AN ANALYTICAL STUDY OF IN VIVO SURVIVAL OF LIMITED POPULATIONS OF ANIMAL RED BLOOD CELLS TAGGED WITH RADIOIRON*

By I. W. BROWN, Jr., and G. S. EADIE

(From the Departments of Surgery and Physiology, Duke University School of Medicine, Durham)

(Received for publication, September 15, 1952)

Until recently, only a few studies (1–6) of red cell life span in a limited number of animal species had been made. These early studies, made by a variety of methods, gave estimates of red cell life span for certain species that varied widely (*e.g.* rabbit 8 to 42 days, dog 20 to 124 days). Recently, Finch and coworkers (7) employing radioiron, and Bale *et al.* (8) employing C^{14} have estimated the life span of the dog red cell to be 107 to 110 and 115 days, respectively. Harrison and associates (9) using radioiron, and Neuberger and Niven (10) using N^{15}-labeled glycine estimated the life span of rabbit red cells to be 62 to 75 days. Valentine and others (11) using N^{15} have estimated the mean life span of the cat red cell at 77 days.

The use of isotopes in tagging erythrocytes for *in vivo* survival studies is subject to certain criticism. Previous investigators (8, 12, 13) have shown that there is no apparent loss or exchange of the isotopes of iron, nitrogen, or carbon when once incorporated into the hemoglobin molecule. However, radioiron is rapidly and almost completely reutilized following red cell breakdown unless suppressed by the competition of greatly increased body stores of non-radioactive iron and continuous high levels of non-radioactive total serum iron (7, 14). N^{15} incorporated in the protoporphyrin of heme and C^{14}-tagged globin are supposedly not reutilized in the formation of new hemoglobin. Nevertheless, red cell survival curves based on these technics, published to date (8, 10, 11, 13, 15), show considerable amounts (10 to 30 per cent of maximal uptake) of both N^{15} and C^{14} persisting in circulating red cells for periods up to 200 days. In addition, the relatively slow uptake and early decline in the N^{15} level (40 to 60 days) lead to great difficulty in estimating with an accuracy the average life span and variations in red cell life.

Recent investigations (7–10) have revealed varying degrees of random destruction of red cells occurring in apparently healthy normal animals—a fact which further complicates *in vivo* survival studies. While isotope reutilization and random destruction

* This investigation was supported by a grant from the National Institutes of Health, United States Public Health Service, and by a grant from the Spanel Foundation.

of cells have not been considered in previous survival studies of animal red cells, it is obvious that these factors, when present, would definitely influence the terminal slopes of survival curves obtained.

Admittedly, the Ashby technic of differential agglutination (16, 17) probably remains superior to all other methods for determining red cell survival. Unfortunately, most animal blood-grouping sera, except for a few sera recently produced and now under study (18, 19), have been incapable of causing sufficiently cohesive agglutination to permit this latter technic to be used.[1] Thus it would appear that the use of radioiron, in conjunction with non-radioactive iron administered intravenously to suppress radioiron reutilization as originally suggested by Finch (7), is perhaps as practical as any of the methods currently available for studies of animal red cell survival.

The present interest in red blood cell preservation indicates a need for employing animals in initial *in vivo* survival studies of red cells processed and preserved by certain methods. This has prompted us to make new studies in normal dogs, cats, and rabbits. We have studied in these species red cell populations of limited age tagged with radiorion in an attempt to more accurately define the average red cell life span, variations of red cell life, random destruction, and the fraction of radioiron reutilized.

Materials and Methods

Animals.—Both sexes of three species of animals which had reached full growth and stable body weights were used. All were housed in individual cages. Mongrel dogs weighing 8 to 12 kilos were maintained on a stock diet of Purina dog chow supplemented with cooked horse meat and bone scraps. The dogs were immunized to canine distempter and examined frequently for any signs of infection, systemic disease, and parasitic infestations including mange and blood larvae of *Dirofilaria immitis*. All dogs were dewormed of intestinal parasites just prior to the experiment and periodically thereafter.

New Zealand white rabbits weighing 3 to 4 kilos and maintained on Purina rabbit chow supplemented with oats and greens were used. All rabbits remained free of ear mites, eye infections, "snuffles," and other signs of disease.

Cats weighing 3 to 4 kilos were employed and maintained on a diet of commercial ("Daily")[2] cat food supplemented with cooked horse meat, raw liver, and milk. All cats were immunized to feline agranulocytosis and remained free of intestinal parasites and other infestations and infections throughout the experimental period. At the conclusion of the experiment all animals were sacrificed and examined by autopsy. Tissue sections for microscopic study were made of various organs from most animals in order to confirm the absence of lesions or disease.

[1] In 1946 Jope (20) reported studies that indicated sulfhemoglobin disappeared from the blood only upon decay of the red cells containing it. Jope suggested sulfhemoglobin as a chemical tag for following red cell survival. Recently, we have made extensive studies, to be reported elsewhere, which fail to support Jope's observations and suggestions.

[2] Obtained from the Great Atlantic and Pacific Tea Company.

