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THE FATE OF C^{14} IN THE TISSUES OF MICE AFTER ADMINISTRATION OF C^{14} METHYL-LABELED GLYCINE

George L. Nardi

January 6, 1949

Berkeley, California

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By

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University of California, Berkeley, California [2]

January 6, 1949

ABSTRACT

1.08×10^6 counts/min. as methyl-labeled (C^{14}) glycine were injected intravenously into mature, A strain, male mice. The animals were sacrificed at 6-hour to 43-day intervals. Determinations of activity of excretions, tissues and carcass were made. Turnover rates of C^{14} were determined.

(1) From 20-70% of the injected activity was found in the exhaled CO_2 of the first 24 hours.

(2) About 1-3% of the injected activity appeared in the first 24-hour urine.

(3) About 10% of the activity appeared in the first 24-hour feces.

Relatively small increments were added to these excretions in ensuing time intervals.

(4) All tissues showed a steady and progressive loss of specific and total activities. The former were quite similar for all tissues at identical time intervals.

---

1 While on leave from the Massachusetts General Hospital, Boston, Massachusetts; Milton Fellow, Harvard University.

2 This paper is based on work performed under Contract No. W-7405-Eng-48 with the Atomic Energy Commission in connection with the Radiation Laboratory, University of California, Berkeley, California.

(5) Turnover rates revealed very similar values for all tissues. Brain was the slowest; bone was as rapid as liver, pancreas and heart.

(6) The turnover rate of the whole body after 43 days was similar to that of the individual tissues. 5.54% of the injected activity was present with a biological half-time of 10.5 days.

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Carbon labeled compounds offer a wide range of experimental possibilities because of the ubiquity of carbon in living organisms. Because of its long half-life (5000 + years) C^{14} offers the additional advantage of an isotope which can be studied over long periods of time. However, this very fact has resulted in an understandable hesitation in its use because of lack of knowledge regarding the effects of prolonged exposure of living tissues to radiation of this sort. It was felt that if the radioactivity were fairly evenly distributed in the organism the total dose could be so calculated so as to keep the radiation to any one tissue within reasonably calculated safety margins. Bloom et. al. (1) using $Ba^{*}CO_3$ injected intraperitoneally into young rats showed by means of autoradiographs that activity tended to localize in bone and remain there long

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(1) W. Bloom, H. J. Curtis and F. C. McLean, Science, 105, 45 (1947).

after soft tissues no longer were active; although no direct weighing and measuring of the activity in various tissues was determined, so the residual activity in bone may have been extremely small. It was felt, therefore, that further work should be done to see if soluble compounds resulted in radioactivity localizing in bone.

Glycine labeled with C^{14} in the methylene position, having an activity of 567,000 counts/min (4.57μc/mg), prepared in this laboratory by Ostwald (2) was injected intravenously into adult, male, A strain mice. Sealed, glass animal cages were used so exhaled CO_2, feces and urine could be collected for measurement. Fifteen animals were injected with a standard dose of 1.08×10^6 counts/min. (one count = 17.6 disintegrations). They were sacrificed at various time intervals from 6 hours to 43 days; the tissues were dried for at least 48 hours in a vacuum desiccator. The dry tissues were then combusted in quartz tubes over copper oxide, and the resultant $\overset{*}{C}O_2$ precipitated as $Ba\overset{*}{C}O_3$, and plated on aluminum discs. (3) Counting was done with thin window end tubes and Nucleometers (Radiation Counter Laboratories), all the counters having been standardized to a single counting tube to obtain comparable results. The moisture removed by vacuum desiccation was collected in a liquid nitrogen trap and wet combusted (4) to determine the presence of any volatile activity in two cases. Less than 1/2 count/min was present in the entire sample, so it was felt reasonable to ignore this

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(2) R. Ostwald, J. Biol. Chem., 173, 465 (1948).

(3) M. Calvin, C. Heidelberger, J. C. Reid, B. M. Tolbert and P. E. Yankwich, "Isotopic Carbon," John Wiley and Sons, Inc., in press.

(4) Pregl and Grant, "Quantitative Organic Microanalysis", page 34, English edition, Blakiston Co., Philadelphia (1946).

as a possible site of activity loss. Reid, (5) using dl-tyrosine labeled with C^{14}, has also found negligible amounts of volatile activity in a similar procedure.

Exhaled Carbon Dioxide: Exhaled CO_2 was collected by drawing through a soda lime tube into the glass cage a current of air which was passed through a sintered glass disc into 1 N sodium hydroxide. By using two columns, samples could be taken at intervals without any loss of activity. The resultant $Na_2\overset{*}{C}O_3$ was precipitated as $Ba\overset{*}{C}O_3$ and examined for total activity. Curve No. 1 shows the total activity in the exhaled breath in a cumulative fashion. In two cases, 100% + of the activity was found in the accumulated breath samples; this is undoubtedly due to experimental error. The animals did not seem to do well in the glass cages, and, therefore, the curves extend only over a few days. As can be seen, there is a wide range among individual animals. Nevertheless, it is obvious that the greater portion of the injected activity is excreted as $\overset{*}{C}O_2$. In the first 24 hours from 8-70% of the activity was lost in the breath. After the first 48 hours, relatively small increments were added. Using tagged dl-tyrosine Reid (5) obtained similar results.

Urine: Most of the activity appeared in the urine within the first 48 hours. (Curve No. 2) About 1-3% of the injected activity appeared at this time. Thereafter, only small additional amounts of activity appeared. Approximately .05%-.1% of the injected activity was found in daily samples after the first 48 hours.

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(5) J. C. Reid and H. B. Jones, J. Biol. Chem., 174, 427 (1948).

Feces: The feces were dried and combusted and measured in a manner similar to the soft tissues. As can be seen from Curve No. 3, approximately 10% of the injected activity is excreted within the first 24 hours. Small daily increments are then found; approximately 1% of the injected dose in each daily sample.

The three curves for exhaled CO_2, urine and feces show similar patterns, i.e., the far greater part of the activity appearing within the first 24 hours, a considerable amount in the second 24 hours and only small increments in the remaining 24-hour periods.

Tissues: The individual dry tissues were combusted and activities determined as previously described. In the case of bone, both femurs and tibiae were used and considered to be 8% of the skeleton from the determination of Morales, et. al. (6.). As a check on bony activity, pieces of skull (parietal bone) were also combusted and were found to have activity identical to that of the leg bone (within limit of experimental error). In the case of muscle no attempt was made to determine the total mass. The activity values are plotted as absolute specific activity on Curve No. 4 (counts/min/mg dry tissue) and as corrected specific activity on Curve No. 5. (Correction for the varying carbon content of the individual tissues was made so as to make the values comparative on an identical carbon content.) As can be seen, there is a very similar drop in the absolute specific activity of all tissues over the time involved. When correction is made for their carbon content as in Curve No. 5 the values fall even closer.

