ERYTHROCYTE LIFE SPAN IN GROWING SWINE AS DETERMINED BY GLYCINE-2-C14*

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In order to interpret ferrokinetic studies in swine (1), it became desirable to determine the life span of normal porcine erythrocytes by a direct method. The purpose of this paper is to present data concerning the life span of normal erythrocytes in young growing swine as determined by measuring the isotope content of heme labelled by the intravenous injection of glycine-2-C14. Previous studies by others (2) have demonstrated that the α-methylene carbon atom of glycine is incorporated into eight of the carbon atoms of heme. Glycine-2-C14 has been used for the determination of the life span of the red cells in the rat (3) and in man (4).

Methods

Five pigs of the Chester-White breed were used in this experiment. Three (14-33, 14-34. 14-36) were from one litter and two (13-89, 13-91) were from a second litter. All the animals were housed in individual cages and were handled by the methods described previously (5). From the 21st day of age the animals were fed the following diet in an amount of 36.4 gm. (152 calories) per kg. of body weight per day: crude casein, 26.1 per cent; sucrose, 57.7 per cent; lard, 11 per cent; and swine salt mix (5), 5.2 per cent. The following vitamins, in crystalline form, were placed in capsules and administered three times a week (milligrams per kilogram of body weight per day): thiamine hydrochloride, 0.25; riboflavin, 0.12; nicotinic acid, 1.20; pyridoxine hydrochloride, 0.20; calcium pantothenate, 0.50; inositol, 0.20; para-aminobenzoic acid, 0.10; biotin, 0.10; pteroylglutamic acid, 0.10; and cobalamine, 0.10. In addition, all of the animals received the following supplements: choline chloride, 10 mg. per kg. per day; vitamin A, 3000 units per kg. per week; vitamin D, 600 units per kg. per week; vitamin E, 1 mg. per kg. per week; and vitamin K, 1 mg. per kg. per week.

Seventy-five μc. of glycine-2-C14 were injected intravenously into three of the swine (14-33, 14-34, 14-36) when they were 57 days of age and into the two other pigs (13-89, 13-91) when they were 75 days of age. Blood samples were collected from the first three animals twice a

week for the first 60 days of the experiment and once weekly thereafter until the 125th day. An accurate record was kept of the volume of blood withdrawn. Blood specimens were obtained from the other two pigs every 2 days for the 1st week and thereafter once a week until the 120th day of the experiment. The volume of packed red cells, hemoglobin, and reticulocyte count were determined in all pigs at the time each blood sample was drawn. The red cell volume was measured every 2 weeks with P^{32} by a modification (6) of the method of Reid and Orr (7).

A solution of hemoglobin was prepared from each blood sample by a modification of the method of Drabkin (8). The blood sample was centrifuged, the plasma discarded, and the red cells were then lysed with distilled water and the lipids extracted with toluene. After standing overnight in the refrigerator, the solution was centrifuged and an aliquot of the clear hemoglobin solution was taken for C^{14} analysis. In addition, crystalline hemin was obtained from blood specimens of three pigs (14-33, 14-34, and 14-36) at weekly intervals for the first 42 days and every 2 weeks thereafter until the 125th day. The crude hemin was isolated by the method of Fischer (9) and recrystallized and purified in the manner described by Shemin, London, and Rittenberg (10).

The hemoglobin solution and the hemin crystals were then combusted to carbon dioxide by the method of Van Slyke and Folch (11) and introduced into a 100 cc. ionization chamber. The rate of voltage change in the ionization chamber was measured with a vibrating reed electrometer and recording potentiometer and the specific activity of the carbon calculated (12).