Technical Methods Employed.—Red cell volumes were determined by the cell dilution technic employing Fe^{59}-tagged (21) red cells from compatible donors of the same species, or by the Evans blue dye–hematocrit method modified after Gibson and Evans (22). When employing the Evans blue dye procedure, a correction factor was used. This was determined for each species by the radioiron-tagged cell dilution technic in order to correct for the difference in venous and total body hematocrit readings. Dye determinations were made on the Beckman DU or Coleman Jr. spectrophotometers. All hematocrit readings were determined by centrifugalizing at 3000 R.P.M. at a relative centrifugal force (R.C.F.) of 1800 at constant temperature (5°C.) for 30 minutes. Special calibrated rubber-tipped hematocrit tubes were employed. These tubes allow the packed red cells from 0.5 cc. to 5 cc. aliquots of whole blood to be determined to the nearest 0.01 cc. and recovered quantitatively and separately from the supernatant plasma. A correction factor for entrapped plasma, determined for our centrifuging conditions by the I^{131} method of Leeson and Reeve (23), was applied to all hematocrit readings. Hemoglobin was determined by the method of Evelyn and Malloy (24) with readings made spectrophotometrically.

All dogs used in transfusion experiments were grouped according to the ABCD dog blood groups of Young (25, 26), to the XYZ dog blood groups of Hamilton (27), and to other immune dog isoantibodies produced in this laboratory (28). Only donor dogs of compatible blood groups were chosen, and donor and recipient dogs were cross-matched before transfusion according to Young's procedure (25) and by the indirect anti-globulin test employing an anti-dog globulin rabbit serum. Similar pretransfusion blood grouping and compatibility studies were made prior to rabbit and cat transfusions. The blood groups of the rabbits and cats were determined by immune blood grouping antisera produced in this laboratory (28). Included among the rabbit blood grouping antisera were rabbit sera containing antibodies identical in specificity to those recently described by Kellner (29) as rabbit "anti-G" and anti-g." Cross-matching of donors and recipients of these species was performed using albumin and serum-suspended red cells and by the indirect anti-globulin test using anti-rabbit globulin cat sera (for rabbits) and anti-cat globulin rabbit sera (for cats).

All recipient animals' sera were tested frequently throughout the experimental period for the presence of immune isoantibodies which might have been stimulated by the transfused blood. Donor blood for transfusion was drawn into cold (1–2°C.) ACD solution in silicone-treated glass containers submerged in an ice bath which immediately chilled the shed blood to 1–2°C. Dog donor blood was stored for 12 hours at 2–5°C. before transfusion, but all transfusions in the other species were administered immediately after the blood was withdrawn from the donor animals.

Radioiron[3] Fe^{55}, of high specific activity (522.7 mc. as Fe^{55}/gm. of Fe and containing less than 0.196 mc. as Fe^{59}/gm. of Fe), was employed in all the survival studies. The Fe^{55} radioactivity of all red blood samples was assayed by a Geiger-Mueller tube sensitive to soft x-rays and shielded to low energy beta irradiation[4] (Fe^{59}) by a beryl-

[3] Obtained from the Oak Ridge National Laboratory.

[4] When Fe^{59} was employed to determine total red cell volume, samples were counted by a thin window Geiger-Mueller tube sensitive to low energy beta irradiation.

lium absorber 150 mg./cm.^{2} in thickness. The efficiency of this tube with the absorber in detecting soft x-rays was approximately 2.0 per cent.

The total red cell volume removed with each blood sample was wet ashed, the hemoglobin iron electroplated, and the radioactivity determined after the method of Peacock *et al.* (30) with some slight modifications and refinements in technic. A standard of the original radioiron injected was prepared and counted with each sample assayed in order to correct for radioactive decay and error due to any slight changes in scalar sensitivity.

Experimental Procedure.—After initial determinations of red cell volume, each animal (excepting those used as transfusion recipients) was given intravenously Fe^{55} in the form of dilute ferric chloride. The dosage of Fe^{55} employed was as follows: dogs 0.06 mg. of Fe representing 7.8×10^5 C.P.M.[5] or approximately 55 μc., rabbits and cats 0.02 mg. of Fe representing 2.6×10^5 C.P.M. or approximately 18.3 μc. Frequent blood samples, representing usually 1 to 2 cc. of packed red cells, were taken from all animals during the initial period of radioiron uptake and later during the period of decline of circulating red cell radioactivity level with less frequent sampling in the interval. The total volume of red blood cells removed at each sampling was determined for each animal using the special centrifuge tubes and hematocrit procedure described previously. The total red cell volume removed at each sampling was used for the radioiron assay. Thus, the amount of circulating red cell radioactivity removed at each sampling, expressed as a ratio of C.P.M. removed in the sample to C.P.M. of the Fe^{55} standard, could be determined by the following equation.

$$\frac{\text{C.P.M. in red cells removed in sample}}{\text{C.P.M. of } Fe^{55} \text{ standard}}$$

The total circulating red cell radioactivity at the time of each sampling, expressed as the ratio of the C.P.M. in the total circulating red cells to C.P.M. of the Fe^{55} standard to correct for radioactive decay, was determined by the following equation:

$$\frac{\text{C.P.M. per cc. red cells in sample} \times \text{animals total red cell volume in cc.}}{\text{C.P.M. of } Fe^{55} \text{ standard}}$$

Because the amount of red cell radioactivity removed by sampling was not insignificant with respect to the total circulating red cell radioactivity, it was necessary to correct for this. The correction used was that suggested by Valentine *et al.* (11) which assumes that the animals' red cell volumes remain relatively constant throughout the experiment. If a_1 is the total circulating red cell radioactivity at the time of the first sample, and if b_1 is the red cell radioactivity removed in that sample, then the total circulating red cell radioactivity at the time of the second sample, a_2, must be divided by

$$\frac{a_1 - b_1}{a_1}$$

[5] C.P.M. here and elsewhere refers to counts per minute of radioactivity determined by the Geiger-Mueller tube employed in these experiments as described under Methods.