The total activities (counts/min) expressed as percent of injected dose are charted in Table I. There is a steady drop in activity of all the tissues over a period of time. After 43 days, the total activity was only a fraction of a percent of the injected activity and this was fairly

Table I

FATE OF C^{14} FROM METHYL-LABELED GLYCINE

% of Administered Dose of C^{14} Remaining in Organ

Time	Bone+	Brain	Heart	Intest.	Kidney	Liver	Stomach	Spleen	Panc.	Colon	Lungs
6 h	2.0	0.3	0.04	10.0	4.9	14.0	3.0	9.0	5.0	1.2	1.4
18 h*	0.5	0.5	0.04	6.0	3.0	6.7	4.3	2.0	1.5	8.5	1.4
25 h	1.3	0.5	---	6.0	2.9	17.0	2.3	0.9	2.3	6.6	1.4
41 h	1.0	0.3	0.5	9.0	2.5	12.0	3.4	1.9	1.0	1.5	0.3
68 h	0.5	0.2	0.4	2.3	1.9	9.0	1.0	0.7	0.8	2.8	0.5
96 h	0.6	0.5	0.4	5.5	2.1	8.0	0.7	0.5	0.5	2.6	0.6
6 d	0.5	0.3	0.4	2.2	1.8	5.3	0.6	0.4	0.6	1.0	0.6
12 d	0.25	0.3	0.1	1.0	0.7	2.0	0.2	0.25	0.3	0.4	0.2
14 d	0.25	0.3	0.2	1.0	0.6	2.2	0.3	0.2	0.3	0.3	0.2
17 d	0.3	0.3	0.2	0.3	0.6	1.4	0.15	0.2	0.2	0.4	0.2
21 d	0.1	0.2	0.2	0.3	0.3	0.6	0.1	0.1	0.1	0.2	0.1
26 d	0.13	0.1	0.1	0.2	0.2	0.6	0.07	0.15	0.08	0.2	0.1
34 d	0.10	0.1	0.09	0.2	0.2	0.3	0.02	0.07	0.06	0.1	0.1
43 d	0.05	0.1	0.05	0.08	0.05	0.2	0.04	0.04	0.1	0.07	0.04

h - hours

d - days

* - this animal may have been sick

+ - both legs, tibia and femur

evenly distributed in all the tissues examined.

In an attempt to determine the actual turnover rates of C^{14} in each tissue the specific activity/mg carbon in each tissue was measured. These values were plotted on semi-log paper and the curves analyzed. The turnover rate, K, was determined for each component of the curve and the value of K obtained from the formula

$$K = \frac{\log_e 2}{1/2\ t}$$

where 1/2 t = the time for values to diminish by 1/2 as determined from the straight line component.

The turnover time is then equal to $\frac{1}{K}$.

The daily percent of injected C^{14} turned over in each tissue was calculated and charted in Table II beside the corresponding K values.

As can be seen, the turnover rates for each component of each tissue are very similar in all cases. The more rapid components show a little more variation; however, the data in these cases are not as reliable for the curve was quite steep and only a few points were available. The slow rate component of liver, bone, pancreas and heart were only slightly slower than the other tissues, the activity having a biological half-time of 15-17 days. Brain had only a single component which was slowest of all but still showed a definite C^{14} turnover with a biological half-time of 21 days.

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(6) H. F. Morales, E. N. Rathbon, R. E. Smith and N. Pace, J. Biol. Chem., 158, 677 (1945).

Table II

C^{14} TURNOVER IN MOUSE TISSUES

	Rapid Component		Medium Component		Slow Component	
Tissue	K_1 x days^{-1}	% Inj.C^{14}	K_2 x days^{-1}	% Inj.C^{14}	K_3 x days^{-1}	% Inj. C^{14}
Liver	negligible	---	0.217	6.7	0.447	1.8
Bone*	0.835	8.24	0.217	6.5	0.0447	3.7
Stomach	0.992	4.02	0.252	0.79	0.0616	0.27
Colon	---	---	0.248	2.6	0.0554	0.52
Intestine	---	---	0.266	7.56	0.0554	1.19
Kidneys	2.31 ? ?	3.39? ?	0.277	2.5	0.0787	1.73
Spleen	2.31	7.83	0.248	0.6	0.0630	0.39
Lungs	0.925	1.95	---	---	0.0603	0.68
Pancreas	0.867	9.14	0.248	1.19	0.0408	0.26
Heart	0.521	1.97	---	---	0.0470	0,37
Brain	---	---	---	---	0.033	0.45

* - liable to correction for % of body weight; tentative figures used.

Entire Organism: The carcass of the 43-day mouse was combusted *in toto*, (carcass tissues examined) and 4.73% of the injected dose found to be still present. The individual tissues from this animal had a sum total activity of 0.81% of the injected dose. Therefore, 5.54% of the initial activity was still present in the entire body after 43 days. If this value is plotted on semi-log paper, a biological half-time of 10.5 days is obtained for the whole animal and a K value of 0.066, practically identical to the K values of the slow components of all tissues when examined and analyzed individually.

If one averages the daily percent turnover of all soft tissues in the three components of Table II, estimated average components for the entire organism may be plotted from which the percent retention of injected C^{14} at any time may be easily seen. This is done on Curve No. 6. At any one time, the percent activity in the organism will be the sum of the three components at that one time.

CONCLUSIONS

C^{14} when injected into mice as methyl-labeled glycine is evenly distributed throughout the animal body. Most of the activity is lost as radioactive CO_2 in the breath in the first 48 hours. After this time there is a slow but steady loss of activity in the urine, feces and breath so that after 43 days only 5.57% of the injected activity remains in the body with an overall biological half-time of 10.5 days. Turnover rates in all tissues are quite similar. There seems to be no selective localization of C^{14} in the bone, or any other tissue. There is no indication of a slower turnover than 10.5* days half-time for bone or any other tissue.

Schubert (7) has shown that growing animals tended to have a much higher concentration and turnover rate of C^{14} than mature animals using insoluble $Ba^{*}CO_3$ intraperitoneally as Bloom did. He, too, was unable to find any selective localization of activity in bone.

Mr. William Siri, in this laboratory, has estimated the doses delivered to organs on the basis of the values obtained in this experiment.

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(7) J. Schubert and W. Armstrong, J. Biol. Chem., in press.

* This is in the same range of effectiveness as P^{32} when used in humans (half-time 14.3 days). The total elimination due to excretion and decay is 8-10 days, one-half removal time.

Assuming a 70 kg. man, the maximum rep/day delivered to the various organs is as follows (when 1 mc C^{14} is administered).

Table III

Tissue	Daily dose to organ in man in rep/day/mc	
	1st day	43rd day
Bone	0.13	0.005
Brain	0.015	0.003
Heart	0.05	0.005
Kidney	0.12	0.0002
Liver	0.14	0.0014
Spleen	0.65	0.002
Pancreas	0.5	0.022
Lung	0.12	0.0025
Al. tract (whole gut)	0.09	0.001

ACKNOWLEDGMENTS

The author wishes to thank Drs. Melvin Calvin and John Lawrence for their suggestion of this problem and their helpful guidance throughout. Thanks are due also to Dr. Hardin B. Jones who helped analyze and interpret the data.

SUMMARY

1.08×10^6 counts/min as methyl-labeled (C^{14}) glycine were injected intravenously into mature, A strain, male mice. The animals were sacrificed at 6-hour to 43-day intervals. Determinations of activity of excretions, tissues and carcass were made. Turnover rates of C^{14} were determined.

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Relatively small increments were added to these excretions in ensuing time intervals.

