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STUDIES OF RED CELL LIFE SPAN IN THE RAT

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Values reported for the life span of the rat red blood cell are listed in Table 1, from which it may be seen that considerable disagreement exists between the results of different investigators. Early attempts to study red cell survival in this species were based on observations of the time taken for the red cell count, or haemoglobin concentration in the circulating blood, to return to normal after the induction of polycythaemia by transfusion or exposure to low oxygen tension or after anaemia by bleeding. Other early studies were based on cyclic changes in reticulocyte count observed after stimulation of erythropoiesis by bleeding or phenylhydrazine administration. These early investigations gave values of 5–18 days for the life span of the red cell, but since they were based on false premises concerning the homoeostatic mechanisms governing red cell volume the results cannot be accepted.

More recent estimations have involved the direct measurement of the survival of isotopically labelled cells in the circulation, and give values of 24–100 days for the life span. These techniques may be classified according as the isotopic label is introduced into red cell precursors in the erythropoietic tissues *in vivo* to label cells of a single age group (^{14}C, ^{59}Fe) or is used *in vitro* to label mature cells of all ages, the labelled blood being then injected into recipient animals (^{51}Cr). In the former type of investigation some difficulty may arise in the interpretation of the data because of the finite time taken for the isotopic label to appear at maximum concentration in the circulating red cells. Shemin & Rittenberg (1946) have described a method of correction for this delay in incorporation, which can be considerable in studies with ^{14}C-labelled glycine (Berlin, Meyer & Lazarus, 1951; Fryers & Berlin, 1952; Berlin, Van Dyke & Lotz, 1953). Intravenously injected ^{59}Fe by contrast is more rapidly incorporated into red cell precursors in the erythropoietic tissues. However, since under normal conditions iron returned to the metabolic pool from destroyed red cells is largely re-utilized for new haemoglobin synthesis (Gibson, Aub, Evans, Peacock, Irvine & Sack, 1947) it is

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usually necessary in studies of life span with ^{59}Fe to give repeated injections of excess non-radioactive iron once the directly labelled red cells have emerged into the circulation, in order to reduce the further incorporation of ^{59}Fe into red cell precursors (Burwell, Brickley & Finch, 1953; Davis, Alpen & Davis, 1955). Allowance must be made for re-utilization in analysing the results of such studies.

TABLE 1. Values reported for red cell life span in the rat

Investigators	Method	Mean life span (days)	^{51}Cr half-clearance time (days)
Schulten (1930)	Recovery after phenylhydrazine administration	15	—
Escober & Baldwin (1934)	Recovery after exposure to low oxygen tension	12–18	—
Creskoff & Fitzhugh (1937)	Recovery after transfusion	5–8	—
Harne, Lutz & Davis (1938)	Cyclic changes in reticulocyte count after haemorrhage	8–9	—
Ponticorvo, Rittenberg & Bloch (1949)	Incorporation of ^{2}H into circulating red cells	100	—
Berlin, Huff & Hennessy (1951)	^{59}Fe turnover by red cells	100	—
Berlin, Meyer & Lazarus (1951)	Survival of ^{14}C-labelled cells	68±8	—
Berlin & Lotz (1951)		64	—
Fryers & Berlin (1952)		60	—
Berlin, Van Dyke & Lotz (1953)		57	—
Burwell, Brickley & Finch (1953)	Survival of ^{59}Fe-labelled cells	45–50	—
Davis, Alpen & Davis (1955)		51	—
Van Dyke, Asling, Berlin & Harrison (1955)	Survival of ^{14}C-labelled cells	53	—
Donohue, Motulsky, Giblett, Pirzio-Biroli, Viranuvatti & Finch (1955)	Survival of ^{51}Cr-labelled cells	—	10±2
Giblett, Motulsky, Casserd, Houghton & Finch (1956)		—	18·4±1·5 (normal)
Hall, Nash & Hall (1957)		—	19·9±3·5 (splenectomized) 8