Since the swine were growing, the determined specific activities of the hemoglobin solution and hemin crystals were corrected for increase in total red cell volume by the use of the following formula:—

$$\text{Sp. act.}_g^t = \frac{\text{Sp. act.}_d^t \times RCV_t}{RCV_0}$$

in which

Sp. act.$_g^t$ = specific activity corrected for increases in red cell volume at any time t

Sp. Act.$_d^t$ = determined specific activity at any time t

RCV_t = red cell volume at any time t

RCV_0 = red cell volume at the time of the initial sample

The values for pigs 14-33, 14-34, and 14-36 were further corrected by the method of Valentine et al. (13) as cited by Brown and Eadie (14) for the amount of isotope removed in the blood samples:—

$$\text{Sp. act.}_t = \text{Sp. act.}_g^t \left[\frac{\text{Sp. act.}_g^{t-1}!}{(\text{Sp. act.}_g^{t-1} - \text{Sp. act.}_r^{t-1})!} \right]$$

in which

Sp. act.$_t$ = specific activity corrected for increase in red cell volume and for isotope lost by previous blood sampling

Sp. act.$_g^t$ = specific activity corrected for increase in red cell volume at any time t

Sp. act.$_g^{t-1}$ = specific activity corrected for increase in red cell volume at previous sampling

Sp. act.$_r^{t-1}$ = isotope removed from the circulation due to the previous blood sample

RESULTS AND MATHEMATICAL INTERPRETATION

The logarithms of the corrected specific activities of samples obtained from pigs 14-33, 14-34, and 14-36, respectively, plotted against time are depicted in Figs. 1, 2, and 3. The data derived from the analyses of hemoglobin solutions

are presented on the left. The hemin data are presented on the right. The specific activity curves for the hemoglobin solution and hemin were essentially
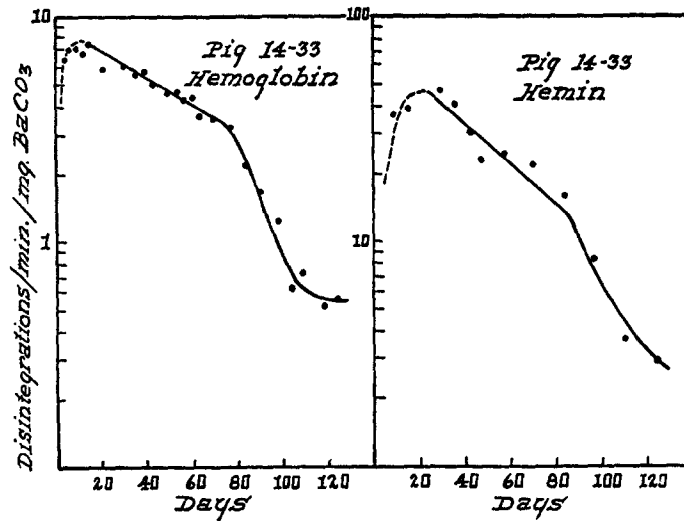

FIG. 1. Specific activity of hemoglobin solution and crystallized hemin of pig 14-33.
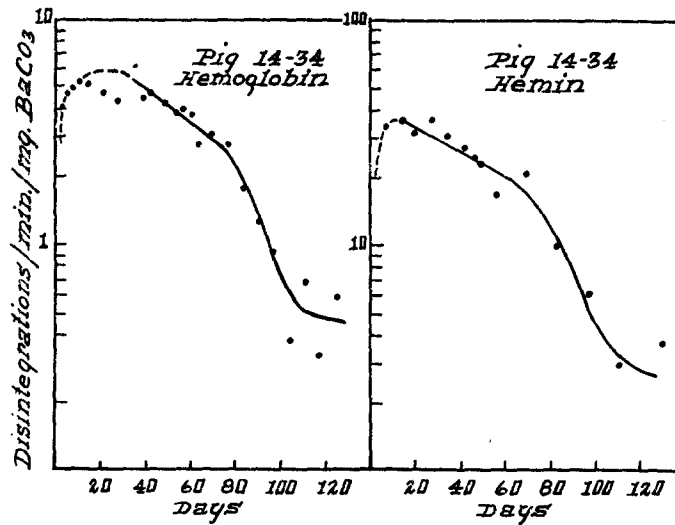

FIG. 2. Specific activity of hemoglobin solution and crystallized hemin of pig 14-34.