Similarly the total circulating red cell radioactivity shown by the third sample must be divided by

$$\frac{(a_1 - b_1)(a_2 - b_2)}{a_1\, a_2}$$

etc.

Once the curve of rapid radioiron uptake neared its plateau, each animal was given intravenously large doses of non-radioactive iron in the form of saccharated iron oxide (proferrin).[6] Dogs were given 100 mg. of Fe as proferrin daily for the first 10 days after the initial uptake period, then 3 to 4 times weekly throughout the remainder of the experimental period. The same procedure was followed in the rabbits and cats except that the individual doses employed in these species was 40 mg. of Fe as proferrin. Recipient animals used in transfusion experiments were also given proferrin intravenously. The same doses for each species as indicated above were given 3 to 4 times per week for at least 3 weeks prior to transfusion and continued thereafter throughout the experimental period.

RESULTS AND DISCUSSION

Theoretical Considerations

The mathematical analysis of total circulating red cell radioactivity data thus obtained must take account of three factors: senescence, random destruction, and reutilization. Random destruction is assumed to be a process which continuously removes a constant fraction of the cells present at any moment, irrespective of age or other characteristics. Kinetically it resembles a chemical first order reaction. Thus, if we start out with N_o isotopically tagged cells of approximately the same age, the number, N, remaining at a time t will be given by the equation

$$N = N_o\, e^{-kt} \tag{1}$$

in which k is the constant of random destruction.

The second assumption is that there is a "normal" distribution of life spans around an average of T days. To formulate this the "probability integral" may be used, following Shemin and Rittenberg (13), but the Verhulst-Pearl (31) growth function is simpler and describes the facts equally well. Neither formula has, of course, any theoretical basis. The growth function gives, in this case, the fraction of the original number of tagged cells that will remain at a time t under this assumption. If there were no random destruction this would be a fraction of the number of cells originally tagged, but under conditions in which there is random decay it is the fraction of cells remaining after random destruction. Hence

$$N = \frac{N_o\, e^{-kt}}{1 + e^{a(t-T)}} \tag{2}$$

[6] Supplied by Sharp and Dohme, Inc.

T is the average life span. α may be called a constant of uniformity: the greater its numerical value, the narrower is the spread around the average life span. This equation, however, would apply only if there were no reutilization of the radioiron into newly formed red cells.

The third assumption is that under the conditions of these experiments a constant fraction, b, of the tagged iron is reutilized in new red cell formation when it is released by red cell destruction. This could only be expected when conditions with respect to iron metabolism are relatively constant. In order to achieve this, in each of our experiments the serum and body non-radioactive iron was maintained at high levels by continual intravenous injections. Although it was clear that the degree of reutilization of radioiron varied somewhat from animal to animal and particularly with the amount of non-radioactive iron administered, there was nothing to indicate that it was not constant in the same animal under the same conditions.

By differentiating Equation 2 we obtain the rate of destruction of tagged cells and therefore the rate of release of radioactive iron.

$$\frac{dN}{dt} = -N_o\, e^{-kt}\, \frac{k + (k + \alpha)e^{\alpha(t-T)}}{[1 + e^{\alpha(t-T)}]^2} \tag{3}$$

The net rate of loss of tagged iron is assumed to be $(1 - b)$ times this. Multiplying by this factor, and integrating,

$$N = \frac{N_o\,(1 - b)e^{-kt}}{1 + e^{\alpha(t-T)}} + N_o\, b \tag{4}$$

since when t is zero N equals N_o, e^{-kt} is 1, and $e^{\alpha(t-T)}$ is negligibly small.

Certain properties of this expression make it possible to fit the data to it and obtain values for the constants. When t is large e^{-kt} is very small and the denominator very large, so that N tends to reach the limit $N_o b$. In practice, N for times immediately following the sharp, S-shaped portion of the curve may be taken as equal to $N_o b$. The early part of the curve, during the initial falling phase, represents a period when $e^{\alpha(t-T)}$ does not differ appreciably from zero, and the equation consequently simplifies to

$$N = N_o\,(1 - b)e^{-kt} + N_o\, b \tag{5}$$

If values for this period are used and the logarithm of $(N - N_o b)$ is plotted against time, the points will fall on a straight line whose slope is k and whose intercept on the Y-axis is log $N_o(1 - b)$. From this, knowing the value of $N_o b$, the values of N_o and b may be obtained separately. To find T and α, values of $N_o(1 - b)e^{-kt}$ are computed for the middle period of the curve and particularly for the period in which the sharp drop usually occurs. They are divided by the corresponding values of $(N - N_o b)$ and 1 is subtracted from the result. The natural logarithm of the number thus obtained is plotted against

time. The points again fall on a straight line whose slope is α and whose intercept on the Y-axis is αT. The value of T can then be obtained by a simple division.