The use of ^{51}Cr in red cell survival studies is complicated by the fact that the ^{51}Cr concentration in the blood falls more rapidly than would be expected from red cell destruction alone, a finding usually explained in terms of a slow elution of the isotopic label from the red cells in the circulation. Measurements of the ^{51}Cr content of the blood must therefore be corrected for elution loss if true survival is to be estimated. Moreover, since cells of all ages are labelled by the ^{51}Cr technique, direct information about the fate of cells of any particular age may be difficult to extract from the data. Since the fall in ^{51}Cr concentration in the circulating blood with time is approximately exponential and therefore yields a straight line when plotted semi-logarithmically, the ^{51}Cr half-clearance time provides a convenient index of the relative rate of red cell destruction under different conditions (Donohue, Motulsky, Giblett, Pirzio-Biroli, Viranuvatti & Finch, 1955; Giblett, Motulsky, Casserd, Houghton & Finch, 1956).

An indirect method of estimating mean red cell life span involves the assumption of a steady state in which the rates of destruction and production of red cells are equal. The rate of production can be calculated from data on the turnover of ^{59}Fe by the erythropoietic tissues, the calculation requiring knowledge of the rate at which intravenously injected ^{59}Fe is cleared from the blood plasma, the plasma iron concentration, the fractional uptake of ^{59}Fe by the red cells and their total iron content (Huff, Hennessy, Austin, Garcia, Roberts & Lawrence, 1950). Accurate values of these parameters are difficult to measure in small animals and the basic assumption of equal destruction and production is invalid in a growing animal whose blood volume is expanding, unless appropriate correction is made. Moreover, the calculations involve the acceptance of a model system for iron metabolism which may be in error. Nevertheless, the method leads to approximate values for the mean red cell life span in the rat in general agreement with the results of direct survival studies with labelled cells (Berlin, Huff & Hennessy, 1951).

Detailed consideration of the results of direct survival studies with cells labelled *in vivo* reveals some confusion regarding the term 'mean life span'. Most workers define this index as the time after which 50% of newly emerging red cells have been destroyed, and measure it as the time for the circulating radioactivity to fall to one half of its maximum value (Davis *et al.* 1955), or if re-utilization is significant, to a level midway between the maximum value and final value (Burwell *et al.* 1953). It should, however, be pointed out that the quantity thus defined is a median life span and will only be equal to the mean if the frequency distribution of life span is symmetrical. Moreover, the time at which the circulating radioactivity falls to the 50% level will only correspond to the median life span if all cells are instantaneously and uniformly labelled and if no elution of the isotopic label from the cells takes place.

Schiødt (1938), Dornhorst (1951) and Eadie & Brown (1953) have emphasized that red cells may be destroyed in various ways, and make an important distinction between senescence and random destruction mechanisms by which cells may be removed from the circulation. Cells may undergo random destruction irrespective of age at any time during their potential life span. Those cells that escape the hazard of random destruction eventually disappear, after an approximately constant life span, as a result of the process of senescence. However, because of the contribution of random destruction the mean life span of the whole population is in general less than the potential life span determined by senescence, and the frequency distribution of life span is asymmetrical. Burwell *et al.* (1953) and Brown & Eadie (1953) show that the relative importance of these two types of destruction mechanism varies widely between species. Brown & Eadie (1953) describe a convenient method of analysing the results of studies with ^{59}Fe in terms of senescence, random destruction and re-utilization effects.

In the studies to be described, red cell survival in the rat has been investigated in terms of the survival of both ^{59}Fe and ^{51}Cr-labelled cells. With ^{59}Fe, the fate of the labelled cells has been studied by assaying the ^{59}Fe content of the circulating blood and of the faeces, and also autoradiographically by measuring the percentage of labelled cells remaining in the circulation. In order to investigate the role of the spleen in red cell destruction, observations have been made on both normal and splenectomized animals. Preliminary details of these studies have been reported elsewhere (Belcher & Harriss, 1956). With ^{51}Cr the radioactive content of the circulating blood only has been followed.

METHODS

All red cell survival studies were made on male brown-hooded rats of the 'August' strain of weight 100–200 g. They were maintained on Medical Research Council diet No. 41 B and water *ad libitum*. In studies on splenectomized animals, splenectomy was performed through a small incision in the abdominal wall on animals of weight 80–100 g at least 4 weeks before the commencement of red cell survival studies.