similar. These values have been corrected for dilution due to increase in red cell mass and for isotope lost due to phlebotomy. Fig. 4 shows similar plots for specific activity of hemoglobin solutions of pigs 13-89 and 13-91. In the

last two instances, however, the specific activities were corrected only for dilution due to increases in red cell volume.
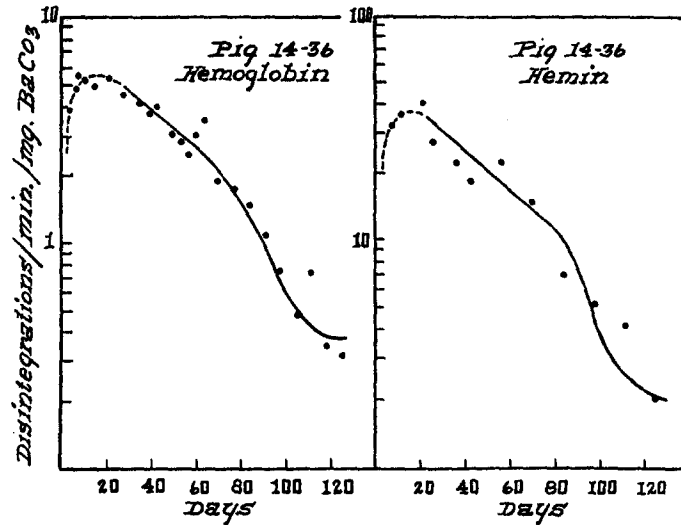

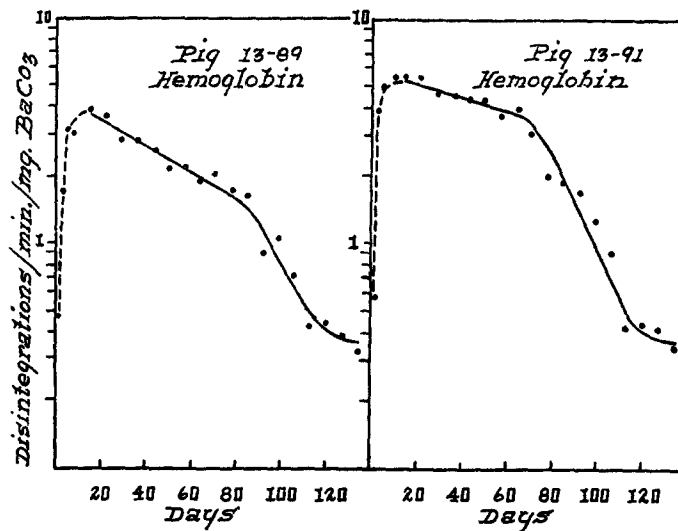FIG. 3. Specific activity of hemoglobin solution and crystallized hemin of pig 14-36.

FIG. 4. Specific activity of hemoglobin solution of pigs 13-89 and 13-91.

It will be seen that following the initial uptake phase, which is illustrated by the dotted line, there was an exponential decrease in specific activity which was terminated by a more precipitous decline. This suggests that the erythro-

cytes of growing swine are destroyed by more than one process. The exponential phase may be ascribed to random destruction, whereas another process which is age-dependent is evidenced by the precipitous terminal decline in specific activity.

Brown and Eadie (14) encountered a similar situation in the rabbit and the cat and they described a mathematical method for handling such data. The number of cells alive at a given time t is expressed as:—

$$N_t = \frac{N_0(1 - b)e^{-kt}}{1 + e^{\alpha(t-T)}} + N_0 b$$

in which

N_t = the number of cells intact at a given time t

N_0 = the number of cells intact at time $t = 0$

b = the constant fraction of the label which is reincorporated into new cells following destruction of the originally labelled cells

k = coefficient of random destruction

α = coefficient of uniformity around T of life span of the cells destroyed by senescent process

