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Kinetic Aspects of Endogenous Carbon Monoxide Production in Experimental Animals

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DISCLAIMER

INTRODUCTION

Previous studies have demonstrated that endogenously-produced carbon monoxide in mammals arises solely from the alpha-methene bridge carbon atom of catabolized heme [1,2], and that one mole of CO is formed for each mole of heme degraded[3-5]. Since there are many different heme-containing compounds within the body, it is obvious that the contribution of each to total endogenous carbon monoxide production will depend not only upon its total heme content, but also upon its rate of turnover. The availability of glycine-2-^{14}C, the specific metabolic precursor for the alpha-methene bridge carbon atom of heme [6], has enabled investigators to study the fate of a cohort of such heme compounds, including hemoglobin heme, the latter comprising more than 90% of the total heme content of the body, by collecting the expired air [7] or peripheral blood [8] for its content of carbon-14 labeled carbon monoxide. This communication is a synthesis of a number of studies performed in this laboratory in an effort to gain insight into the kinetic behavior of the several heme-containing "compartments" within the body, through the study of endogenous production of ^{14}CO in experimental animals following injection of tracer doses of glycine-2-^{14}C.

MATERIALS AND METHODS

Animals studied included male buffalo or Sprague-Dawley rats, female LAF_1 mice, or male Japanese quail (Coturnix japonica, courtesy of Dr. Hans Abplanalp, Dept. of Poultry Sciences, University of California, Davis.) These animals were treated with a variety of experimental procedures, including transfusion-induced plethora, phenylhydrazine, repeated venesection, phenobarbital, allylisopropylacetamide (AIA), cross-transfusion, or gastrectomy, using established techniques.

Endogenous ^{14}CO production was studied using a method previously presented [7]. Animals were injected with 10-50 microcuries of glycine-2-^{14}C (specific activity 15-28 millicuries per millimole) intravenously, and placed immediately into plastic metabolism cages. Air was either continuously evacuated from such cages by a diaphragm pump, or forced through the cages by

pressure from a cylinder of compressed air, at a flow rate of 300-400 ml per minute. The air leaving these cages was then, in succession, dried by passage through anyhdrous $CaSO_4$, the carbon dioxide removed by passage through soda-lime, and the ^{14}CO oxidized to $^{14}CO_2$ by passage through a Hopcalite cannister (Mine Safety Appliances, Pittsburgh,Pa.) The $^{14}CO_2$ thus generated, representing endogenously-produced ^{14}CO without significant contamination from endogenously-produced $^{14}CO_2$ [7], was then trapped in an ethanolamine-containing solution [9], aliquots of which were counted by liquid scintillation. By means of a bank of ethanolamine-containing absorbing tubes and air valves, attached to a timing device, breath of experimental animals could be collected continuously in collection periods of from 1-5 hours each. For the study of the "early labeled peak" (ELP) of CO production, samples were collected continuously for the first 48 hours following glycine-2-^{14}C injection, and then daily for the next 3-5 days. For the study of the "late peak", animals were placed in the metabolism cages, and air flow started about 15-30 minutes before sample collection was started, in order to wash the non-equilibrated room air out of the chamber and dead space of the apparatus. Single 5-hour collections were then performed 1-4 times a week over the time period of about 7-150 days after injection of the labeled glycine.

Liquid scintillation samples were counted in duplicate for a sufficient length of time to allow a maximum uncertainty of $\pm$ 1 cpm (low activity samples) or $\pm$ 2% (high activity samples) at 95% confidence. Sample counting efficiency was determined using a toluene-^{14}C internal standard, and production rates for ^{14}CO were expressed in terms of cpm/hour or dpm/hour .

In most cases, the result for each sample collection period was considered as a single data point obtained at the midpoint of the collection period. However, most experiments were designed with the same sample collection schedule for the control and experimental animals, so that data points within any series of experiments would be directly comparable. "Late peak" data were fitted to appropriate mathematical formulae using a least-squares fitting program

and a high speed digital computer. Calculations derived from such best-fit parameters were then performed using a small digital computer, separately programmed for each task.

RESULTS AND DISCUSSION

A. The "Early Labeled Peak" (ELP) in Control Animals:

The production of ^{14}CO, normalized with respect to the weight of the animal and the dose of injected glycine-2-^{14}C in a normal LAF_1 mouse, normal buffalo rat, and normal quail is shown in Figure 1 for the first 2 days after labeled glycine injection. It can be seen that the general shape of the ELP is similar in all three animals, with peak ^{14}CO excretion rates 1-3 hours after isotope injection, and a smooth decline thereafter. Although there was always some variability in individual data points, in no instance did the ELP in normal animals show a distinct secondary rise during the first week after isotope injection.

In an attempt to separate the ELP of the LAF_1 mouse into erythropoietic and non-erythropoietic components, mice were studied either in the untreated state, or following prolonged transfusion-induced plethora. In the latter state, erythropoiesis is completely suppressed, as evidenced by the lack of significant ^{59}Fe incorporation into circulating RBC, absence of recognizable erythroid precursors in the bone marrow, and absence of reticulocytes from the peripheral blood [10]. Figure 2 indicates that the "late peak" of the hypertransfused mice was completely abolished, although the ELP was still present, although at a reduced magnitude. This indicates that the "late peak" is completely dependent upon the presence of active erythropoiesis, while at least a portion of the ELP is independent of erythropoiesis. The ELP in the 2 groups of mice is seen in more detail in Figure 3; it can be seen that the ELP is very similar in both groups, although there appears to be a more rapid decline in the ELP of the plethoric mice as compared to the controls. Simple subtraction of like data points yielded a difference curve shown in Figure 3 as a finely dashed line, which was roughly symmetrical and maximal at about 12-22 hours after isotope injection.

When similar experiments were performed in plethoric rats, no significant differences in the slope or timing of the ELP were noted between the control and hypertransfused rats. In the latter group the entire ELP was diminished in magnitude, the lowest magnitude being observed in the animal which attained the highest hematocrit (Table I). In the rat with a hematocrit of 65% on the day of isotope injection, the magnitude of the ELP was 40% of the control. Since erythropoiesis is not completely suppressed in the rat by hypertransfusion [10], this gives a rough upper limit to the non-erythropoietic component of the ELP of about 40% in the rat. This value agrees well with estimates obtained using the excretion of labeled bilirubin in normal and hypertransfused dogs [11].

B. The Non-Erythropoietic Component of the ELP:

It is generally accepted that the non-erythropoietic component of the ELP is associated with the turnover of tissue heme compounds, especially the heme compounds of the liver [12-14]. The magnitude of the ELP for bilirubin in intact rats was shown to be very similar to the labeled bilirubin formed in the isolated perfused rat liver, suggesting that this organ alone can account for most of the ELP [12]. In addition, Robinson has shown that the initial portion of the ELP is greatly diminished in magnitude following surgical removal of the liver [15]. Experiments in the hypertransfused mice (Figure 3)also showed that the initial portion of the ELP for CO is relatively unchanged in the presence or absence of erythropoiesis. Thus, it appears that the initial portion (first 2-3 hours) of the ELP is most closely associated with turnover of hepatic hemes.

Further evidence suggesting that the initial portion of the ELP is asociated with hepatic heme turnover can be obtained from studies employing phenobarbital or the porphyrogenic drug allylisopropylacetamide (AIA), drugs known to increase hepatic heme turnover. In fasted rats treated with 80 mg per kg of phenobarbital for 5 days, the ELP for CO was increased 2-fold (Figure 4 [5]). Similarly, treatment with AIA (300 mg per kg for 5 days) increased the mag-

nitude of the ELP 2-3-fold, with the major alteration in the ELP occurring during the initial few hours following injection of the labeled glycine (Figure 4[5]). Since these animals were fasted throughout the study, erythropoiesis was markedly depressed, as noted by decreased hemoglobin heme specific activity in circulating RBC 2 days after glycine-2-^{14}C injection (Table II). Thus, in these experiments the magnitude of the obtained ELP must closely approximate non-erythropoietic components of the ELP alone.

Results in the phenobarbital-treated rats agree well with results previously obtained through study of labeled bilirubin production in rats using labeled glycine or labeled delta-aminolevulinic acid to label heme cohorts [13,15-17], and also with non-isotopic studies of endogenous CO production performed in human subjects by Coburn [18]. The major heme compounds contributing to the ELP have not yet been definitively established, however. It has been suggested that an "unassigned" heme pool may be involved in this process [13], perhaps explaining why the magnitude and turnover rate of the ELP are greater than can be accounted for by the known amounts and turnover rates of specific hemoproteins in the liver. Kinetic studies by Marver et al. [19] have shown that administration of AIA does not lead to increased heme content in the liver, although the turnover rate of liver hemes appears to be increased by the drug. Thus the increased magnitude of the ELP for ^{14}CO seen in these animals [5] may be due to the drug-induced acceleration of the turnover of this "unassigned" heme pool.