Studies with ^{59}Fe

Rats were injected intravenously by tail vein with 10 μc ^{59}Fe (specific activity 1–3 μc/μg Fe) as ferric chloride in 0·2 ml. 1% (w/v) sodium citrate solution. In measurements of the ^{59}Fe content of circulating blood, intramuscular injections of an iron-dextran complex (Imferon, Bengers Laboratories Ltd., 0·1 ml. containing 5 mg Fe/100 g body weight) were made into the femoral muscles 24 hr after ^{59}Fe administration, and thereafter daily or every 2 days to reduce re-utilization of ^{59}Fe. At weekly intervals 0·02 ml. blood samples were withdrawn from the cut tail of each animal into blood pipettes and diluted to 5 ml. with ammoniated distilled water in matched test tubes for haemoglobin estimation. Haemoglobin was estimated colorimetrically as oxyhaemoglobin; the diluted blood samples were then transferred to sample tubes of diameter $\frac{5}{8}$ in. (15 mm) for ^{59}Fe assay. ^{59}Fe content was measured in a well-type scintillation counter (efficiency for ^{59}Fe, 20%; background, 6 counts/sec) in terms of an ^{59}Fe standard.

In measurements of ^{59}Fe content of faeces, animals were maintained in metabolism cages. Daily faecal samples were collected and transferred to sample tubes of diameter $\frac{3}{4}$ in. (19 mm) for ^{59}Fe assay. ^{59}Fe content was measured in a well-type scintillation counter with a larger well than that used for the assay of blood samples (efficiency for ^{59}Fe, 10%; background, 8 counts/sec).

In autoradiographic studies blood smears were made from tail vein blood on glass slides at weekly intervals. Autoradiographs were prepared from these smears by the stripping film technique, using Kodak AR. 10 autoradiographic stripping film, exposed for 1 month and processed. On each autoradiograph the percentage of cells showing an uptake of ^{59}Fe, as evidenced by an increase above background level in the number of developed grains in the emulsion overlying the cell, was determined by examining 1000 cells under the phase-contrast microscope. The mean grain count per radioactive cell, defined as the mean increase above background level in the number of developed grains in the emulsion within an area of diameter three times the mean cell diameter, was also determined by performing grain counts over 20 cells. In studies of mean grain count autoradiographs of all smears taken from any single animal were prepared on the same day and exposed and processed together. In measurements of percentage of labelled cells autoradiographs were not always prepared simultaneously.

Studies with ^{51}Cr

These studies were made on groups of up to five litter mates, one animal of each litter serving as donor, and the remainder as recipients. Two millilitres of blood was withdrawn by cardiac puncture from each donor into a syringe moistened with heparin (Pularin; Evans Medical Supplies Ltd.)

and transferred to a graduated test tube. 1 ml. of acid-citrate dextrose solution and 1 ml. of a solution of isotonic saline containing 50 μc ^{51}Cr (specific activity 20–50 μc/μg Cr) as sodium chromate were added with gentle mixing. The mixture was allowed to stand for 30 min at room temperature and was then centrifuged at 2500 rev/min for 10 min at a radius of 15 cm. The supernatant was removed and discarded, the cells resuspended in isotonic saline and the volume readjusted to 2 ml. Labelling was 85–95 % complete and the labelled cell suspension contained 0·4–1·2 μg Cr/ml. 0·5 ml. portions of this suspension were injected intravenously by tail vein into recipient animals. At daily intervals thereafter, 0·02 ml. blood samples were withdrawn from the cut tails of the recipients into blood pipettes and diluted to 5 ml. with ammoniated distilled water for haemoglobin estimation as already described. The diluted samples were then transferred to sample tubes of diameter $\frac{5}{8}$ in. for ^{51}Cr assay in the well-type scintillation counter (efficiency for ^{51}Cr, 5 %; background, 6 counts/sec) in terms of a ^{51}Cr standard.