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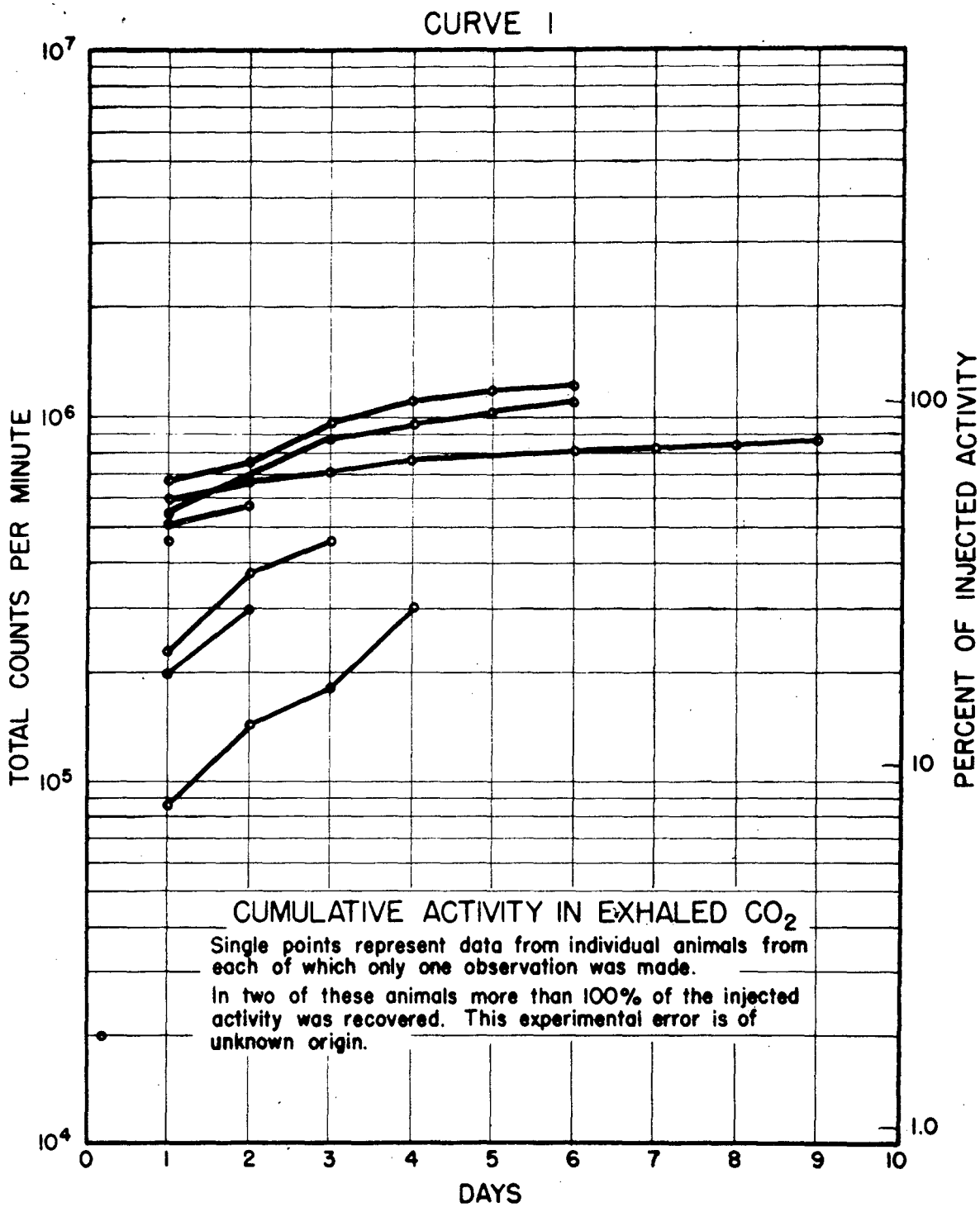