C. The Erythropoietic Component of the ELP:

Previous theories concerning the genesis of the erythropoietic component of the ELP have implicated such sources as : heme formed in excess of globin in developing erythrocytes, loss of a certain fraction of hemoglobin-containing cytoplasm accompanying normoblast nucleus extrusion [20], and death of a small fraction of developing RBC within the bone marrow. The first 2 of these processes would constitute a "chemical death" of erythropoietic heme, while

only the last would constitute what is generally thought of as "ineffective erythropoiesis" i.e. production of RBC which never circulate in the peripheral blood.

Bassis has shown using electron microscopy [20] that the normoblast nucleus is extruded with up to 8% of the total cytoplasm still attached. Since this fragment of cytoplasm may contain a similar fraction of total RBC hemoglobin, as well as some or all of the cell organelles containing non-hemoglobin hemes, this process would naturally lead to a component of the ELP which would be directly proportional to the rate of erythropoiesis. If erythropoietic and non-erythropoietic components of the ELP in the rat are approximately in the ratio of 3:2 (Section A), then one would expect 92 units of heme destruction due to mature RBC destruction ("late peak"), 8 units of heme destruction due to hemoglobin loss associated with nuclear extrusion, and 5 units of heme destruction from the non-erythropoietic component(s) of the ELP. This would yield an ELP/ total heme turnover ratio of 13/105, or 12%, which is only about one-half of the ratio as actually measured in the normal buffalo rat (see Section C, Table III).

Israels et al.[21] have shown that the ELP in the duck appears to have an erythropoietic component; in this animal with circulating nucleated RBC, nuclear extrusion does not occur. It is known, however, that there is a reticulocyte phase in avian erythrocytes, so that partial functional loss of the RBC nucleus seems probable. Thus, heme-containing organelles in the developing RBC may be lost at some stage, resulting in an erythropoietic component. Figure 5 shows that when the quail is made anemic with 2 consecutive daily injections of phenylhydrazine, the ELP magnitude is increased about 3-fold, although a definite alteration in the shape of the ELP does not seem to have occurred.

These results point to an important facet of the erythropoietic component of the ELP which has not been stressed until recently [13]-- the presence of non-hemoglobin hemes within developing erythrocytes. These may have

turnover rates quite similar to hepatic hemes, for example, so that at least a portion of the erythropoietic component of the ELP would be kinetically indistinguishable from the non-erythropoietic component. Thus, stimulation of erythropoiesis in animals might increase the initial portion of the ELP by increasing the non-hemoglobin heme content of developing erythroid tissue. This behavior was seen in the quail stimulated with phenylhydrazine (Figure 5), and also in rats treated with 3 consecutive daily injections of phenylhydrazine or 3 consecutive daily phlebotomies (Figure 6). In these animals the increase in magnitude of the ELP was associated with an increase in the initial portion of this curve. It thus appears that the erythropoietic component of the ELP probably consists of a number of phenomena. It should be noted, however, that studies of the ELP for bilirubin have generally not shown an increase for this initial phase following stimulation of erythropoiesis [16,22]. Further studies concerning this point seem indicated.

When there are conditions leading to death of developing RBC within the sites of production, an erythropoietic component of the ELP becomes clearly evident. Figure 7 shows the ELP in a normal rat and in a severely iron-deficient rat secondary to gastrectomy*. A secondary rise in the ELP can be seen in the latter animal, occurring about 10-16 hours after isotope injection. This result is similar to those reported by Robinson, who studied the ELP for bilirubin in rats made deficient in iron by dietary means [23]. In these animals, the shape of the ELP was found to be markedly altered, with the greatest alteration occurring 8-13 hours after glycine injection.

Since it is known that the maximum uptake of iron by the developing erythrocytes in the bone marrow occurs about 6-8 hours after ^{59}Fe injection in the rat [24], and that the maximum rate of appearance in the circulation of labeled reticulocytes occurs at about 24 hours after injection [25], it would be probable that death of RBC in the marrow during maturation would be associated with increased production of bilirubin or CO between about 6 and 24 hours

* Kindly provided by Dr. Samuel Lepovsky, Dept. of Poultry Husbandry, University of California, Berkeley.

after isotope injection. The observed results in the iron deficient rats, in which a secondary rise in the ELP was noted 10-16 hours(Figure 7) and 8-13 hours [23] after isotope injection strongly implicate the presence of "ineffective erythropoiesis".

Unpublished results from this laboratory and the Jackson Memorial Laboratory in certain strains of congenitally anemic mice [26] have also shown a secondary rise in the ELP in the time range of 6-24 hours after isotope injection. Figure 8 compares the ELP in a normal mouse with the ELP in two sets of mice with congenital hemolytic anemia. The two disorders whose results are depicted in this figure have much in common with the thalassemic disorders in man, in which ineffective erythropoiesis is prominent [27]. The ELP in these animals was noted to be markedly increased, with maximum production of ^{14}CO 12 hours after injection of glycine-2-^{14}C, in keeping with the above arguments. Similar results were also found in mice with the Steel defect [26].

The results shown in Figure 3 also suggested that this phase of ^{14}CO production could be detected in normal LAF_1 mice, although by the magnitude of the subtraction curve it could not account for more than 10-20% of the ELP. It is therefore possible that there may be a small amount of ineffective erythropoiesis present in the mouse. The same subtraction technique, when applied to results in hypertransfused and normal rats, did not uncover an alteration in this phase of the ELP, however. It might thus be concluded that if there is ineffective erythropoiesis in these animals, it constitutes only a small fraction of total heme turnover.

D. Definition of the Time Limits for the ELP:

Inspection of the ELP in the hypertransfused mice (Figure 2) indicates that the production of ^{14}CO continues well beyond the first week following injection of labeled glycine. While most investigators have used arbitrary time limits for the ELP of about 3-5 days in animals and 1-2 weeks in man, Figure 2 indicates that ^{14}CO production in observable for more than 60 days in the complete absence of erythropoiesis. Berlin et al.[29] have shown that

significant amounts of activity persist in the free glycine pool in human subjects given glycine-2-^{14}C for many months, and that this continual persistence of label may be responsible for most of the non-ideal behavior seen in heme labeling curves in such subjects. Since the continual ^{14}CO production noted in the hypertransfused mice was also seen in the normal mice, but was not seen in mice or rats cross-transfused with labeled RBC, it is suggested that this continuing production of labeled CO is due to persistence of label in the glycine pool, with continual cycling of labeled glycine into successive heme cohorts.

If this "slow" component of CO production, which was found to have a half-time of about 50 days in the LAF$_1$ mouse and 100 days in the buffalo rat,[28] is subtracted from total ^{14}CO production, then the slowest remaining component of ^{14}CO production in mice or rats with suppressed erythropoiesis has a half-time of about 1-2 days. Such components are also seen in normal animals and in animals with increased erythropoietic rate when the phase of ^{14}CO production due to destruction of circulating RBC is also subtracted out. Thus, the processes associated with the ELP of the initially labled heme cohorts appear to last not more than 1 week. Along with a successful model for the kinetics of CO production from circulating RBC (see Section F), it should then be possible to define the ELP by means of a sum of exponential terms, which may be quantitatively more meaningful than arbitrary time intervals.

E. ^{14}CO Production and Destruction of Reticulocytes:

The preceding sections have dealt with destruction of tissue hemes and with death of RBC in the sites of production, including "chemical death" of hemoglobin heme. Three other modes of destruction of hemoglobin heme in RBC have been studied, namely:

1. Death of RBC upon entering the circulation (reticulocyte death),
2. Age-independent RBC destruction (random hemolysis), and
3. Age-dependent RBC death (senescence).

Studies in human subjects have demonstrated that in certain diseases RBC are trapped and destroyed in the spleen shortly after entry of these cells into the circulation. This phenomenon is best demonstrated by organ monitoring during iron kinetics studies, in which splenic activity is shown to increase (trapping of RBC) as the activity in the bone marrow decreases (release of RBC into the circulation) [30]. Kinetic arguments would suggest that death of reticulocytes should start at or slightly after entry of such cells into the circulation, and should end shortly after all labeled cells from the initial cohort have entered the circulation. For the rat and mouse, this phase should encompass the time interval of 24-96 hours after glycine-2-^{14}C injection. In a strain of congenitally anemic mice (mk/mk), following a normal ELP, there was noted to be a secondary rise in ^{14}CO production starting at about 24 hours, and ending about 72 hours after isotope injection.[26] When the spleen was removed from these animals prior to glycine injection, this added "peak" of ^{14}CO production was entirely abolished. Figure 8 suggests that this phenomenon also occurs in congenitally anemic mice (ha/ha). In these mice, as well as in the nb/nb mice there is evidence for "ineffective erythropoiesis" in that ^{14}CO production is increased in the time period of about 12-18 hours after isotope injection. However, as opposed to the situation in the nb/nb mice in which ^{14}CO production dropped rapidly after 18 hours, in the ha/ha mice ^{14}CO production continued at a markedly increased rate for more than 48 hours, returning towards the curve in the nb/nb mice at 72 hours. Moreover, the "difference curve" between the ha/ha and nb/nb mice was of the same general shape and timing as the added "peak" seen in the mk/mk mice. These preliminary results suggest that the phase of reticulocyte death is not present in normal animals, but if present under abnormal circumstances is a discrete event which can be distinguished from other processes of RBC destruction by timing and response to splenectomy.