RESULTS

Studies with ^{59}Fe

Measurements of ^{59}Fe content of circulating blood. In early studies of the disappearance of intravenously injected ^{59}Fe from the circulating blood (Belcher & Harriss, 1956) rats were given 1 μc ^{59}Fe by injection into a tail vein and 0·5 ml. samples of blood were withdrawn at weekly intervals by cardiac puncture. It was found, however, that significant amounts of blood

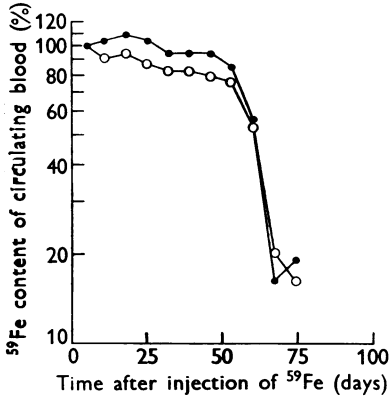

Fig. 1

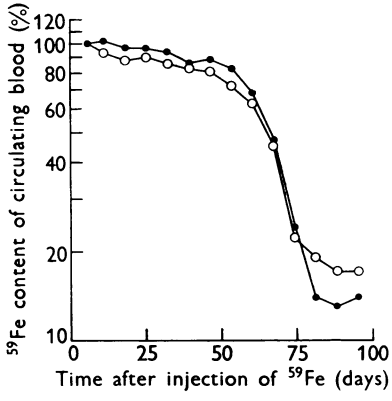

Fig. 2

Fig. 1. Variation of ^{59}Fe content of blood of two normal rats following injection of ^{59}Fe-citrate; semi-log. scale.

Fig. 2. Variation of ^{59}Fe content of blood of two splenectomized rats following injection of ^{59}Fe-citrate; semi-log. scale.

might be lost by haemorrhage after sampling by this method, and that such loss of blood could lead to over-estimation of the rate of disappearance of labelled cells from the circulation. The method was therefore abandoned in favour of sampling from the cut tail, 10 μc ^{59}Fe being injected and 0·02 ml. samples of blood being withdrawn at weekly intervals.

Figure 1 shows the results of such studies on two normal animals given intramuscular injections of iron-dextran to suppress re-utilization on alternate

days throughout the period of study. The animals tolerated the iron-dextran injections well, and no significant disturbance of physiological function has been observed in rats at this dosage level (Golberg, Smith & Martin, 1957). Similar data for two splenectomized animals are shown in Fig. 2. In order to express the results in terms of the total ^{59}Fe content of the circulating blood, the measured ^{59}Fe contents of successive blood samples from each animal have been corrected for expansion of blood volume with growth during the course of the experiment and also for the small amounts of ^{59}Fe removed in sampling. The initial samples were taken 4 days after injection of ^{59}Fe, by which time it may be assumed that all the directly labelled cells have entered the circulation. The corrected values of ^{59}Fe content are expressed as percentages of that of the initial sample, using the formula:

$$\frac{N_t}{N_1} = \frac{\mathrm{Fe}_t}{\mathrm{Fe}_1} \times \frac{RCV_t \times \mathrm{Ht}_1}{RCV_1 \times \mathrm{Ht}_t} \times \frac{N_1}{N_1 - N_s};$$

where N_t = total amount of ^{59}Fe in circulating red cells at time t,
N_1 = total amount of ^{59}Fe in circulating red cells at time of initial sample,
N_s = total amount of ^{59}Fe removed in previous samples,
Fe_t = observed ^{59}Fe content of blood sample at time t,
Fe_1 = observed ^{59}Fe content of initial blood sample,
RCV_t = red cell volume at time t,
RCV_1 = red cell volume at time of initial blood sample,
Ht_t = venous haematocrit at time t, and
Ht_1 = venous haematocrit at time of initial blood sample.

Values of red cell volume and venous haematocrit for rats of different weights were obtained from tables based on the published data of Belcher & Harriss (1957).

Figures 1 and 2 reveal a loss of ^{59}Fe from the circulation in both normal and splenectomized animals during the first 40 days after injection, followed by a phase of more rapid loss during which senescence predominates. The ^{59}Fe content of the blood finally flattens at a level determined by the degree of re-utilization still occurring despite the repeated injection of iron-dextran. Such data may conveniently be analysed by the method of Brown & Eadie (1953) on the assumptions that the early loss of ^{59}Fe is due to a random destruction process which obeys an exponential law and that the potential life span has a normal distribution which can be approximated by the Verhulst–Pearl growth function. The disappearance of ^{59}Fe from the blood can then be represented by an equation of the form

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

where N_t = total amount of ^{59}Fe in circulating red cells at time t after injection,

N_0 = total amount of ^{59}Fe in circulating red cells at time 0,

b = fraction of ^{59}Fe which is re-utilized when released in red cell destruction,

k = coefficient of random destruction,

T = mean potential life span, and

α = coefficient of uniformity of potential life span.