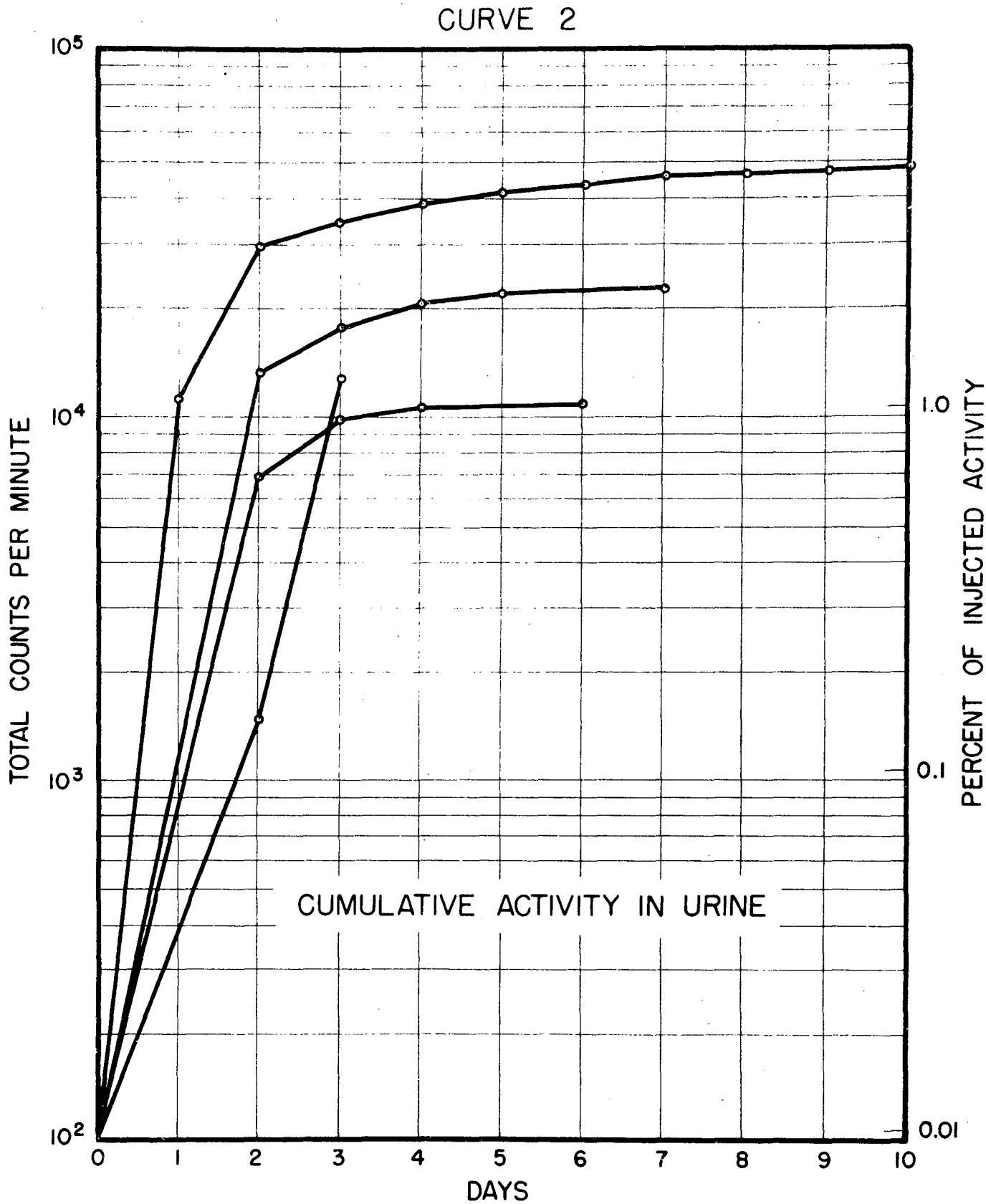
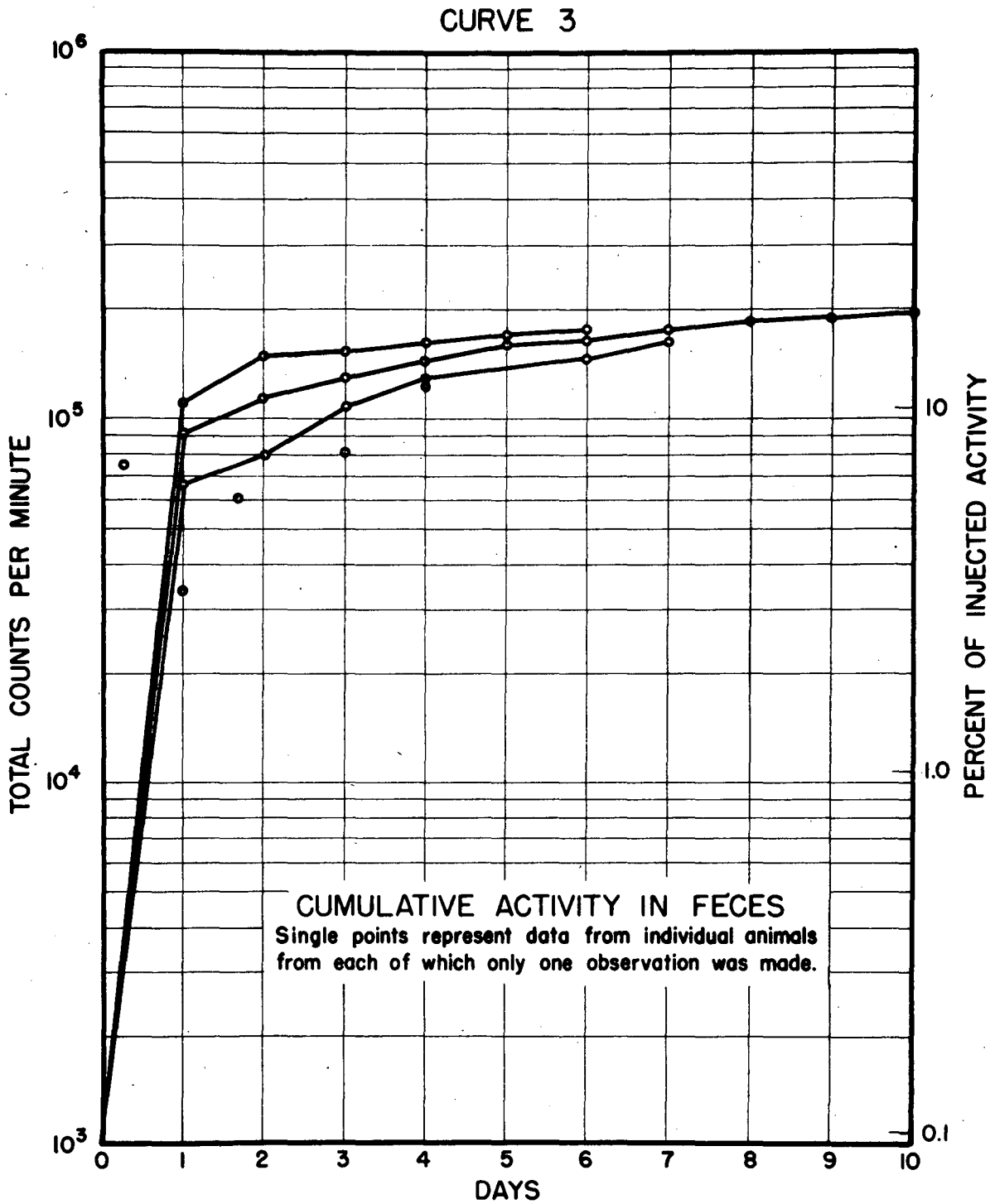