F. Death of Mature Circulating RBC:

Labeling of a cohort of circulating RBC has been studied using a variety

of methods, such as RBC labeling with ^{59}Fe, ^{51}Cr, DFP-^{32}P, and glycine-2-^{14}C [31], and studies of ^{14}CO production have in general been consistent with results obtained by these methods [26]. Such studies indicate only 2 modes of death for circulating RBC-- random hemolysis and senescence. By random hemolysis is meant an age-independent process(es) by which a certain fraction of all circulating RBC are destroyed per unit of time. By definition, this process would exclude any destruction of labeled reticulocytes out of proportion to their concentration in the blood. (The processes of reticulocyte destruction and random destruction have, however, been shown to co-exist in the mk/mk mouse [26]. Splenectomy abolishes the former, but only slightly reduces the latter.) Senescence can be defined as the death of RBC subsequent to the ageing of some critical portion of the RBC, and is the dominant mode of RBC death in most normal animals and in man. In this process, RBC appear to be destroyed in a gaussian distribution about some period of time, called the mean potential lifespan. The mean potential lifespan varies widely, and ranges from about 23 days in the quail to 120 days in man. It appears to be closely related to the body weight of the animal [32] and to its rate of heat production [33].

The RBC labeling pattern following injection of a cohort label such as glycine-2-^{14}C has been well established. Eadie and Brown give the following formula for heme activity (H(t)) as a function of time (t) after isotope injection : [34]

$$H(t) = \frac{Ce^{-kt}}{1 + e^{a(t-T)}} \qquad (1)$$

where (C) is the size of the labeled cohort, (k) the rate of random hemolysis, (T) the mean potential lifespan, and (a) the coefficient of uniformity of lifespans about (T). The parameter (a) is a measure of the distribution of lifespans about (T), and is analagous to the width of a gaussian distribution as measured by the parameter (sigma). Equation (1) represents the activity in the cohort at any time (t), and the first derivative of this

equation represents the rate of change of heme activity with time. Since this activity can change only when labeled cells leave the circulation and are replaced by unlabeled cells, this first derivative should represent the rate of destruction of cells in the cohort. If (C) represents the total carbon-14 activity in hemoglobin heme of circulating RBC, and if only the alpha-methene bridge carbon atom of heme gives rise to CO, then the rate of ^{14}CO production arising from destruction of labeled RBC (CO(t)) will be 1/8 th of the first derivative of Equation (1), since glycine can potentially label 8 of the carbon atoms of heme [6]. This is shown in Equation (2) :

$$CO(t) = \frac{Ce^{-kt}(k + (k+a)e^{a(t-T)})}{8(1 + e^{a(t-T)})^2} \qquad (2)$$

Figure 9 shows the behavior of Equations (1) and (2) using an arbitrary set of values for the above parameters.

When washed labeled RBC were cross-transfused into normal compatible rat hosts, and the total amount of ^{14}CO expired over the first 100 days was determined, it amounted to 95 $\pm$ 5 % of the amount of activity calculated to be present in the alpha-methene bridge carbon atoms of the hemoglobin heme in the injected RBC. Moreover, the curve of ^{14}CO excretion rate versus time after initial glycine injection could be well approximated by Equation (2), as shown in Figure 10. Such experiments demonstrate the applicability of these equations in predicting the behavior of a cohort of RBC, and appear to confirm the previous demonstrations that CO arises *in vivo* from only one of the eight potentially labeled carbon atoms of heme.

The applicability of this method of determining RBC survival from ^{14}CO production will be the subject of future communications [28].

C. Fractionation of Total CO Production into Component Parts:

Fractionation of total CO production can be estimated using the following assumptions:

1. The "late peak" can be described by Equation (2).
2. The ELP can be described by all of the ^{14}CO produced during the first 2 to 3 days after glycine injection, and by a single exponential function after this period of time.
3. The single slow exponential (half-time 50-100 days) seen in mice and rats with suppressed, normal, or increased erythropoiesis represents the persistence of label in the glycine pool, does not reflect the destruction of the initial heme cohorts, and thus all of the ^{14}CO arising from this source must be omitted from the calculations.
4. The ratio of the non-erythropoietic portion of the ELP to total ELP is 2:5.

With these assumptions, the average fractionation of total ^{14}CO production in the buffalo rat is that shown in Table III. From previously noted arguments, it may be stated that there do not appear to be significant amounts of ineffective erythropoiesis or splenic reticulocyte destruction in normal animals. It is then possible, using these further assumptions, to determine the fraction of all labeled RBC being destroyed by the modes of destruction outlined in this communication, by quantitating the following phases of ^{14}CO production:

1. "Ineffective erythropoiesis": definite peak within 8-15 hours after glycine injection.
2. Splenic destruction of reticulocytes: definite peak occurring 24-96 hours after glycine injection.
3. Random hemolysis: as obtained from Equation (2).
4. Senescence: as obtained from Equation (2).

Table IV indicates the results of this fractionation in 6 normal buffalo rats and in a single sprague-dawley rat with iron deficiency secondary to gastrectomy. When such calculations are applied in animals with alterations in erythropoiesis, they are able to give important information concerning the

alterations in RBC survival which may be present. For example, in the iron deficient rat, such calculations indicate that the phases of "ineffective erythropoiesis" and random hemolysis were markedly increased (Table IV). This model has been applied to the study of RBC survival in mice with various congenital anemias, and will be the subject of separate communications[26].

SUMMARY

Endogenous ^{14}CO production was studied in experimental animals following the injection of glycine-2-^{14}C. As had been noted previously for labeled bilirubin and stercobilin, an "early labeled peak" and a "late peak" were noted, the latter being exclusively related to the destruction of the initial cohort of red blood cells labeled specifically in the alpha-methene bridge carbon atoms of hemoglobin heme. A mathematical formula was presented by which this "late peak" could be described, and included parameters describing the size of this cohort, the rate of random hemolysis, and the phase of senescent death.

The "early labeled peak" (ELP) was shown to consist of a portion associated with erythropoiesis, and a component unassociated with erythropoiesis, with the latter comprising approximately 40% of the total ELP in normal rats. This non-erythropoietic component could be increased in magnitude following treatment with phenobarbital or allylisopropylacetamide (AIA), agents which are known to increase non-hemoglobin heme turnover in mammalian liver.

The magnitude of the ELP could be increased following phlebotomy, phenylhydrazine, iron deficiency anemia, and in certain congenital anemias in the mouse. Evidence was produced to show that such stimuli increased different portions of the ELP, suggesting that the erythropoietic component of the ELP consists of a number of discrete processes, including turnover of non-hemoglobin hemes in developing erythroid precursors, "ineffective erythropoiesis", and early destruction of reticulocytes by the spleen. The latter two processes were felt to be minimal or absent in normal animals.

By quantitation of these various phases of ^{14}CO production, the contribution to total endogenous carbon monoxide production from the above processes

could be estimated, as could the fractionation of the various modes of death of the initial cohort of labeled red blood cells.

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TREATMENT	HEMATOCRIT ON DAY OF GLYCINE-2-^{14}C INJECTION (%)	RELATIVE "EARLY LABELED PEAK" MAGNITUDE (0 to 50 hours)
CONTROL	45	1.00
HYPERTRANSFUSED	56	0.64
HYPERTRANSFUSED	65	0.40

TABLE I

ALTERATION OF "EARLY LABELED PEAK" MAGNITUDE FOLLOWING HYPER-TRANSFUSION.

TREATMENT GROUP	SPECIFIC ACTIVITY OF HEMOGLOBIN HEME OF CIRCULATING RED BLOOD CELLS 2 DAYS AFTER INJECTION OF 50 uC OF GLYCINE-2-^{14}C (dpm/mg)			
CONTROL, NON-FASTED	5953 *	5598	4352	
CONTROL, FASTED	753	1052		
PHENOBARBITAL, FASTED	422	1715		
ALLYLISOPROPYLACETAMIDE, FASTED	428	144	212	302

* Each number indicates the result in a single animal.