This expression assumes that the labelled cells appear instantaneously in the circulation at the start of their life span at zero time and also that re-utilization of ^{59}Fe follows instantaneously after red cell destruction. Due allowance must therefore be made for the finite time of incorporation of ^{59}Fe into the circulating cells in applying it to experimental data.

TABLE 2. Detailed analysis of results of ^{59}Fe studies

Condition of animal	Method of investigation	Iron-dextran dosage interval (days)	Mean potential life span T (days)	Coefficient of uniformity α (days^{-1})	Coefficient of random destruction k (days^{-1})	Coefficient of re-utilization b
Normal	Assay of ^{59}Fe in blood	2	59	0·29	0·32	0·16
		2	59	0·46	0·63	0·18
	Autoradiography	None	62	0·28	0·69	0
		None	60	0·20	0·31	0
Splenectomized	Assay of ^{59}Fe in blood	2	64	0·22	0·50	0·18
		2	66	0·25	0·46	0·14
	Autoradiography	None	68	0·29	0·22	0
		None	69	0·27	0·60	0

Detailed results of analyses of the individual data of Figs. 1 and 2 are presented in Table 2. In normal animals the mean potential life span averaged 59 days and rate of random destruction 0·48% per day; in splenectomized animals the corresponding figures were 65 days and 0·48% per day. In deriving these values a period of 1 day has been allowed for the interval between the injection of ^{59}Fe and the appearance of the isotopic label in the circulating red cells (Baxter, Belcher, Harriss & Lamerton, 1955).

A number of similar investigations were made with rats injected daily with iron-dextran. Iron-dextran dosage at this level was not well tolerated and these animals showed anorexia and arrested growth during the period of study. Random destruction appeared somewhat increased, but the mean potential life span was not significantly different from the value obtained in animals given iron-dextran on alternate days.

Autoradiographic studies. It may be argued that the injection of large amounts of iron to reduce re-utilization results in far from normal conditions that may adversely affect the circulating red cells. This difficulty is avoided in the use of high resolution autoradiographic techniques to study the survival

of ^{59}Fe-labelled cells. If, after injection of ^{59}Fe, serial blood smears are taken and autoradiographs are prepared from these smears by the stripping film method (Doniach & Pelc, 1950) the directly labelled cells can readily be identified by virtue of the increased number of developed grains in the emulsion overlying them. The percentage of labelled cells remaining in the circulation can thus be determined by examining one thousand cells. The advantage of the autoradiographic technique lies in the fact that only cells labelled directly after injection of ^{59}Fe at a time when the specific activity of the plasma iron is high give recognizable autoradiographs. Red cell precursors incorporating re-utilized ^{59}Fe do so at such low specific activity that the mature red cells to which they give rise are not recognizable autoradiographically in terms of an increase in grain density. The survival of ^{59}Fe-labelled cells can thus be studied without the administration of excess iron and with the minimum of disturbance of physiological function.

Figures 3 and 4 show the results of autoradiographic studies of red cell survival in two normal and two splenectomized rats each given 10 μc ^{59}Fe intravenously. The measured percentages of labelled cells have been corrected for the increase in blood volume with growth, as already described. Negligible amounts of blood were removed in the preparation of blood smears. Such data may be analysed as already described by the method of Brown & Eadie (1953), the coefficient of re-utilization, b, being here taken as zero. Results of such analyses of the data of Figs. 3 and 4 are included in Table 2. In normal animals the mean potential life span averaged 61 days and the rate of random destruction 0·50 % per day; in splenectomized animals the corresponding values were 68·5 days and 0·41 % per day. These values are seen to be in good agreement with the results of measurements of the ^{59}Fe content of the circulating blood and confirm the increased potential life span observed after splenectomy.