TABLE I

Results of Mathematical Analysis of Data of Animals Studied

Species	Animal No.	Average life span (T)	Coefficient of uniformity (α)	Uptake of total Fe^{55} utilized		Coefficient of random destruction (k)	Fraction of radioiron reutilized (b)
		days		*per cent*	*in days*		
Rabbit	1	69	0.26	84	2	0.0088	0.145
	2	76	0.28	95	2	0.0067	0.151
	3	66	0.20	88	2	0.0121	0.199
	4	64	0.15	65	2	0.0173	0.201
	5	59	0.12	63	2	0.0122	0.212
	6	69	0.22	86	2	0.0114	0.148
	7	67	0.26	84	2	0.0157	0.154
	8	71	0.25	84	2	0.0081	0.184
	9	64	0.26	81	2	0.0114	0.129
	10	72	0.20	86	2	0.0089	0.134
	11*	58 (38‡ + 20§)	0.28	51	2	0.0110	0.149
	12*	76 (38‡ + 38§)	0.17	55	2	0.0382	0.163
Average....		67.6	0.22	76.8	2	0.0135	0.164
Cat	1	72	0.26	67	3	0.0121	0.179
	2	72	0.18	38	3	0.0251	0.138
	3*	66 (20‡ + 46§)	0.18	—	—	0.0027	0.183
	4*	65 (20‡ + 45§)	0.26	—	—	0.0133	0.160
	5*	67 (20‡ + 47§)	0.22	—	—	0.0468	0.077
Average....		68.4	0.22	—	—	0.0200	0.147
Dog	1	106	0.18	88	6	0.0000	0.558
	2	105	0.22	86	6	0.0000	0.588
	3	111	0.25	70	2	0.0042	0.317
	4*	108 (30‡ + 78§)	0.42	—	—	0.0134	0.228
	5*	105 (30‡ + 75§)	0.22	—	—	0.0039	0.258
Average....		107	0.258	—	—	0.0043	0.389

* Transfusion recipients.

‡ Life span in donor.

§ Life span in recipient.

For convenience T has been calculated from the time of the period of administration of radioactive iron. If the greater part of the uptake has occurred within 48 hours, it is sufficiently accurate to subtract 1 day from this value of

T. The rate of uptake affects also the value of α. The more rapid the uptake of radioiron, the greater the value of this constant. The results of this analysis when applied to the total circulating red cell radioactivity data of the animals studied are given in Table I.

Analysis of Data

Fig. 1 shows typical curves of circulating red cell radioactivity levels in the normal dog, rabbit, and cat after intravenous injection of radioiron. As noted by previous investigators (7, 9, 32), the rise in concentration of circulating red cell radioactivity is extremely rapid until approximately three-fourths or more of the ultimate level is reached. This is particularly true if extremely small doses of iron of high specific activity are administered as in these experiments. This is shown in Figs. 1 and 2 by dotted lines which simply join the actual points determined and have no theoretical significance.

Following the initial period of rapid utilization of iron (usually 2 to 4 days) there follows a period of slower uptake lasting 5 to 7 days, representing in most animals 5 to 20 per cent of the total amount of radioiron utilized. This has been neglected in the mathematical derivation of our curves, and the total uptake is assumed to occur at the same rate as the initial rapid phase. This neglect has two effects. In the first place, the cells formed in the slow radioiron uptake phase will outlive, on the average, the cells formed earlier, and will be present to a large extent in the blood after the end of the major senescence phase, that is, the period of sharp drop. We have considered that any radioactivity remaining at this time is due to reutilized radioactive iron, but, since these cells from the original uptake period still persist, this is not strictly true. The apparent degree of reutilization is therefore increased. Secondly, the S-shaped part of the curve resulting from the distribution of life spans around an average will be rendered somewhat skew by an apparent increase in life spans of those cells living longer than the average. Finally, the average life span will be apparently increased to a slight extent. These effects are relatively small. This is shown with respect to skewness by the fact that the experimental points do not diverge from the theoretical lines (solid lines Figs. 1, 2, and 5) which are not skew by more than can be attributed to experimental error and the usual biological variations in the daily state of the animals such as slight fluctuations in total red cell volumes.

From the time of maximum concentration of circulating red cell radioiron the data fit the equation, as shown in Fig. 1, as closely as could be expected. Moreover, during the fitting, when values for $\log N_o(1 - b)$ were plotted against time, in every case they fell on a straight line, as postulated previously. This was also invariably the case when