TABLE II

EFFECT OF FASTING, PHENOBARBITAL, AND ALLYLISOPROLYLACETAMIDE ON INCORPORATION OF GLYCINE-2-^{14}C INTO CIRCULATING RED BLOOD CELL HEMOGLOBIN HEME.

PROCESS	PERCENT OF TOTAL ^{14}CO PRODUCTION	
"Early Labeled Peak "	24	
Erythropoietic portion		14
Non-erythropoietic portion		10
" Late Peak "	76	
Random hemolysis		23
Senescence		53
	100	100

TABLE III

FRACTIONATION OF TOTAL ^{14}CO PRODUCTION INTO COMPONENTS IN THE NORMAL BUFFALO RAT.

MODE OF RED BLOOD CELL DEATH	PERCENT OF INITIAL COHORT DESTROYED BY SPECIFIC PROCESS	
	AVERAGE OF 6 NORMAL BUFFALO RATS	IRON-DEFICIENT RAT
"Ineffective erythropoiesis"	0.0	24.9
Splenic destruction of reticulocytes	0.0	0.0
Random hemolysis	35.2	71.4
Senescence	64.8	3.7
	100.0	100.0

TABLE IV

FRACTIONATION OF PROCESSES OF RED BLOOD CELL DESTRUCTION IN 6 NORMAL BUFFALO RATS AND IN A SINGLE SPRAGUE-DAWLEY RAT WITH IRON DEFICIENCY ANEMIA SECONDARY TO GASTRECTOMY

FIGURE LEGENDS

FIGURE 1: Excretion rate of ^{14}CO (dpm/hour/50 microcuries of injected glycine-2-^{14}C/100 grams body weight, ordinate) versus time after injection of labeled glycine (hours, abscissa) in a normal LAF_1 mouse (open circles), Japanese quail (open triangles), and buffalo rat (open squares).

FIGURE 2: Excretion rate of ^{14}CO (cpm/hour, ordinate) in a group of normal LAF_1 mice (open circles) and a group of hypertransfused LAF_1 mice (closed circles) versus time after glycine-2-^{14}C injection (days, abscissa). Note that the "Late peak" is absent in the hypertransfused mice, although the "early labeled peak" is still present. Reprinted from Science (J.R.Goldsmith and S.A.Landaw, Science, volume 162, pages 1352-1359, December 20, 1968). Copyright 1968 by the American Association for the Advancement of Science.

FIGURE 3: "Early labeled peak" for ^{14}CO in the normal LAF_1 mouse (open triangles, solid line) and the hypertransfused LAF_1 mouse inwhich erythropoiesis has been totally suppressed (open circles, dashed line). Data the same as for Figure 2. The difference curve, presumably representing the component of the ELP associated with erythropoiesis, is shown as the finely dashed line and open squares. (Ordinate: ^{14}CO excretion rate (cpm/hour/animal), abscissa: time after injection of glycine-2-^{14}C (hours).)

FIGURE 4: "Early labeled peak" for ^{14}CO (ordinate and abscissa same as for Figure 3) in 3 starved Sprague-Dawley rats, one untreated (open squares), one treated with phenobarbital (open triangles), and one treated with the porphyrogenic drug allyl-isopropylacetamide (AIA) (open circles). The relative magnitude of the ELP for the first 49 hours for each of the three animals is shown in the upper right portion of the figure.

FIGURE 5: "Early labeled peak" for ^{14}CO (ordinate and abscissa same as for Figure 3) in two male Japanese quail, one untreated (open triangles) and one treated with 2 daily consecutive injections of phenylhydrazine (open circles). Note that the magnitude of the ELP of the latter is increased 3-fold, although the shape of the curves is quite similar.

FIGURE 6: "Early labeled peak" for ^{14}CO (ordinate and abscissa same as for figure 3) in three male buffalo rats, one normal (open squares), one treated with three consecutive daily phlebotomies (open traingles), and one treated with three consecutive daily injections of phenylhydrazine (open circles). The relative magnitudes of the ELP (first 48 hours) and the "late peak" for the three animals are shown in the upper right portion of the figure.

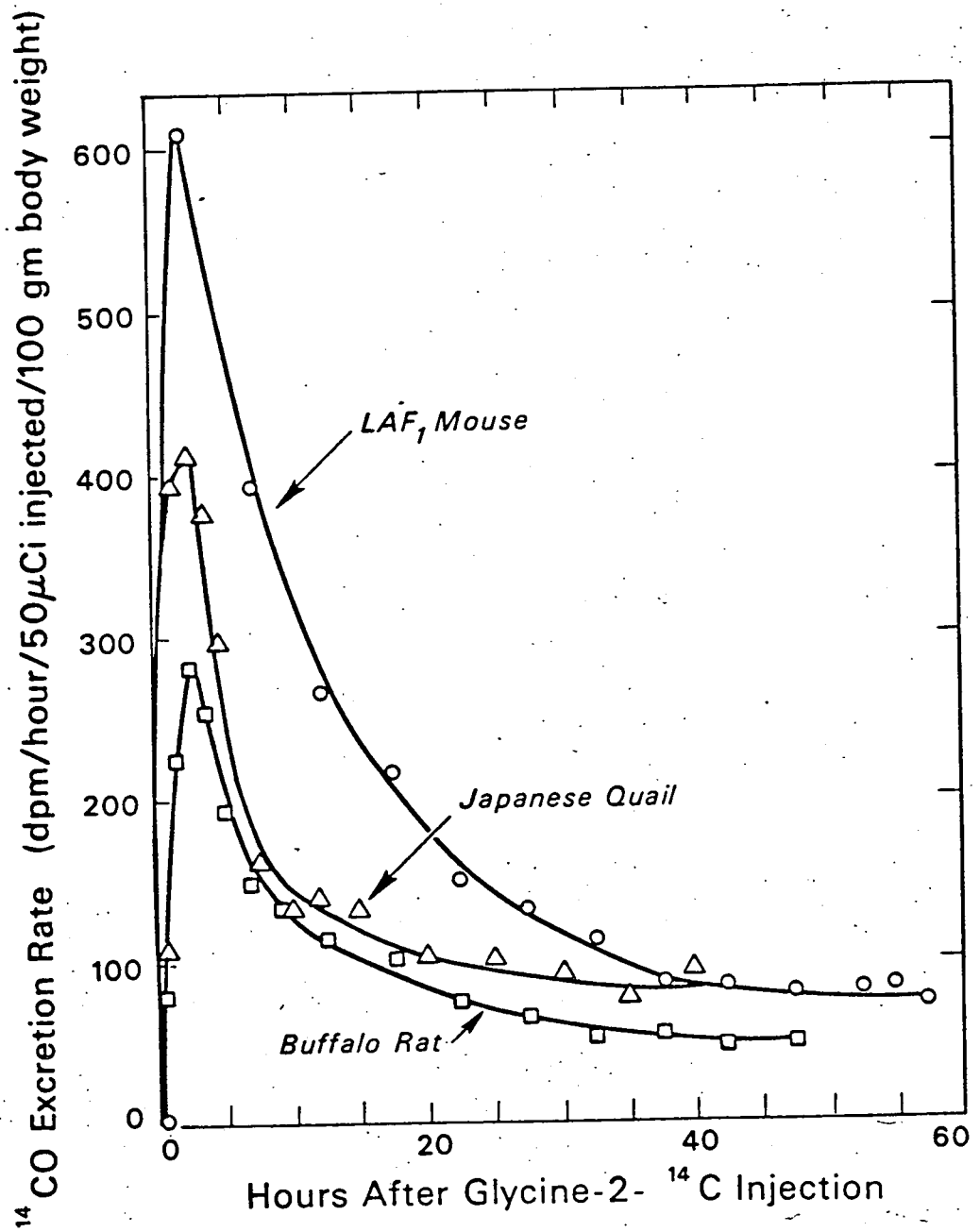
FIGURE 7: ^{14}CO "Early labeled peak" in a normal male buffalo rat (open circles, dashed line) and a gastrectomized male Sprague-Dawley rat with iron deficiency anemia (open circles, solid line). Note the presence of a secondary rise in the ELP in the iron deficient rat at about 10 to 16 hours after injection of glycine-2-^{14}C.

FIGURE 8: ^{14}CO "Early labeled peak" in a single normal female mouse (dotted line), 3 female mice homozygous for "normoblastic" (nb/nb, dashed line), and 3 female mice homozygous for "hemolytic" (ha/ha, solid line). Note the marked increase in the magnitude of the ELP in the two strains of anemic mice, with the maximum time of ^{14}CO excretion shifted from 1 to 2 hours to about 12 to 18 hours, suggesting the presence of "ineffective erythropoiesis". The difference between the ELP in the two anemic mouse strains

Figure 8 (Cont.):
is probably due to the presence of splenic destruction of reticulocytes in the "hemolytic" (ha/ha) mice.

