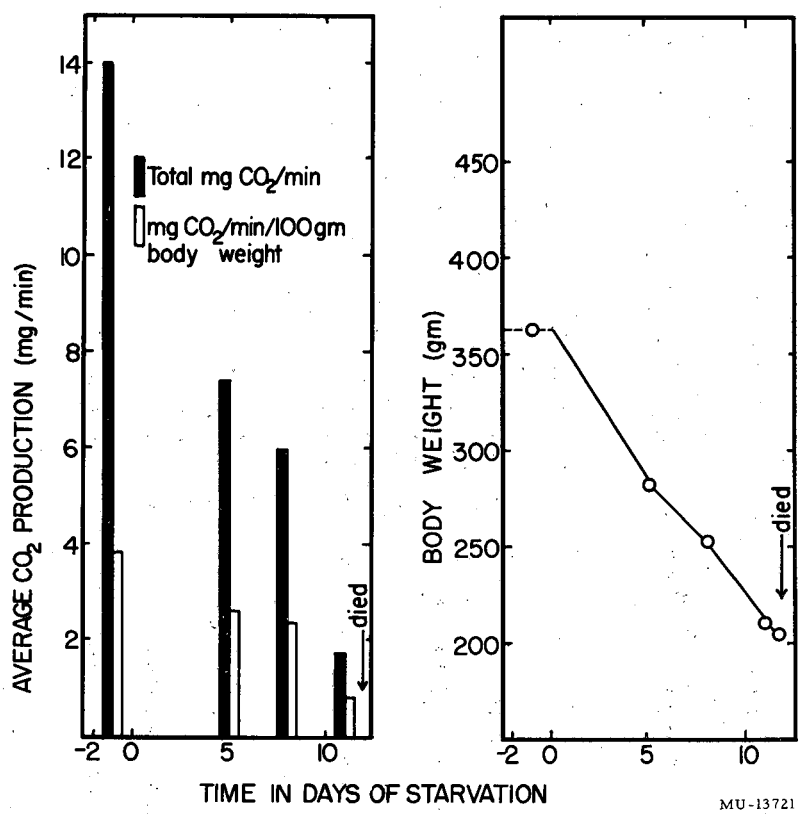

Total mg CO_2/min
mg CO_2/min/100gm body weight
AVERAGE CO_2 PRODUCTION (mg/min)
14
12
10
8
6
4
2
died
-2
0
5
10
BODY WEIGHT (gm)
450
400
350
300
250
200
died
-2
0
5
10
TIME IN DAYS OF STARVATION
MU-13721

Fig. 10.