$$\text{Log } \frac{N_o(1 - b)e^{-kt}}{N - N_o b}$$

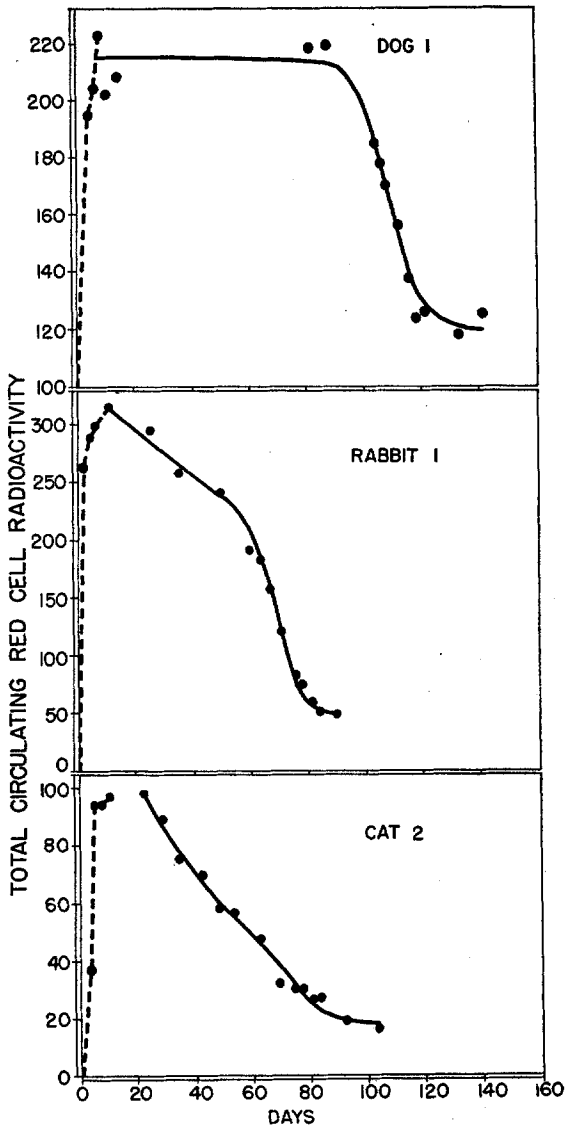

FIG. 1. Plots of total circulating red cell radioactivity (solid circles) against time for three typical normal animals following the tagging of limited populations of their red cells by intravenous Fe^{55}. The close fit to each theoretical curve of survival (solid line) derived by the mathematical treatment explained in the text, is shown for each animal. Note the varying degrees of random destruction of red cells for the three animals. The broken lines, which have no theoretical significance, merely indicate the rate of uptake by new red cells of the radioiron utilized.

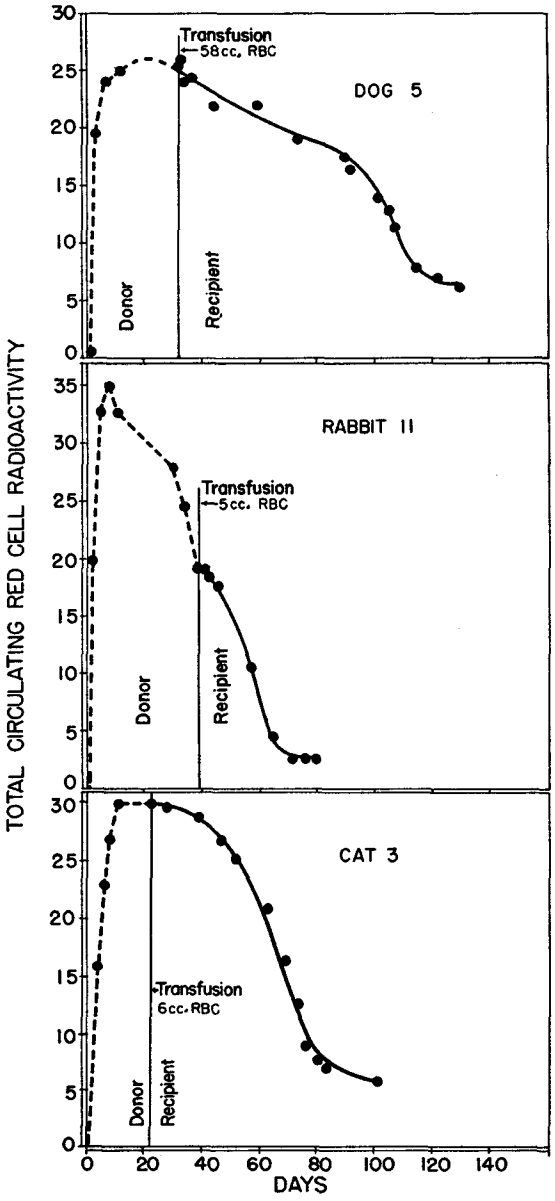

FIG. 2. Plots of total circulating red cell radioactiviy (solid circles) against time in three typical transfusion experiments. Values for total red cell circulating radioactivity of the donors have been multiplied by a factor in order to convert them to the same scale as used for the recipients. Note the close fit of the theoretical survival curves (solid lines), derived according to the mathematical treatment explained in the text, for the period of survival in each recipient.

was plotted against time. These findings increased confidence in the validity of the equations.

From Table I it will be seen that the average life span of the rabbit erythrocyte is 67.6 days with a standard error of the mean of 1.52 days. This agrees well with the previous values of 75 days (Harrison *et al.* (9)) and 65 to 70 days (Neuberger and Niven (10)). For the cat the value of 72 days is slightly shorter than the average value of 77 days given by Valentine *et al.* (11), but only half their values were between 72.5 and 81.5 days. They did not, however, take account of reutilization, and this tends to lengthen the apparent life span. The value for the dog, 107 days, is somewhat shorter than the 115 days given by Bale *et al.* (8), but practically identical with the 106 to 109 day life span for dogs reported by Finch *et al.* (7).

The life span as determined by transfusion experiments was found to be the same as when followed in the same animal (Fig. 2). The life spans of tagged homologous red cells transfused into two rabbits were 56 and 76 days, the average being 66 days which is as close as can be expected to the average of 67.7 obtained in animals whose own cells were originally tagged. The transfused cats had an average red cell life span of 66 days which is within the expected range, but slightly shorter than the 72 days of the non-transfused cats. Homologous cells transfused into dogs had almost exactly the same life span as those in non-transfused dogs. Thus, transfusions of fresh normal animal red cells confirm the values for life spans obtained without transfusion. Averaging the values for the transfusion experiments with the values of the non-transfused animals gives the following average life spans with the respective standard errors of the mean: rabbit, 67.6 ± 1.94; cat, 68.4 ± 1.50; and dog, 107 ± 1.14. Only in the case of the cat, in which the number of animals is small, is there a change in the average value, but even here the standard error does not indicate any unduly wide range of life spans as compared with other animals studied.