FIGURE 9: Theoretical behavior of a cohort of red blood cells according to Equations (1) and (2) in text. Solid line indicates the total activity in red blood cell hemoglobin heme (dpm, left ordinate, Equation (1)). The solid squares indicate the proposed production rate of ^{14}CO arising from the destruction of the labeled RBC (dpm per hour, right ordinate, Equation (2)). In this case the rate of random hemolysis (k) has been set at zero, to indicate the destruction of the cohort by senescence alone. Note the gaussian shape of the ^{14}CO "late peak", with a maximum at the time of the mean potential lifespan (T). The parameter (Lambda, λ), not indicated in the text , is related to the rate of entry of RBC into the circulation, and has been set at infinity to indicate instantaneous entry of labeled cells into the circulation for the purpose of clarity.

FIGURE 10: "Late peak" for ^{14}CO in a normal buffalo rat cross-transfused with glycine-2-^{14}C-labeled RBC from a normal, compatible rat donor. The solid circles indicate collected data points, while the solid line is the least-squares best fit of the data points to Equation (2). The parameters of RBC sruvival obtained from this best-fit curve are shown in the upper left portion of the figure. Red blood cells were transfused 24 hours after injection of glycine-2-^{14}C into the initial donor.



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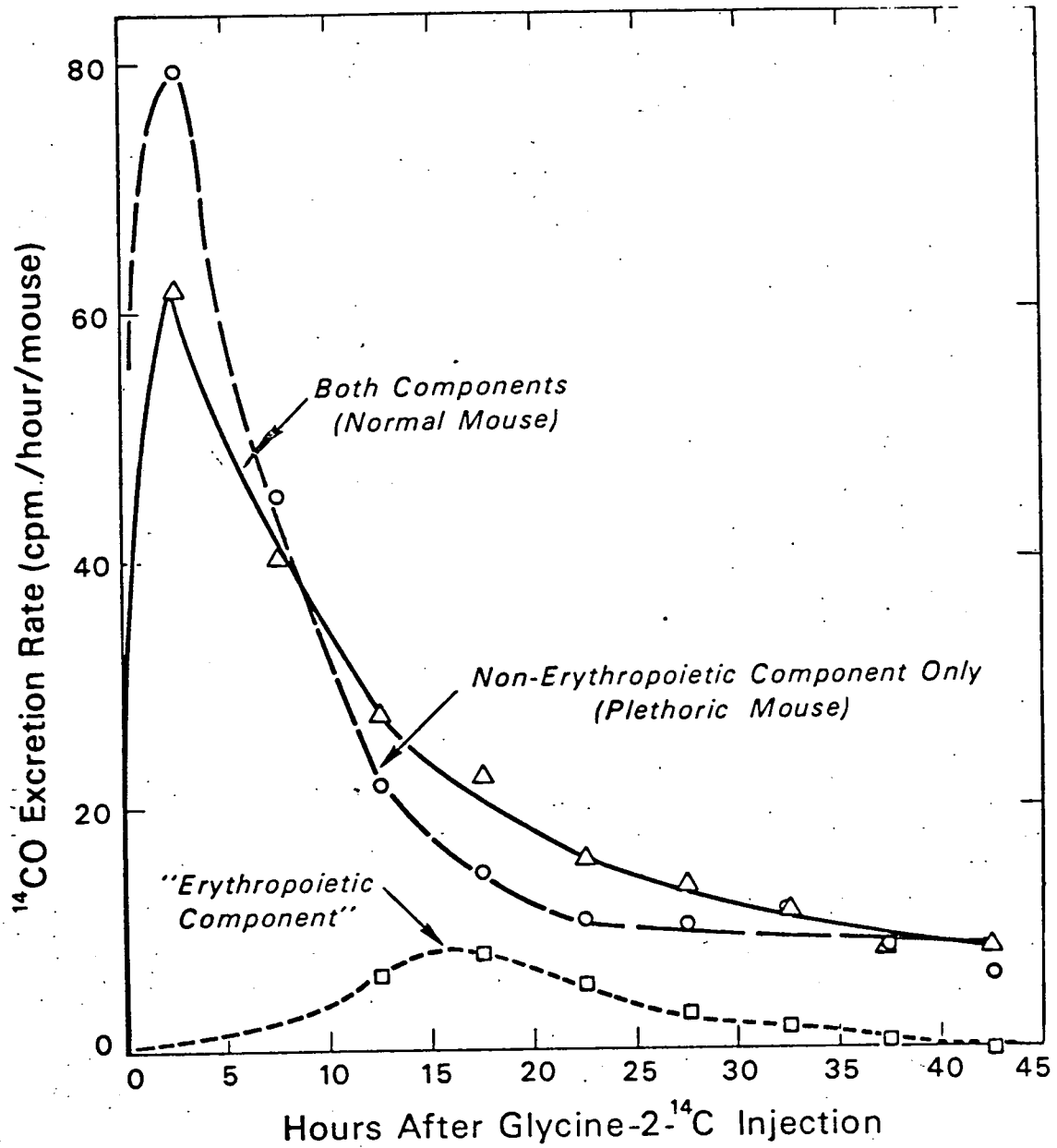

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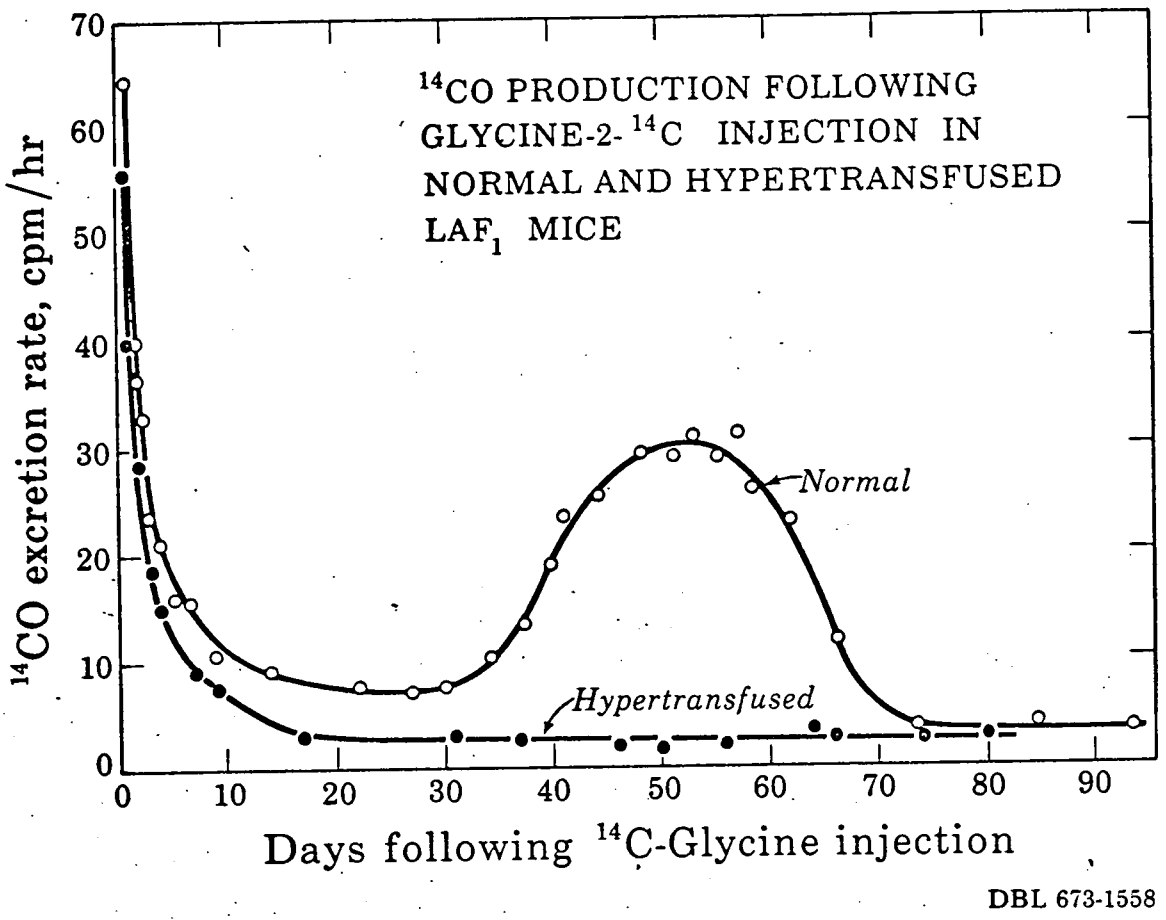
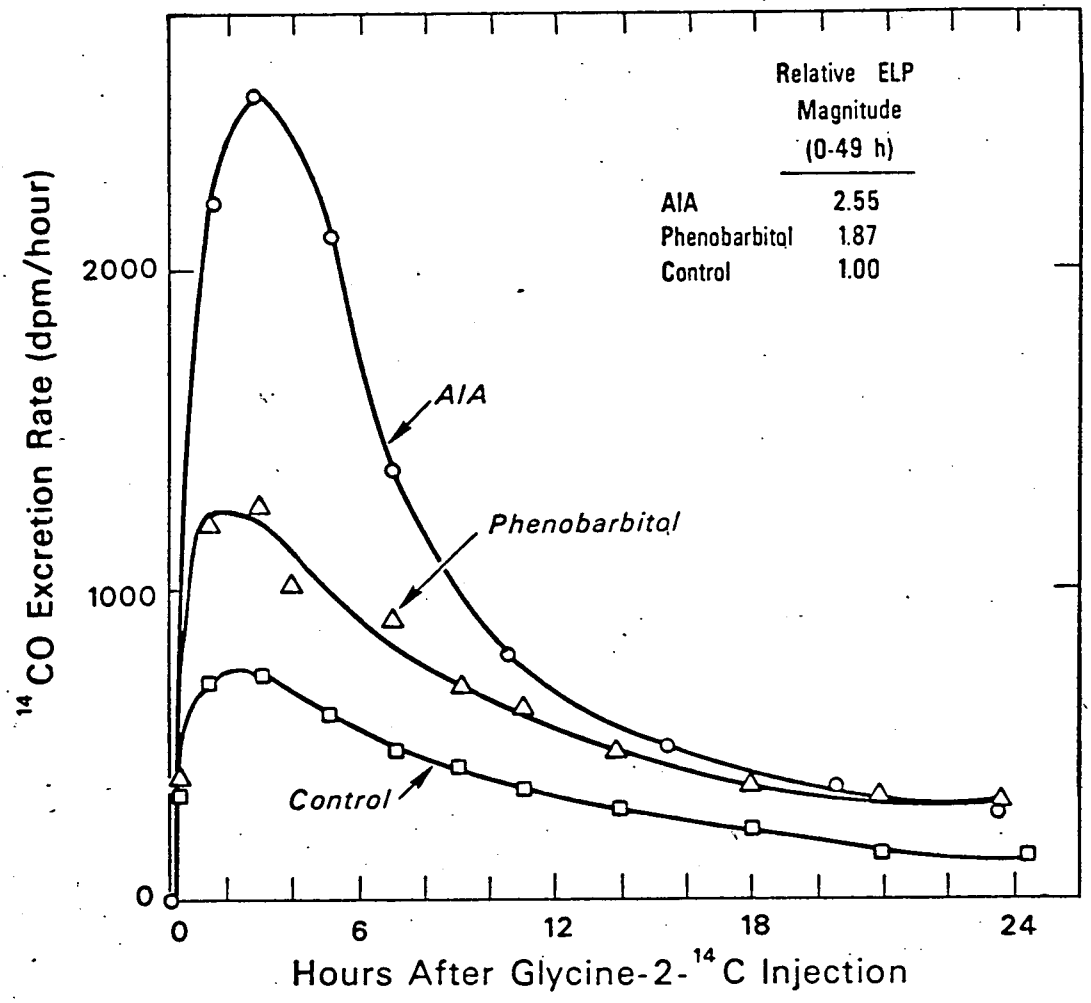