It thus appears that the injection of iron-dextran on alternate days has little, if any, effect on the pattern of red cell survival and that the finding of a significant random loss of ^{59}Fe from the circulation is normal in the rat. This random loss has been attributed to a random destruction of circulating cells but could equally be explained in terms of a slow turnover of iron by the labelled cells in the circulation. In order to investigate this question, the variation in average grain count over ^{59}Fe-labelled cells throughout their life span was studied in a number of animals by counting grains over 20 labelled cells selected at random on the serial autoradiographs prepared for survival studies.

Figure 5 shows the results of such measurements on a rat on which red cell survival studies were also made by the autoradiographic method and by measurements of the ^{59}Fe content of the circulating blood. This animal was given daily injections of iron-dextran and random destruction was high (0·97 % per day as measured in terms of the ^{59}Fe content of the blood, 0·82 % per day

as measured autoradiographically) yet there was no significant change in grain count over the labelled cells even after 63 days, by which time 70% of the labelled cells had been destroyed. The net grain count over the labelled cells averaged 25 grains and the background count 8 grains.

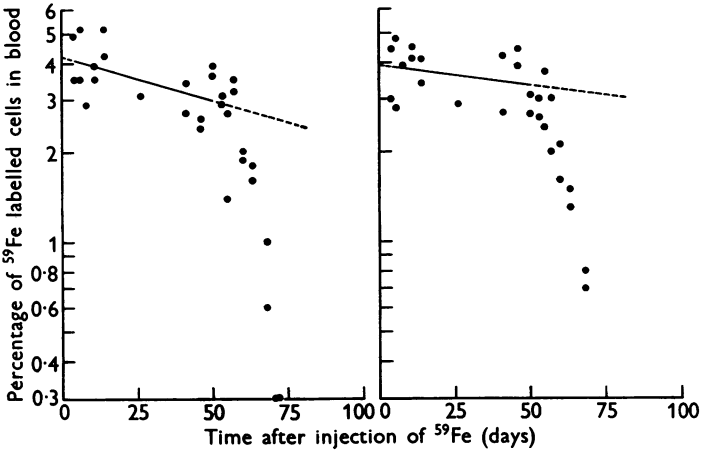

Fig. 3. Variation of percentage of ^{59}Fe labelled red cells in blood of two normal rats following injection of ^{59}Fe-citrate; semi-log. scale.

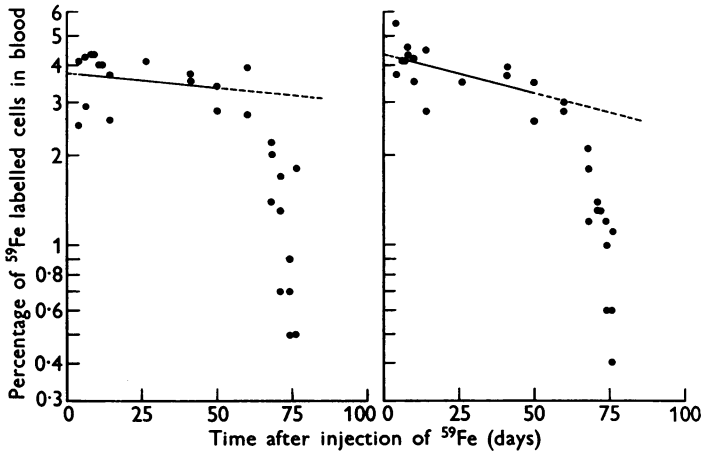

Fig. 4. Variation of percentage of ^{59}Fe labelled red cells in blood of two splenectomized rats following injection of ^{59}Fe-citrate; semi-log. scale.

In none of the animals studied in this way was a significant change in grain count observed during the life of the labelled cells. Hence, the early loss of ^{59}Fe cannot be explained in terms of a slow turnover of iron by the circulating cells, and must represent a real loss of cells from the circulation.

Studies of faecal excretion of ^{59}Fe. Another method of study of red cell survival with ^{59}Fe in the rat, which avoids the necessity for repeated injection of excess iron, is based on the measurement of ^{59}Fe excretion in the faeces. ^{59}Fe returned to the metabolic pool as a result of red cell destruction is largely re-utilized under normal conditions, but a small fraction is excreted. Excretion is mainly in the faeces, although small amounts of ^{59}Fe appear in the urine.