T = average potential life span of cells destroyed by senescent process

The nature of this expression permits the ready evaluation of the constants. When t is large the denominator becomes very large and e^{-kt} is small. Thus, the value of N_0, following the rapid senescent decline, is taken to be equal to $N_0 b$. When t is small $e^{\alpha(t-T)}$ is small and

$$N_t = N_0(1 - b)e^{-kt} + N_0 b$$

When the logarithm of $(N_t - N_0 b)$ is plotted against time the points will fall on a straight line, the $Y-$ intercept of which is ln $[N_0(1 - b)]$ and the slope of which is $-k$. From this, with a knowledge of $N_0 b$, the values of N_0 and b may be obtained separately. The data were interpreted in this region by the method of least squares with the appropriate weighting factor, Y_i^2 (15), due to the weighting implied by the logarithmic transformation. The variance was assumed to be constant over the linear region before the onset of the senescent phase. Thus, the method of least squares gives the following expression from the coefficient of random destruction (16):—

$$-k = \frac{\Sigma Y_i^2 \Sigma Y_i^2 t_i \ln Y_i - \Sigma Y_i^2 t_i \Sigma Y_i^2 \ln Y_i}{\Sigma Y_i^2 \Sigma Y_i^2 t_i^2 - [\Sigma Y_i^2 t_i]^2}$$

$$\ln [N_0(1 - b)] = \frac{\Sigma Y_i^2 t_i^2 \Sigma Y_i^2 \ln Y_i - \Sigma Y_i^2 t_i^2 Y_i^2 t_i \ln Y_i}{\Sigma Y_i^2 \Sigma Y_i^2 t_i^2 - [\Sigma Y_i^2 t_i]^2}$$

in which

$$Y_i = N_t - N_0 b$$

In order to determine T and α, values of

$$\ln \left[\frac{N_0(1 - b)e^{-kt}}{N_t - N_0 b} - 1 \right]$$

were plotted against time. The slope of the straight line so obtained is α, and the Y intercept is $-\alpha T$. By simple division of αT by α, T is obtained. As large values of t are approached, the specific activities approach background levels, and the variance becomes large. Thus, the

assumption of constant variance is no longer valid, and the slope and Y intercept must be evaluated graphically.

The results of the mathematical interpretations of the red cell survival data derived for the five growing pigs are summarized in Table I. It is apparent that the coefficients of random destruction differed appreciably among the pigs studied. This would indicate that the rates of random destruction are quite variable among young growing swine. There is also some difference between

TABLE I

Pig no.	Measurement	Coefficient of random distribution k	Coefficient of uniformity of life span α	Fraction reutilized b	Average potential life span T	"Corrected" average potential life span	"Mean" survival time	Cells destroyed randomly
					days	*days*	*days*	*per cent*
14-33	Hemoglobin solution	0.015	0.16	0.058	91	88	65	90
	Hemin	0.021	0.12	0.035	99	85	56	96
14-34	Hemoglobin solution	0.019	0.14	0.045	91	80	67	95
	Hemin	0.014	0.12	0.057	89	82	65	87
14-36	Hemoglobin solution	0.023	0.11	0.038	91	86	54	98
	Hemin	0.021	0.16	0.036	95	88	56	96
13-89	Hemoglobin solution	0.015	0.11	0.075	100	95	63	91
13-91	Hemoglobin solution	0.007	0.10	0.062	86	80	72	60
Mean................		0.017	0.13	0.051	93	86	62	89

the coefficients of random destruction of the hemoglobin solution and hemin plots on the same pig. This is probably due to the relatively small number of hemin samples analyzed and the errors inherent in C^{14} determination. This variation is not consistent in its pattern. Hence, it would appear likely that any label accounted for in the hemoglobin solution is handled in a manner identical to that in the heme moiety.

In all the swine there was a precipitous decrease of specific activity toward the end of the cell life span. On linear plots, this has a sigmoid shape, and has been interpreted as representing a phase of cell decline which is due to an age-dependent process. The parameter that describes the variation in life span of red cells around T is α. Had only cells in the same stage of develop-

ment been labelled instantaneously, the coefficient would be larger and primarily a measure of the distribution of cell thresholds to senile death. However, the magnitude of α is a resultant of the interaction of the two processes since incorporation of isotope proceeds over a definite time interval.