^{14}CO PRODUCTION FOLLOWING
GLYCINE-2-^{14}C INJECTION IN
NORMAL AND HYPERTRANSFUSED
LAF$_1$ MICE
Normal
Hypertransfused
^{14}CO excretion rate, cpm/hr
Days following ^{14}C-Glycine injection
0
10
20
30
40
50
60
70
80
90

DBL 673-1558



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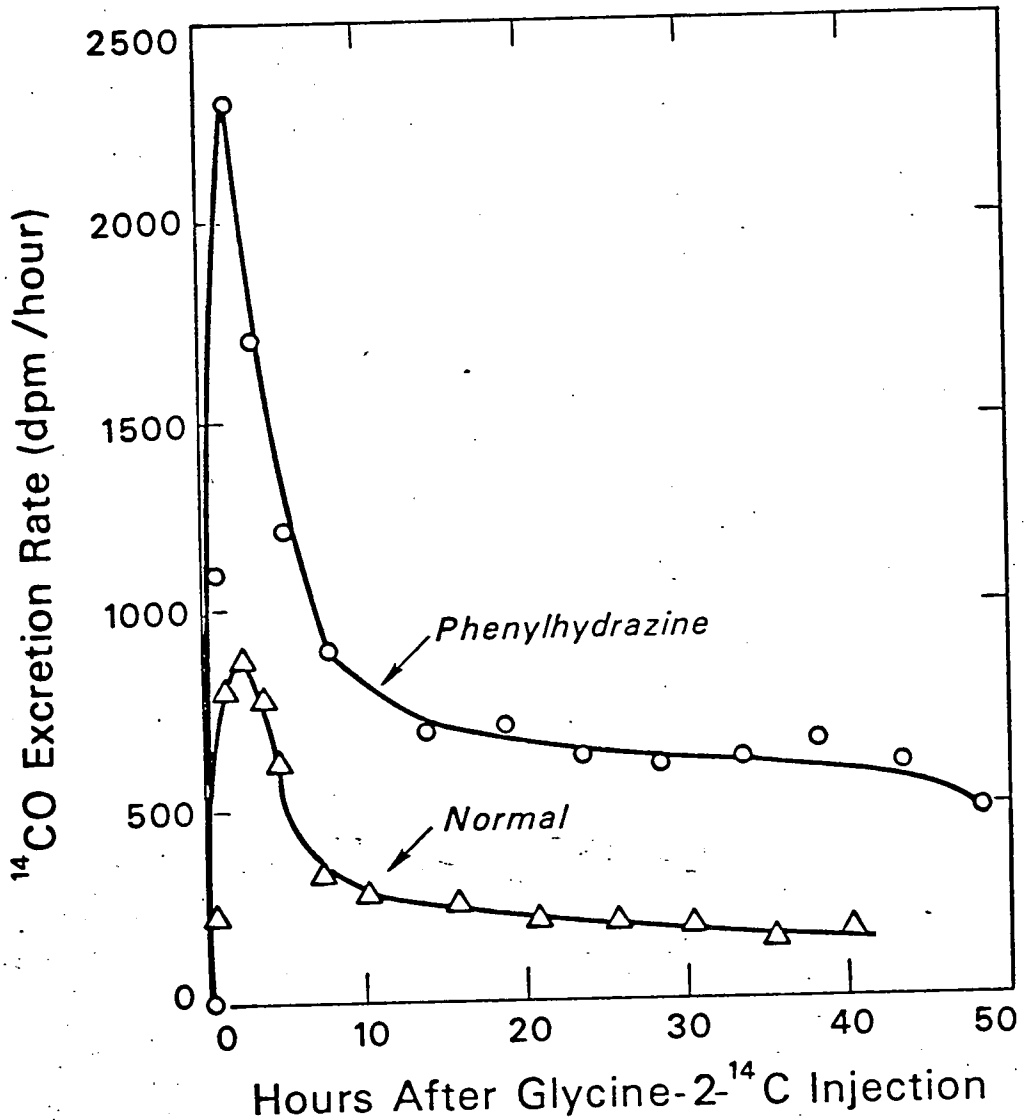