Small variations in the coefficients of uniformity of life span are largely independent of the rate of uptake of radioiron, as shown by comparison with the column (Table I) giving percentages of the total radioiron uptake occurring in the first 48 hours. Apparently, therefore, another factor such as individual biological variation affects this coefficient. The differences in the coefficient for cats 1 and 2, however, are due, to a significant extent, to the former factor. Cat 1 utilized 67 per cent of its total uptake of radioiron in 3 days, while cat 2 utilized only 38 per cent of its total radioiron uptake in the same period of time. As will be noted from Table I, the coefficients of uniformity for the cat and rabbit agree quite closely. This coefficient for the dog is slightly greater averaging 0.258. This indicates the variation of red cell life in the dog is slightly less than in the former species.

The fraction of cells remaining when senescence is the sole cause of cell

disintegration would be, in the absence of reutilization,

$$\frac{1}{1 + e^{\alpha(t-T)}}$$

Fig. 3 illustrates the theoretical variation in red cell life span for the dog based on the average life span value (T) of 107 days and an average coefficient of uniformity (α) of 0.258. This latter value implies that 95 per cent of tagged dog red cells of the same age would die in 107 $\pm$ 14 days. This value for α,

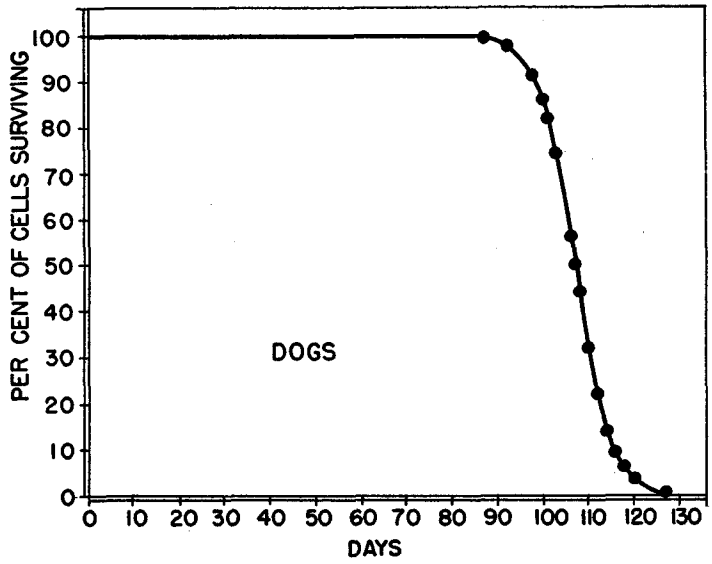

FIG. 3. Illustrating the relatively small theoretical variation in red cell life span of the dog calculated for an average life span of 107 days, and from an average coefficient of uniformity of 0.258 (explanation in text).

however, is an underestimate, as explained above, because the uptake of radioiron is not instantaneous. For this reason, the variation of $\pm$14 days is too high, and the true value is probably 1 or 2 days less. We are not aware of any previous data on variations in animal red cell life. However it is of interest that this latter variation of $\pm$12–14 days for the dog agrees quite closely with Mollison's "rough estimate" (33) of 12 days, under the same circumstances, for human red cells.[7] Mollison's estimate for human red cells would indicate a value for

[7] The radioiron-tagged red cell survival curves of Finch *et al.* (7) of human reticulocytes from a pernicious anemia patient and reticulocytes deficient in iron, both transfused into patients with aplastic anemia, closely resemble our animal curves exhibiting random destruction. These latter investigators considered the life span of these

α of 0.305. The average coefficients of uniformity for the rabbit and cat are identical. In these species 95 per cent of tagged populations of red cells of the same age would theoretically disappear during a period of 17 days before and after the average life span. Therefore, their theoretical variations of red cell life span are the same when plotted with respect to their average red cell life (Fig. 4).

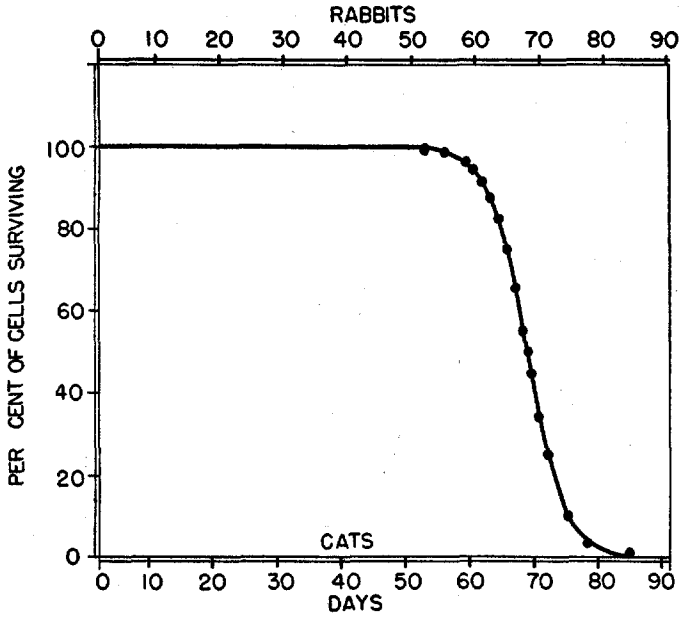

FIG. 4. Illustrating the theoretical variation in red cell life span for the rabbit and cat based on a coefficient of uniformity of 0.22.