A constant fraction of the isotope liberated when cells die by either a random or age-dependent process is assumed to be reincorporated into newly formed erythrocytes. The mean value for the constant obtained by this treatment was 0.051, a value that is one-third as great as that reported in the cat and rabbit by Brown and Eadie (14) who employed radioactive iron to tag the cells and followed this with injections of non-radioactive iron to limit reincorporation of the isotope. The "reutilization constant" obtained by this treatment may actually be excessive. Berlin (17) found that when C^{14}-labelled hemin was injected intraperitoneally into rats, less than 0.01 per cent of the carbon was reutilized. In addition, Berlin, Hewitt, and Lotz (18) presented evidence that after the injection of glycine-2-C^{14} into man, labelled glycine persisted over a time interval sufficiently prolonged to account for the terminal plateau. Thus, the apparent "reutilization" of C^{14} encountered in this study may be due in a large part to continued synthesis of hemoglobin from labelled glycine which persisted from the time of administration.

The average potential life span represents the average life span of cells that were destroyed by an age-dependent process. This value was "corrected" by subtracting the time required for greater than 80 per cent of the label to appear in the erythrocytes.

The "mean survival time," determined by the method of Neuberger and Niven (19), represents the time interval between the appearance and disappearance of cells containing half of the maximum label determined at the appropriate time during the study.

The last column in Table I lists the per cent of labelled cells destroyed by a random process during a time interval of 125 days. This value is somewhat excessive since it is calculated as though 100 per cent of the label were present at zero time.

DISCUSSION

The coexistence of a random and an age-dependent process of erythrocyte destruction in apparently normal growing swine was an unexpected finding. In health the life span of the red corpuscles of man (20), rat (3), cat (13), and dog (14, 21) seems to be terminated almost entirely by a destructive process that operates on cells that have aged to a certain stage. The only exceptions to this were found by Brown and Eadie (14) in the cat and the rabbit and by Neuberger and Niven (19) in the rabbit. Using radioactive iron (Fe^{55}) to label the erythrocytes, Brown and Eadie (14) showed that the red cells of the cat and the rabbit are exposed to both a random and an age-dependent process.

On the other hand, Valentine *et al.* (13), by the use of N^{15}-labelled glycine, found that the erythrocytes of the cat are destroyed solely by an age-dependent process. The explanation for the discrepancy between the findings of these two groups of workers is not apparent.

No good explanation is available for the presence of the two processes in swine. The animals were healthy and normal as evidenced by normal growth, and normal values for hemoglobin, red cells, reticulocytes, and volume of packed red cells. The possibility of C^{14} turnover through a non-heme compartment of the red cell is ruled out by the similarity of the curves and constants for hemoglobin and heme specific activity in the swine in which the specific activity in both of these substances was measured. Neuberger and Niven (19) showed by the use of N^{15}-labelled glycine that the life span of erythrocytes which are rapidly produced in rabbits subjected to acute hemorrhage is considerably shortened. Berlin and Lotz (22) confirmed this work in the rat by the use of glycine-2-C^{14}. In addition, they showed that the cells produced after hemorrhage are destroyed in an exponential manner. It is tempting to speculate that the random destruction observed in this study was due to the rapid production of erythrocytes necessitated by the rapid rate of growth of young swine. It is unfortunate that it was not possible to study the life span of erythrocytes in mature swine.

The "corrected" average potential life span of porcine erythrocytes, which represents the life span of the cells destroyed by an age-dependent process, was 86 ± 11.5 days. The mean cell survival time, which is the figure which should agree most closely with the survival time derived from ferrokinetic data, was 62 days. This is in close agreement with the mean erythrocyte survival as determined by the ferrokinetic method (1).

SUMMARY

Red blood cell survival studies were performed on five normal growing swine by following the C^{14}-specific activity of hemoglobin and heme after the administration of glycine-2-C^{14}. The erythrocytes of normal growing swine appear to be destroyed both by a random and an age-dependent process. Random destruction accounts for the larger portion of the cells which are destroyed.

The "mean" red cell survival time was 62 days. This represents the interval from the time of incorporation of 50 per cent of the maximal amount of labelling achieved to the time when the level had decreased once more to the 50 per cent amount. The " 'corrected' average potential life span" of the red cells was 86 ± 11.5 days. This figure was obtained by subtracting the number of days required to attain 80 per cent of the maximal labelling from the average survival time of red cells destroyed by an age-dependent process as distinguished from random destruction.

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