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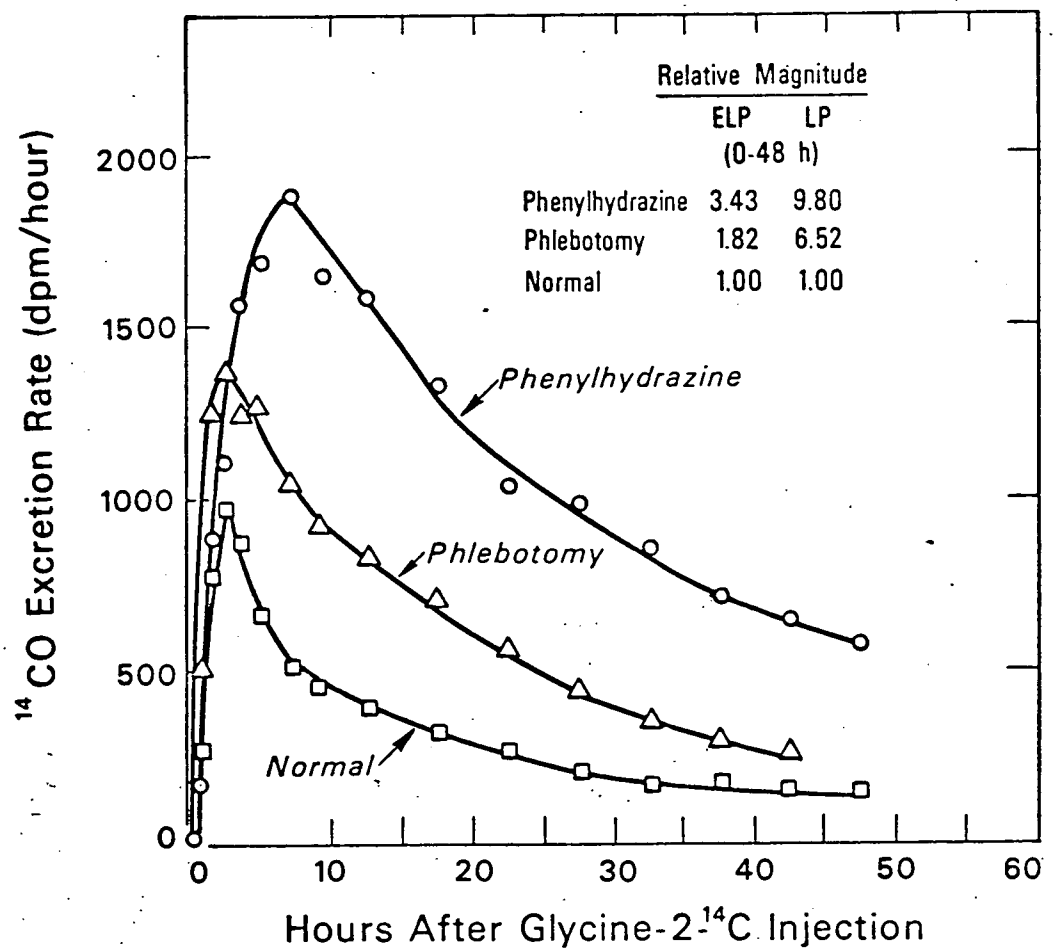