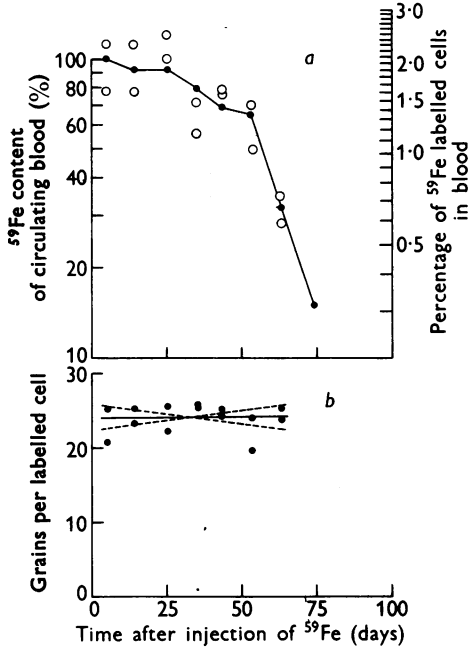

Fig. 5. (*a*) Variation of ^{59}Fe content of blood and percentage of ^{59}Fe labelled red cells in blood of normal rat following injection of ^{59}Fe-citrate; semi-log. scale. ●, ^{59}Fe content of blood; ○, percentage of labelled cells.

(*b*) Mean grain count over labelled cells of same rat as in (*a*). Full line shows regression of mean grain count on time after ^{59}Fe injection. Dotted lines show fiducial limits to the slope of the regression line for $P = 0{\cdot}05$.

Hence, changes in the rate of red cell destruction are paralleled by changes in the daily ^{59}Fe output in the faeces (Foreman, Huff, Oda & Garcia, 1952; Baxter *et al.* 1955).

Figure 6 shows results of faecal excretion studies in three normal animals given 10 μc ^{59}Fe subcutaneously. Figure 7 shows similar studies on three splenectomized animals. The excretion curves may be divided into three phases. The initial fall in faecal ^{59}Fe content probably represents clearance of ^{59}Fe from the labile iron pool. This phase is followed by a phase of relatively constant daily output during which random destruction of red cells predominates. The daily output then increases by a factor of two or three to a

broad maximum during which cells are destroyed as a result of senescence. The maximum due to senescence is not always clearly defined but is usually recognizable.

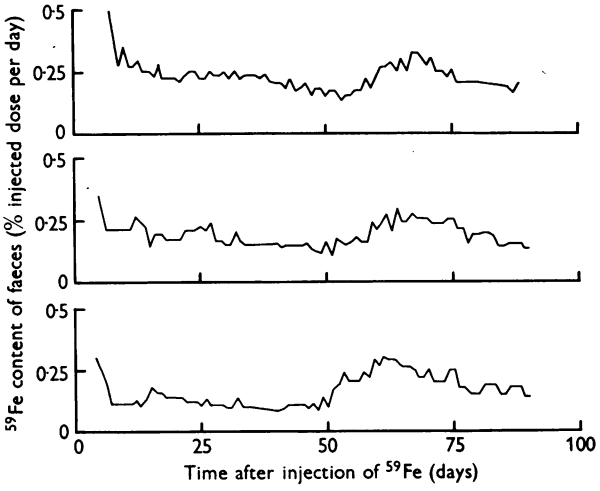

Fig. 6. ^{59}Fe excretion in faeces of three normal rats following injection of ^{59}Fe-citrate.

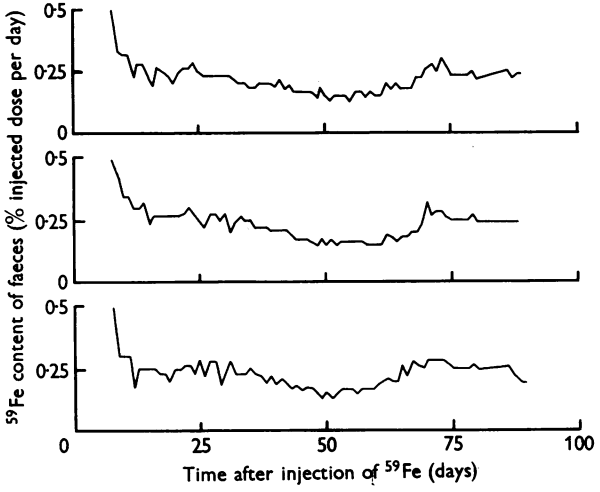

Fig. 7. ^{59}Fe excretion in faeces of three splenectomized rats following injection of ^{59}Fe-citrate.