The coefficient of random destruction is about the same for cats and rabbits, but it is considerably smaller for the dog. In two dogs it was zero within the limits of error of the methods employed. After transfusion of the same donor

tagged reticulocytes to be relatively normal in spite of a progressive loss of circulating radioactivity commencing immediately following transfusion. In order to consider these latter cells normal, it would have to be assumed that some normal red cells have relatively short life spans. This assumption is not supported by survival curves of normal human cells obtained by Ashby counts (33) and differs from our present findings of animals cells in which the variation of red cell life is relatively small. Perhaps these observations of Finch *et al.* (7) are better explained by the recent work of Hamilton *et al.* (34) which reveals that transfused normal red cells (as well as the patient's own red cells) disappear in a random fashion if subjected to the circulation of a patient with inadequately treated pernicious anemia.

dog's blood into two different recipient dogs, it was 0.0039 in one, approximately the same as the coefficient of random destruction found for the untransfused dog 3 (Table I). In the second transfused dog this coefficient was somewhat greater. Evidence is insufficient here to draw more detailed conclusions. However, from these and other experiments (7–10) it appears that animals of these three species, unlike man, may exhibit varying degrees of random

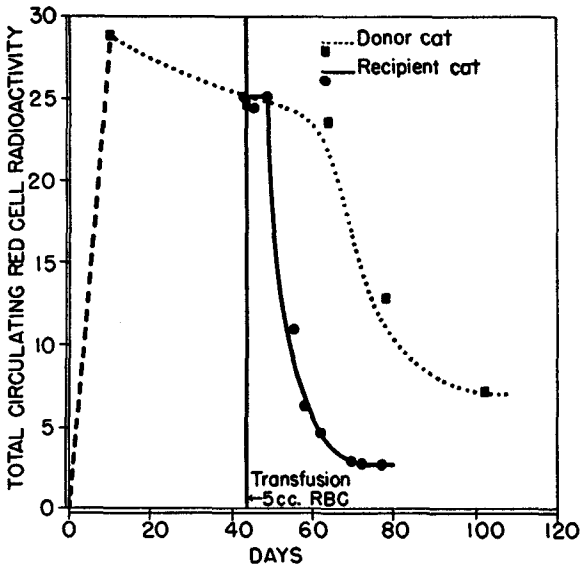

FIG. 5. Plot of total circulating red cell radioactivity against time for both donor and recipient animals (cats). Values for the donor have been multiplied by a factor to reduce them to the same scale as the recipient values. The close fit of the theoretical survival curves in each animal is shown (donor cat, dotted line, recipient cat, solid line). The rapid random destruction of the donor cells occurring approximately 6 days after transfusion resulted from isosensitization of the recipient by the transfused blood.

destruction of normal red cells even when in apparently normal health. As yet, we have found no explanation for this random red cell destruction in our animals.

Fig. 5 illustrates the application of the mathematical treatment described to an abnormally rapid random destruction of transfused normal red cells encountered in one of our earlier experiments prior to finding suitable cat blood grouping sera. As shown by the curve (solid line Fig. 5), there was a sudden rapid elimination of the transfused red cells after a period of normal survival in the recipient cat of approximately 6 days. Although no isoagglutinins to the donor's cells could be found in the recipient cat prior to transfusion, co-

incident with the rapid elimination of donor cells, the recipient cat developed potent isoantibodies which strongly agglutinated the donor cells in high titer. The theoretical curve of elimination (solid line Fig. 5) was obtained by the mathematical analysis of the data employing a random destruction coefficient of approximately 10 times (0.201) that usually encountered in cats (0.02). There was no evidence of any significant loss of cells by senescence, and it was obvious that the sudden and rapid elimination of donor cells was caused by the isoagglutinin provoked by the transfusion. This latter curve should be compared with the theoretical curve of survival obtained for the donor animal (dotted line Fig. 5) in which red cell life span was normal and senescence was the chief cause of cell disappearance. This demonstrates the possible application of the mathematical treatment described to studies of red cell survival by radioiron in animals, such as red cell preservation experiments in which varying rates of red cell destruction might be encountered.

The size of the fraction of radioiron reutilized in new red cell formation depends in large part on the excess stores and continual supply of non-radioactive iron administered intravenously. It is unlikely that complete suppression of reutilization of radioiron would have resulted from larger and more frequent injections of non-radioactive iron. It should be pointed out that the size of the excess iron stores is only one factor to be considered. Perhaps the frequency of administration of non-radioactive iron in maintaining a near saturated serum iron-binding capacity is of equal or greater importance.

SUMMARY

Animal red blood cell *in vivo* survival curves, obtained by the radioiron tagging of populations of approximately the same age followed by the administration of non-radioactive iron to suppress radioiron reutilization, have been subjected to mathematical analysis on the basis of the three following assumptions:—

(*A*) Red blood cells disappear from the circulation as the result of senescence: there is an average life span around which the life spans of individual cells are distributed in the usual way.

(*B*) Red blood cells may be removed from the circulation by a process of random destruction which continuously removes a constant fraction of the cells present at any moment irrespective of age or other characteristics.