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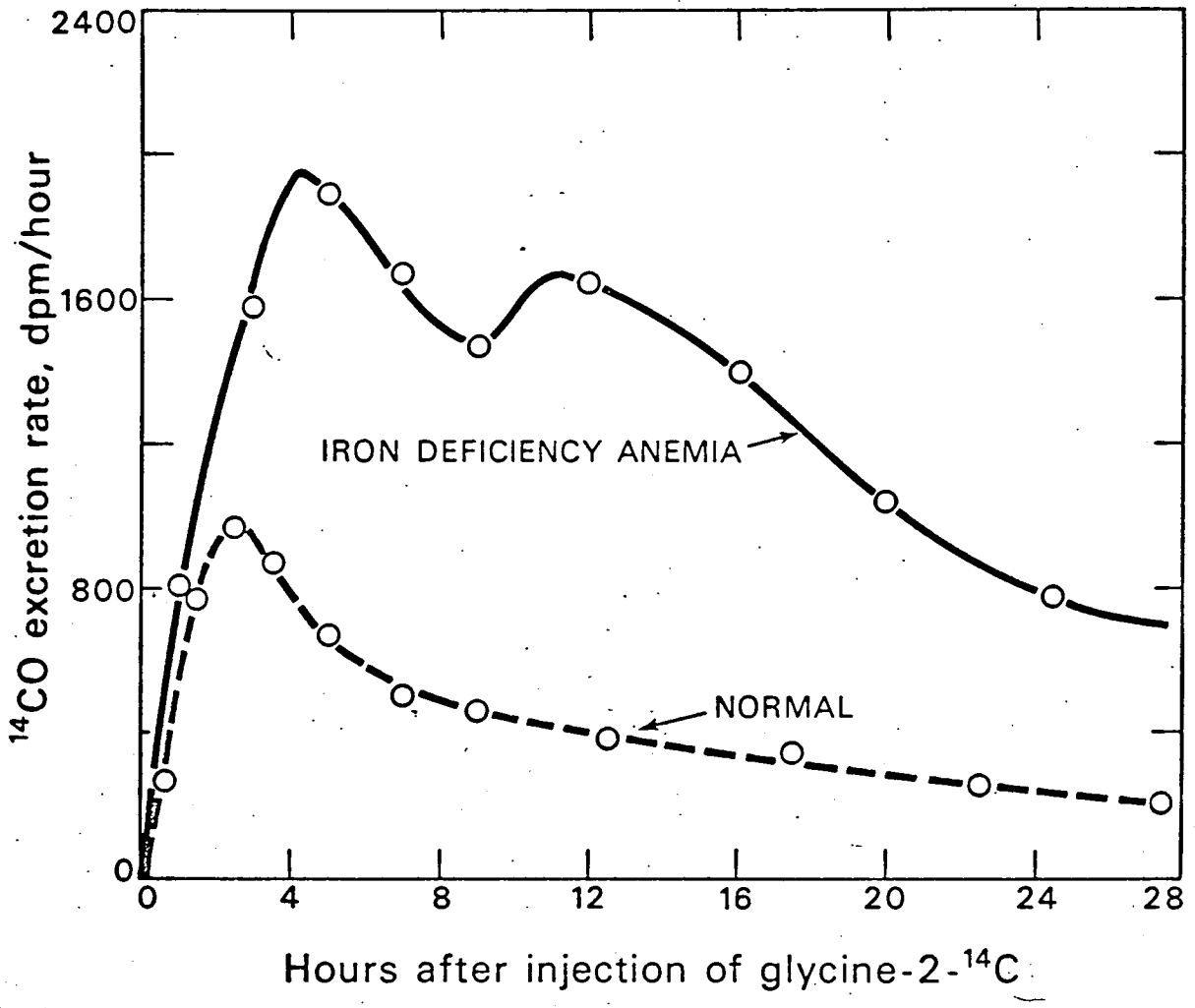
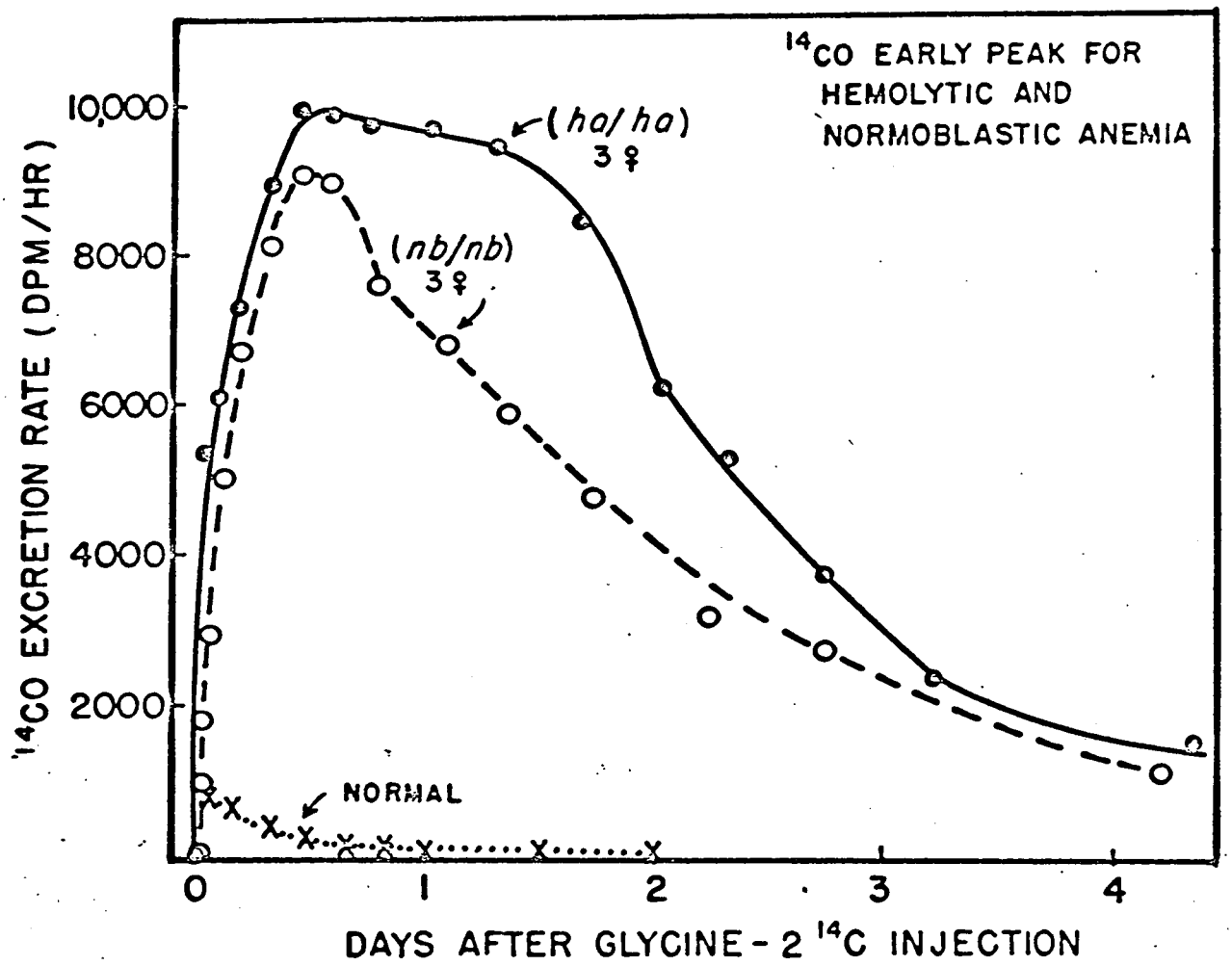
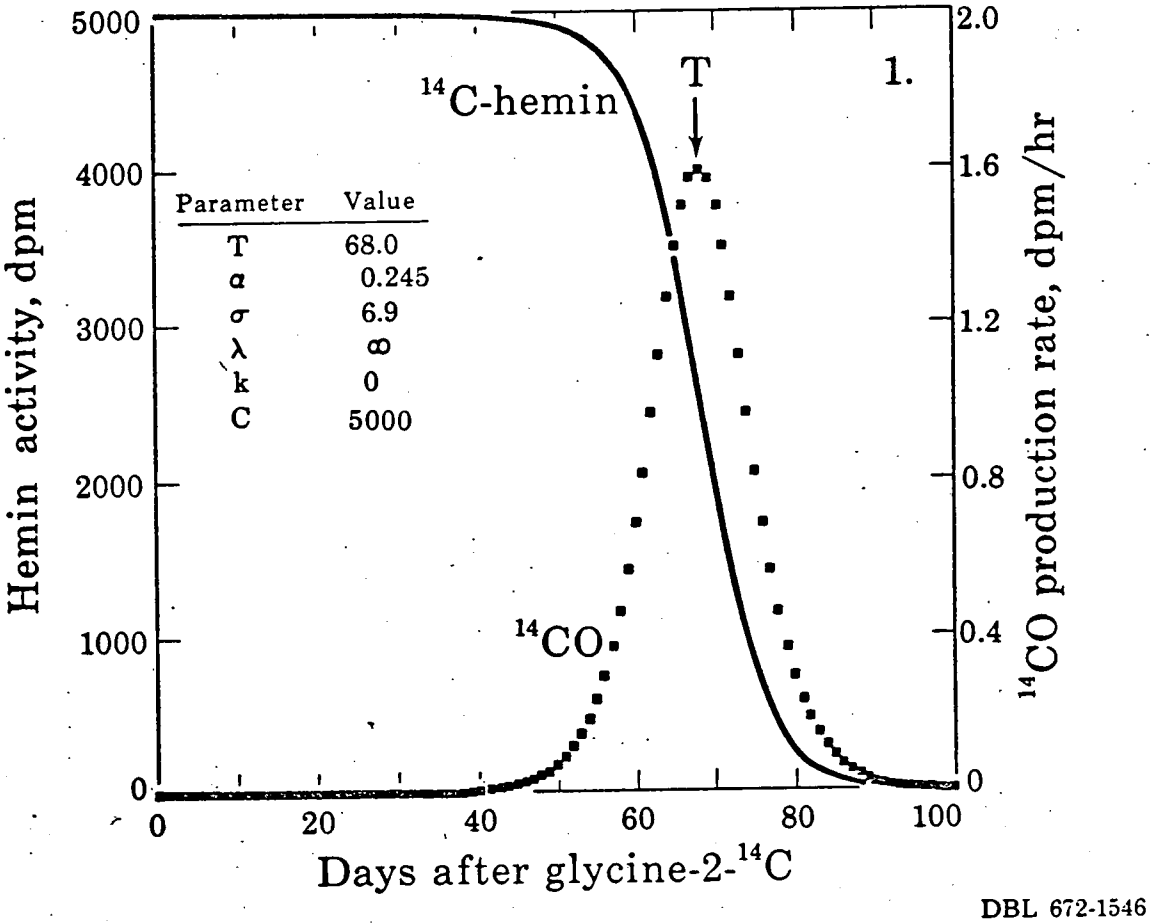
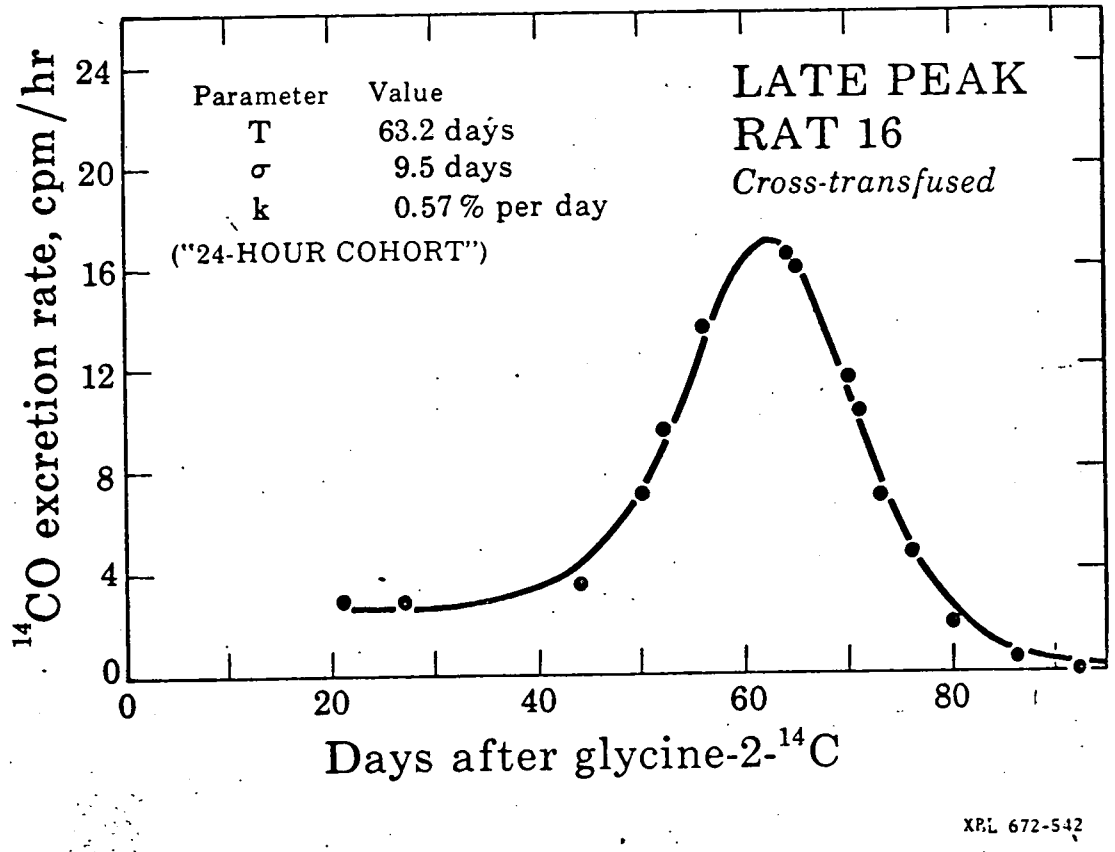

2400
1600
800
0
^{14}CO excretion rate, dpm/hour
IRON DEFICIENCY ANEMIA
NORMAL
0
4
8
12
16
20
24
28
Hours after injection of glycine-2-^{14}C

DBL 684-4658


^{14}CO EARLY PEAK FOR
HEMOLYTIC AND
NORMOBLASTIC ANEMIA
(ha/ha)
3♀
(nb/nb)
3♀
NORMAL
^{14}CO EXCRETION RATE (DPM/HR)
10,000
8000
6000
4000
2000
0
1
2
3
4
DAYS AFTER GLYCINE-2 ^{14}C INJECTION


5000
4000
3000
2000
1000
0
Hemin activity, dpm
2.0
1.6
1.2
0.8
0.4
0
^{14}CO production rate, dpm/hr
^{14}C-hemin
T
1.
Parameter Value
T 68.0
α 0.245
σ 6.9
λ ∞
k 0
C 5000
^{14}CO
0
20
40
60
80
100
Days after glycine-2-^{14}C
DBL 672-1546


LATE PEAK
RAT 16
Cross-transfused
Parameter Value
T 63.2 days
σ 9.5 days
k 0.57 % per day
("24-HOUR COHORT")
^{14}CO excretion rate, cpm/hr
Days after glycine-2-^{14}C
0
4
8
12
16
20
24
0
20
40
60
80
XBL 672-542

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