CURVE I
TOTAL COUNTS PER MINUTE
10^7
10^6
10^5
10^4
PERCENT OF INJECTED ACTIVITY
100
10
1.0
0
1
2
3
4
5
6
7
8
9
10
DAYS
CUMULATIVE ACTIVITY IN EXHALED CO_2
Single points represent data from individual animals from each of which only one observation was made.
In two of these animals more than 100% of the injected activity was recovered. This experimental error is of unknown origin.


CURVE 2
TOTAL COUNTS PER MINUTE
10^5
10^4
10^3
10^2
PERCENT OF INJECTED ACTIVITY
1.0
0.1
0.01
CUMULATIVE ACTIVITY IN URINE
0
1
2
3
4
5
6
7
8
9
10
DAYS

OZ-339


CURVE 3
10^6
10^5
10^4
10^3
TOTAL COUNTS PER MINUTE
10
1.0
0.1
PERCENT OF INJECTED ACTIVITY
0
1
2
3
4
5
6
7
8
9
10
DAYS
CUMULATIVE ACTIVITY IN FECES
Single points represent data from individual animals from each of which only one observation was made.

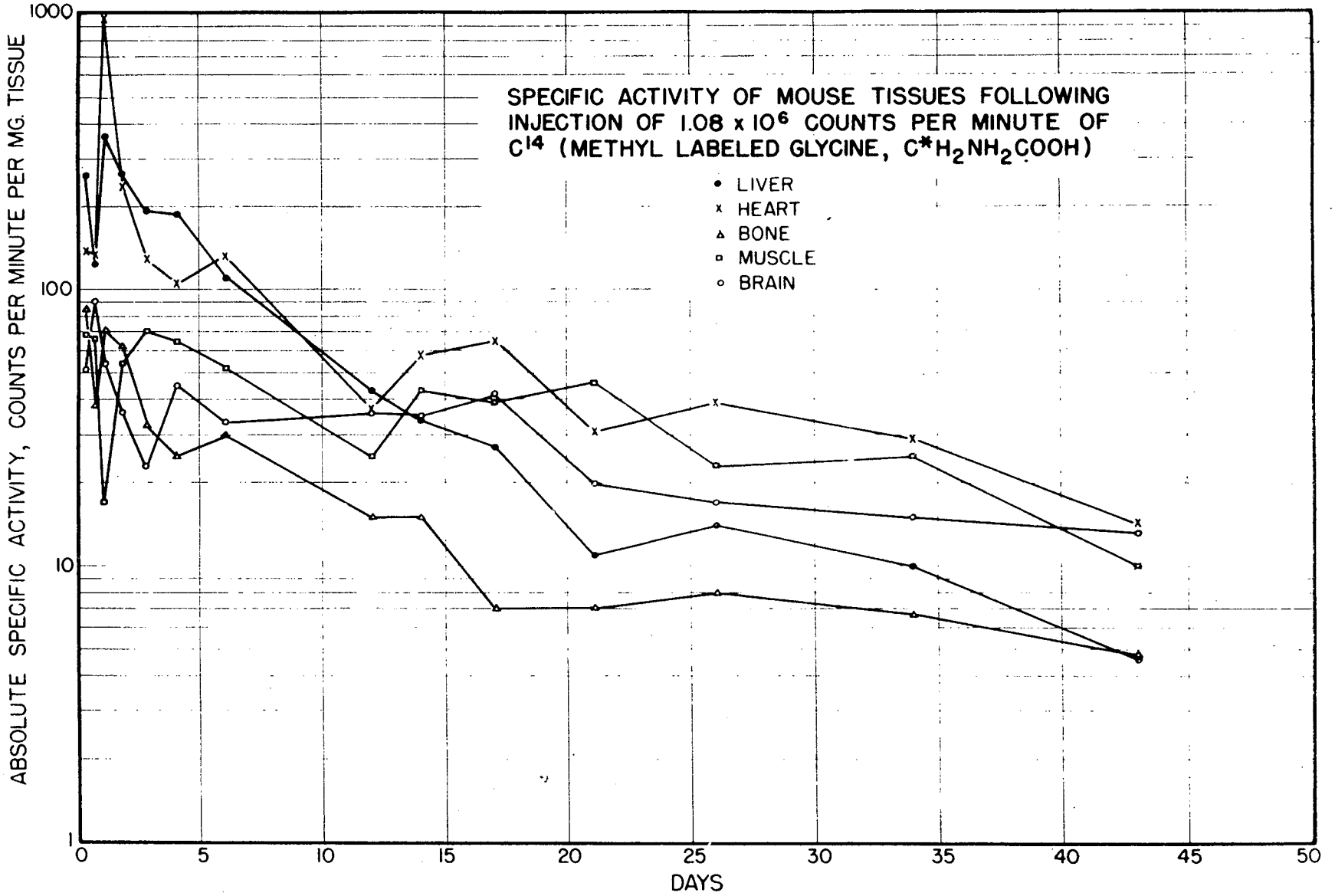

SPECIFIC ACTIVITY OF MOUSE TISSUES FOLLOWING INJECTION OF 1.08 x 10⁶ COUNTS PER MINUTE OF C¹⁴ (METHYL LABELED GLYCINE, C*H2NH2COOH)
LIVER
HEART
BONE
MUSCLE
BRAIN
ABSOLUTE SPECIFIC ACTIVITY, COUNTS PER MINUTE PER MG. TISSUE
1000
100
10
1
0
5
10
15
20
25
30
35
40
45
50
DAYS

FIG. 4a

OZ-345

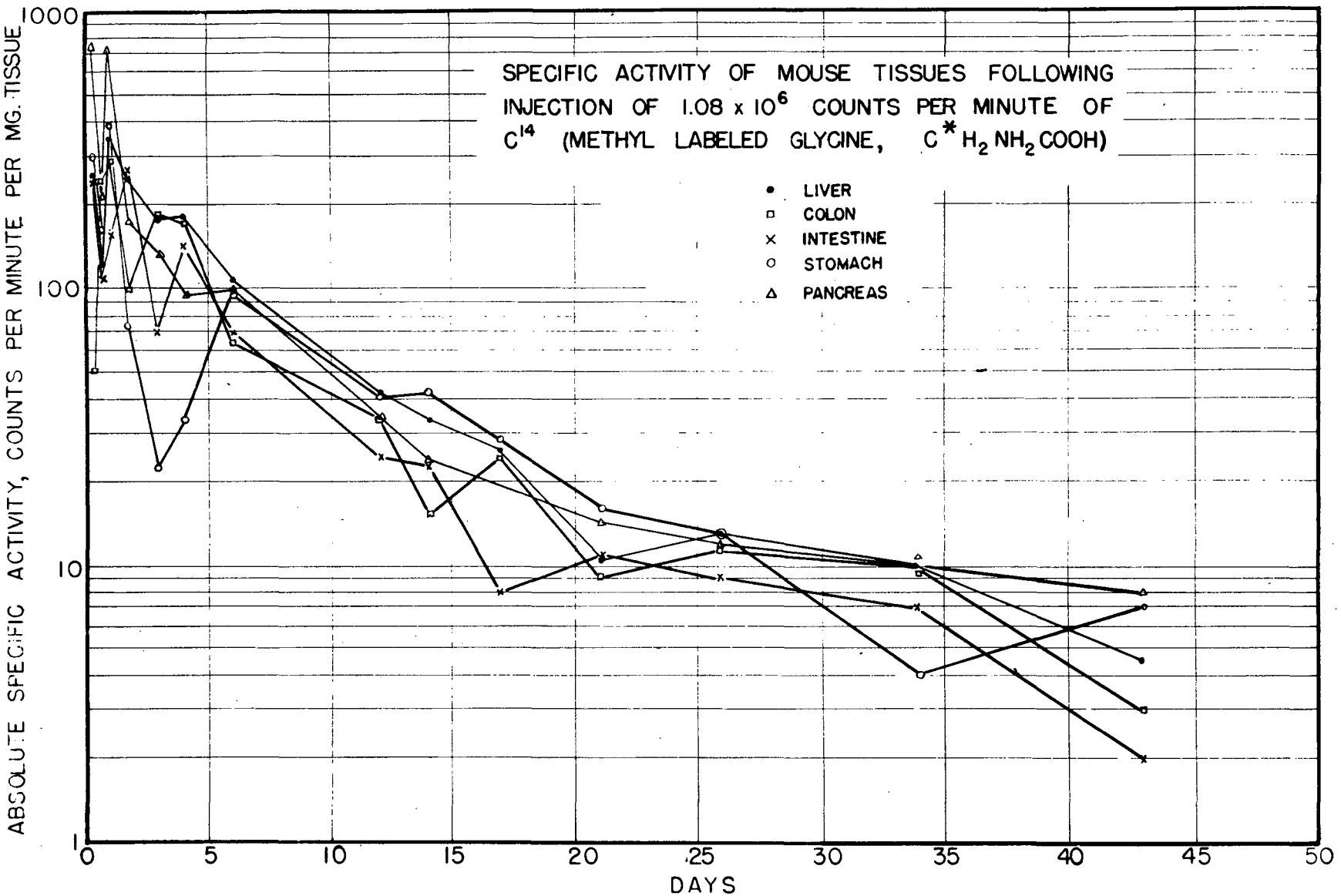

SPECIFIC ACTIVITY OF MOUSE TISSUES FOLLOWING INJECTION OF 1.08 x 10^6 COUNTS PER MINUTE OF C^14 (METHYL LABELED GLYCINE, C*H2NH2COOH)
LIVER
COLON
INTESTINE
STOMACH
PANCREAS
ABSOLUTE SPECIFIC ACTIVITY, COUNTS PER MINUTE PER MG. TISSUE
1000
100
10
1
0
5
10
15
20
25
30
35
40
45
50
DAYS