(*C*) Under the conditions of the experiments described, a fraction of the radioiron, constant for each animal, is reutilized in new red cell formation when released by red cell destruction.

This mathematical analysis indicates the following average life spans with the respective standard errors of the mean: dog 107 days $\pm$ 1.14; rabbit 67.6 days $\pm$ 1.94; cat 68.4 $\pm$ 1.50.

The mathematical treatment presented has permitted a consideration of

the theoretical variation of red cell life spans which was found in these experiments to be relatively small for all three species studied. In the rabbit and cat 2.5 per cent of tagged populations of red cells of the same age would theoretically have disappeared by senescence 17 days before the average life span was reached. The variation of red cell life in the dog was slightly less.

Animals of the three species studied, in spite of apparently normal health, exhibited varying degrees of random destruction of both autogenous and transfused fresh normal homologous red cells. As yet, we have no explanation for this random loss of cells occurring in apparently healthy normal animals.

The method of mathematical analysis presented is applicable to animal red cell survival studies employing radioiron in which differing rates of random destruction are operating in the removal of red cells.

BIBLIOGRAPHY

1. Eaton, R., and Dameron, F. L., *South Med. J.*, 1930, **23,** 311, 395.
2. Schulten, H., *Folia haematol.*, 1930, **42,** 158.
3. Escobar, R. A., and Baldwin, F. M., *Am. J. Physiol.*, 1934, **107,** 249.
4. Kurtz, P. L., *Am. J. Physiol.*, 1937, **119,** 24.
5. Hawkins, W. B., and Whipple, G. H., *Am. J. Physiol.*, 1938, **122,** 418.
6. Davis, J. E., *J. Lab. and Clin. Med.*, 1943, **28,** 848.
7. Finch, C. A., Wolff, J. A., Rath, C. E., and Fluharty, R. G., *J. Lab. and Clin. Med.*, 1949, **34,** 1480.
8. Bale, W. F., Yuile, C. L., DeLaVergne, L., Miller, L. L., and Whipple, G. H., *J. Exp. Med.*, 1949, **90,** 315.
9. Harrison, B. A., Burwell, E. L., and Finch, C. A., *Fed. Proc.*, 1951, **10,** 357.
10. Neuberger, A., and Niven, J. S. F., *J. Physiol.*, 1951, **112,** 292.
11. Valentine, W. N., Pearce, M. L., Riley, R. F., Richter, E., and Lawrence, J. S., *Proc. Soc. Exp. Biol. and Med.*, 1951, **77,** 244.
12. Hahn, P. F., Bale, W. F., Ross, J. F., Hettig, R. A., and Whipple, G. H., *Science*, 1940, **92,** 131.
13. Shemin, D., and Rittenberg, D., *J. Biol. Chem.*, 1946, **166,** 627.
14. Finch, C. A., Gibson, J. G., 2nd, Peacock, W. C., and Fluharty, R. G., *Blood*, 1949, **4,** 905.
15. London, I. M., Shemin, D., West, R., and Rittenberg, D., *J. Biol. Chem.*, 1949, **179,** 463.
16. Ashby, W., *J. Exp. Med.*, 1919, **29,** 267.
17. De Gowin, E. L., Sheets, R. F., and Hamilton, H. E., *J. Clin. Inv.*, 1950, **29,** 693.
18. Brown, I. W., Jr., unpublished data.
19. Young, L. E., personal communication.
20. Jope, E. M., *Brit. J. Ind. Hyg.*, 1946, **3,** 136.
21. Gibson, J. G., 2nd, Weiss, S., Evans, R. D., Peacock, W. C., Irving, J. W., Jr., Good, W. M., and Kip, A. F., *J. Clin. Inv.*, 1946, **25,** 616.
22. Gibson, J. G., 2nd, and Evans, W. A., Jr., *J. Clin. Inv.*, 1937, **16,** 301.
23. Leeson, D., and Reeve, E. B., *J. Physiol.*, 1951, **115,** 129.
24. Evelyn, K. A., and Malloy, H. T., *J. Biol. Chem.*, 1938, **126,** 655.

25. Young, L. E., Ervin, D. M., and Yuile, C. L., *Blood*, 1949, **4,** 1218.
26. Christian, R. M., Ervin, D. M., and Young, L. E., *J. Immunol.*, 1951, **66,** 37.
27. Hamilton, A. S., *Am. J. Physiol.*, 1949, **154,** 525.
28. Brown, I. W., Jr., and Zoellner, B., unpublished data.
29. Kellner, A., and Hedal, E. F., *Am. J. Path.*, 1952, **28,** 539.
30. Peacock, W. C., Evans, R. D., Irvine, J. W., Jr., Good, W. M., Kip, A. F., Weiss, S., and Gibson, J. G., 2nd, *J. Clin. Inv.*, 1946, **25,** 605.
31. Pearl, R., Introduction to Medical Biometry and Statistics, Philadelphia, W. B. Saunders Co., 3rd edition, 1940, 459.
32. Hahn, P. F., Ross, J. F., Bale, W. F., and Whipple, G. H., *J. Exp. Med.*, 1940, **71,** 731.
33. Mollison, P. L., Blood Transfusion in Clinical Medicine, Springfield, Illinois, Charles C Thomas, 1951, 104.
34. Hamilton, H. E., Sheets, R. F., and Janney, C. D., *J. Clin. Inv.*, in press.

The authors acknowledge the technical assistance of L. L. Dozier, Jr.