FIG. 4b

OZ-340

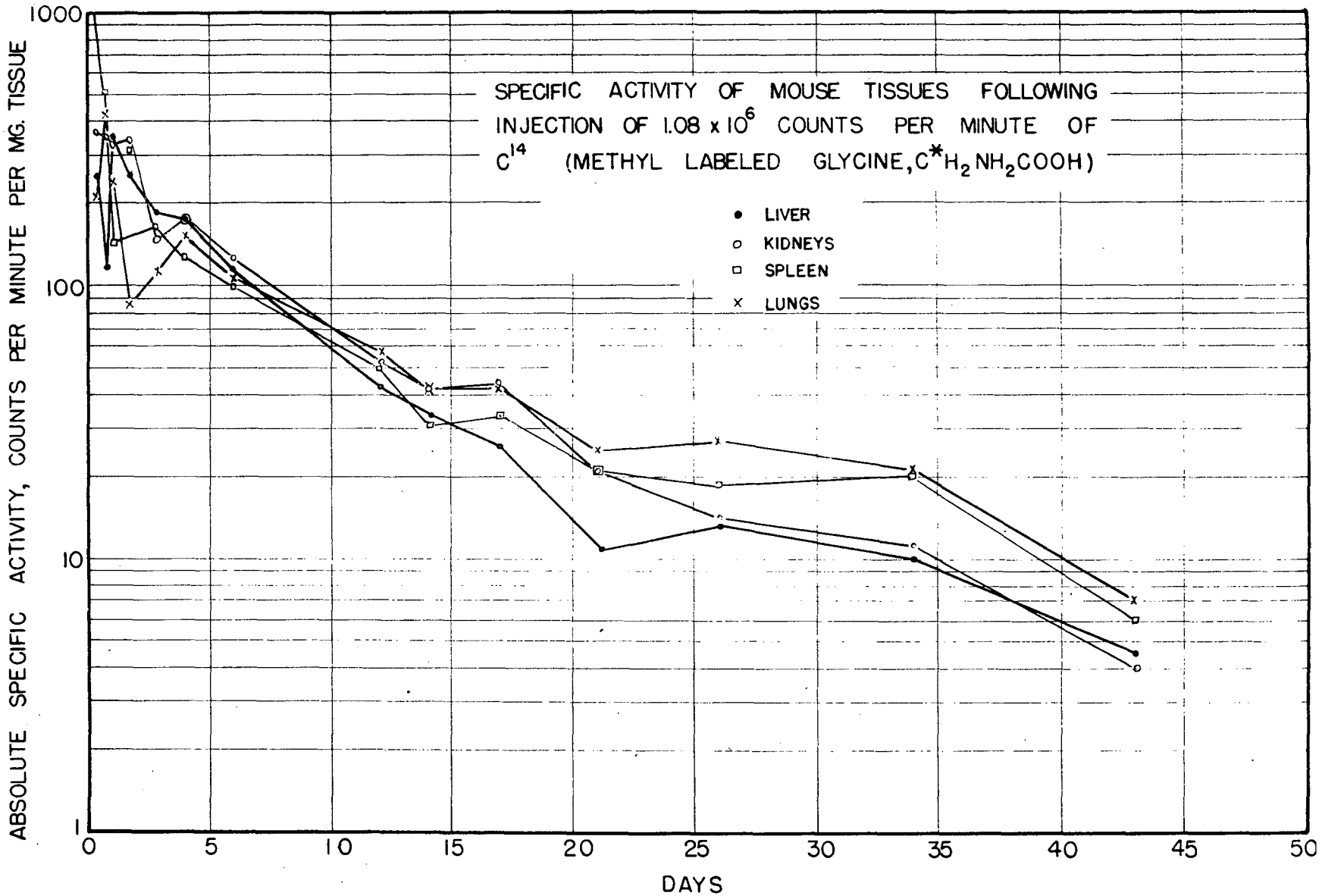

SPECIFIC ACTIVITY OF MOUSE TISSUES FOLLOWING INJECTION OF 1.08 x 10^6 COUNTS PER MINUTE OF C^14 (METHYL LABELED GLYCINE, C*H2NH2COOH)
LIVER
KIDNEYS
SPLEEN
LUNGS
ABSOLUTE SPECIFIC ACTIVITY, COUNTS PER MINUTE PER MG. TISSUE
1000
100
10
1
0
5
10
15
20
25
30
35
40
45
50
DAYS

FIG. 4c

OZ-341

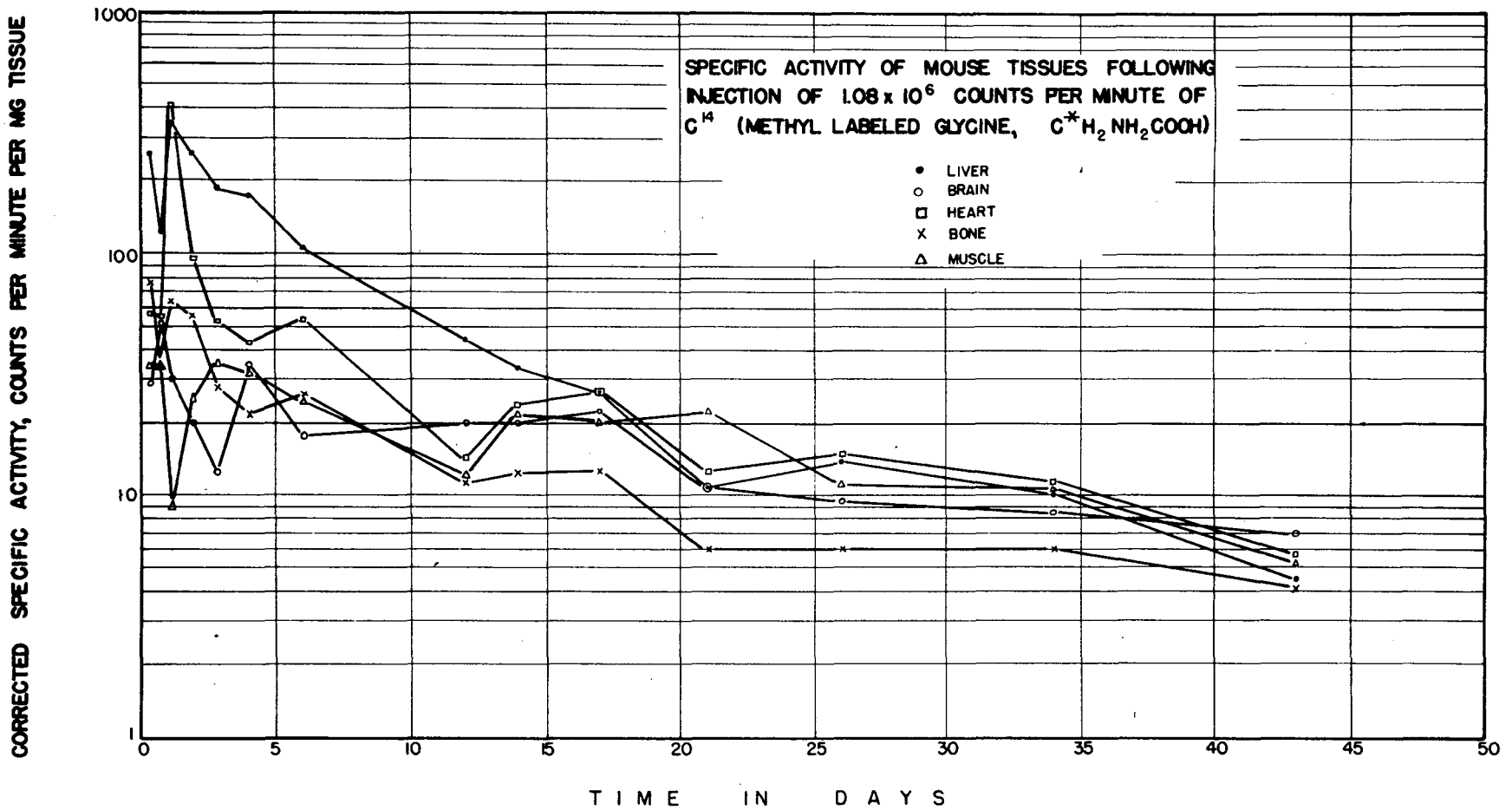

SPECIFIC ACTIVITY OF MOUSE TISSUES FOLLOWING INJECTION OF 1.08 x 10^6 COUNTS PER MINUTE OF C^14 (METHYL LABELED GLYCINE, C*H2NH2COOH)
LIVER
BRAIN
HEART
BONE
MUSCLE
CORRECTED SPECIFIC ACTIVITY, COUNTS PER MINUTE PER MG TISSUE
1000
100
10
1
0
5
10
15
20
25
30
35
40
45
50
TIME IN DAYS

FIG. 5a

OZ-342

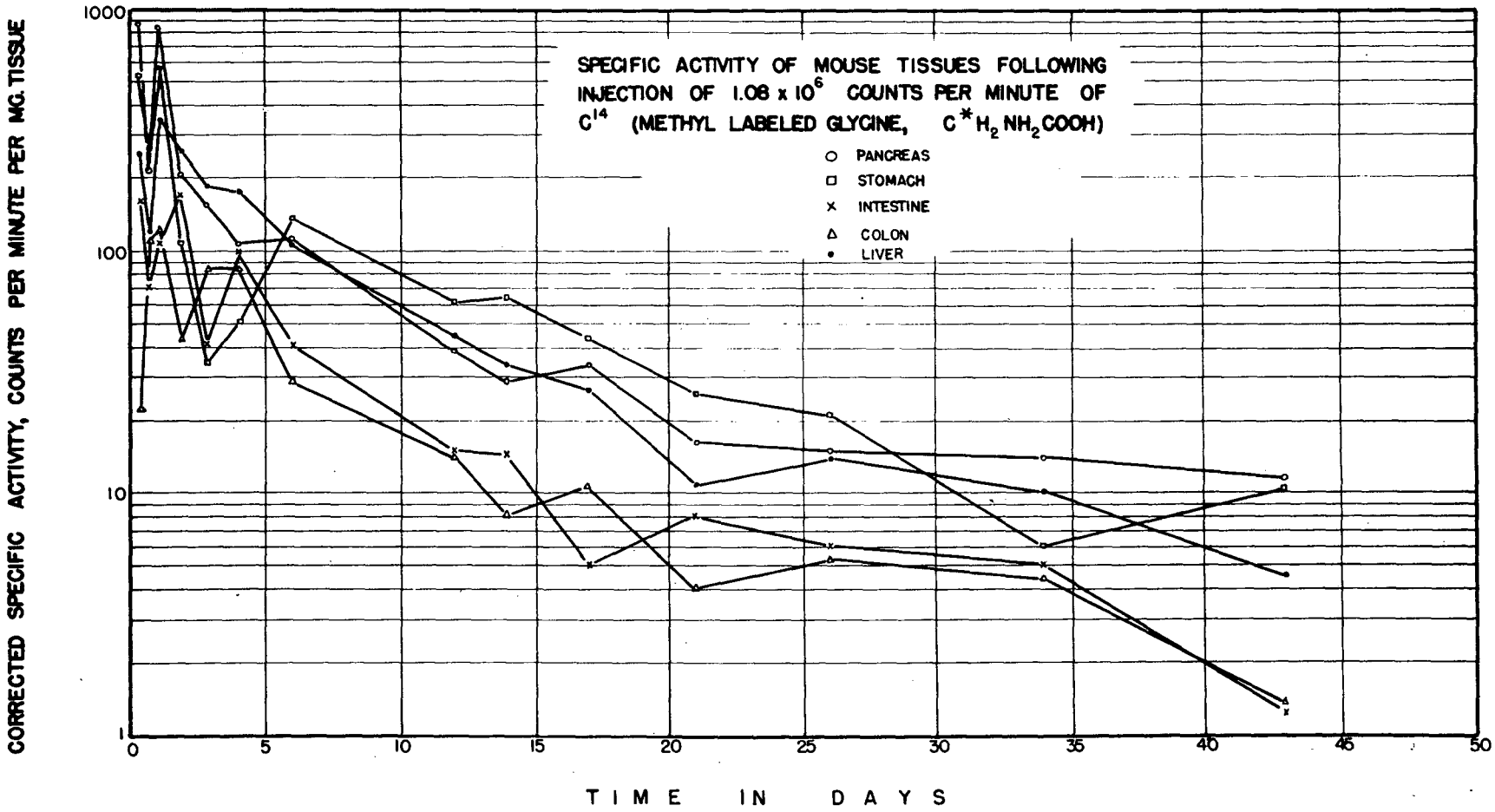

SPECIFIC ACTIVITY OF MOUSE TISSUES FOLLOWING INJECTION OF 1.08 x 10^6 COUNTS PER MINUTE OF C^14 (METHYL LABELED GLYCINE, C*H2NH2COOH)
PANCREAS
STOMACH
INTESTINE
COLON
LIVER
CORRECTED SPECIFIC ACTIVITY, COUNTS PER MINUTE PER MG. TISSUE
1000
100
10
1
0
5
10
15
20
25
30
35
40
45
50
TIME IN DAYS

FIG. 5b

OZ-343

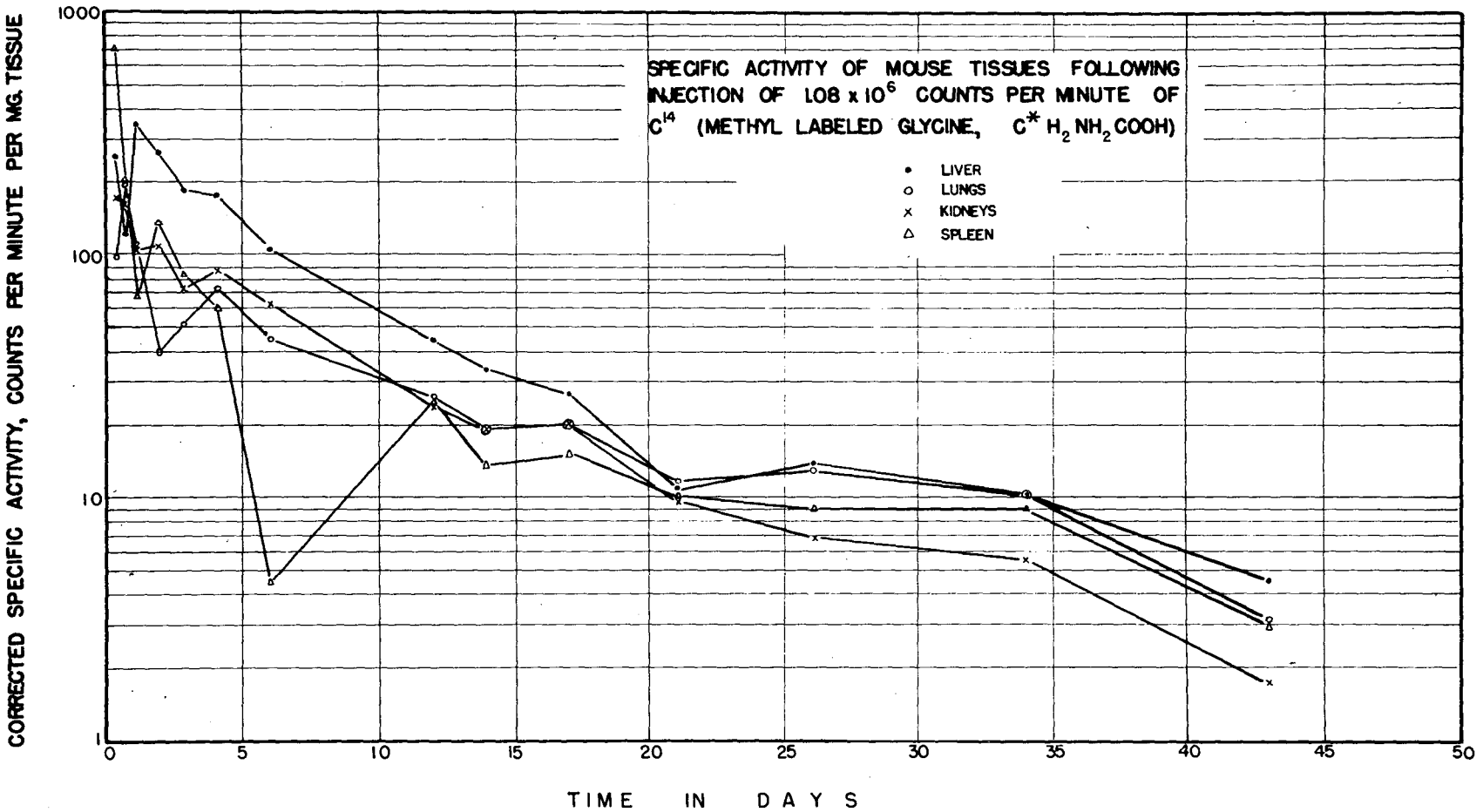
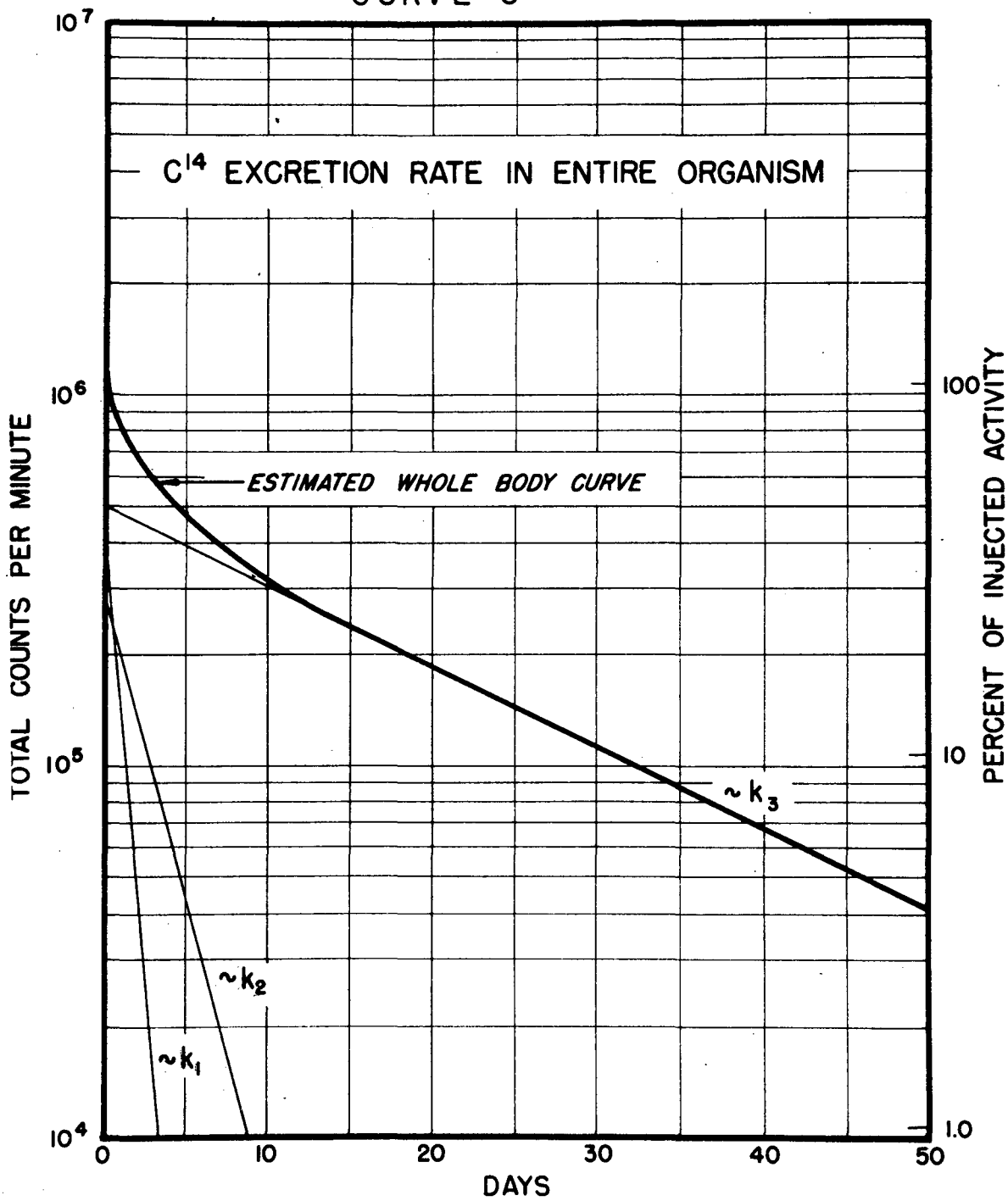

SPECIFIC ACTIVITY OF MOUSE TISSUES FOLLOWING INJECTION OF 1.08 x 10^6 COUNTS PER MINUTE OF C^14 (METHYL LABELED GLYCINE, C* H2 NH2 COOH)
LIVER
LUNGS
KIDNEYS
SPLEEN
CORRECTED SPECIFIC ACTIVITY, COUNTS PER MINUTE PER MG. TISSUE
1000
100
10
1
0
5
10
15
20
25
30
35
40
45
50
TIME IN DAYS

FIG. 5c

CURVE 6


C^{14} EXCRETION RATE IN ENTIRE ORGANISM
ESTIMATED WHOLE BODY CURVE
$\sim k_3$
$\sim k_2$
$\sim k_1$
10^7
10^6
10^5
10^4
TOTAL COUNTS PER MINUTE
100
10
1.0
PERCENT OF INJECTED ACTIVITY
0
10
20
30
40
50
DAYS