Because of random destruction and re-utilization effects, the maximum due to senescence has a slight positive skewness and the mode is displaced relative to the time of maximum red cell destruction by about one day. Further small corrections are necessary for the time of incorporation of ^{59}Fe into the circulating red cells and for the interval between destruction of labelled cells and

the appearance of radioactivity in the faeces. On the basis of red cell uptake studies (Baxter *et al.* 1955) the former is taken as 1 day; since the faecal ^{59}Fe content is maximal 3 days after injection of iron, the latter interval is estimated at 3 days. Results for twelve normal animals corrected in this way indicated a mean potential life span of 58 days with a standard deviation of $\pm 2{\cdot}1$ days. Eight splenectomized animals showed a mean potential life span of 68 days with a standard deviation of $\pm 4{\cdot}1$ days.

Studies with ^{51}Cr

Survival studies with ^{51}Cr-labelled cells were carried out by taking blood from donor animals, labelling *in vitro* with ^{51}Cr, and injecting into litter-mate recipients. Each recipient animal received 0·5 ml. of labelled cell suspension containing 10–15 μc ^{51}Cr, the chromium content of the labelled cell suspension being 0·4–1·2 μg/ml. 0·02 ml. blood samples were taken daily from the cut tails of the recipients and haemoglobin concentration and ^{51}Cr content assayed.

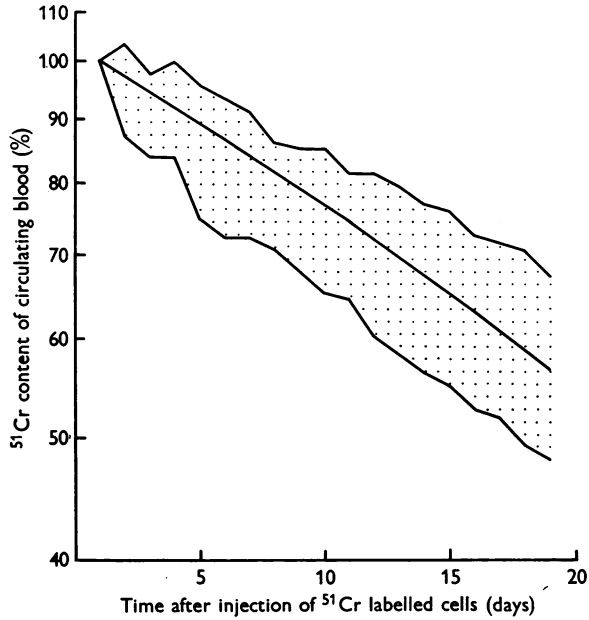

Fig. 8. Variation of ^{51}Cr content of blood of normal rats following injection of ^{51}Cr labelled red cells; semi-log. scale. The envelope shows the range of survival in twenty-three animals. The full line represents the survival calculated according to the equation

$$N_t = N_0 \frac{(e^{-kt} - e^{-kT}).e^{-k_e t}}{(1 - e^{-kT})}$$

Figure 8 shows the range in ^{51}Cr-survival observed in twenty-three normal animals, the data being corrected as described above for the increase in blood volume with growth and for the amounts of ^{51}Cr removed in successive samples.

In a few instances survival was initially within the normal range, but after an interval of 8–15 days the remaining labelled cells rapidly disappeared from the circulation and the blood haemoglobin concentration fell precipitately, probably because of an immune response of the recipient to the injected cells. Such results have been excluded from the analysis of the data.

If it is assumed that the data can be represented exponentially, the mean ^{51}Cr half-clearance time may be estimated as 20·7 days with a standard deviation of $\pm 2{\cdot}5$ days. However, although ^{51}Cr survival curves approximate to exponential form over their initial part (Donohue *et al.* 1955) they cannot properly be represented as either exponential or linear functions of time. Dornhorst (1951) has shown that if red cells which have a constant potential life span but which are also subject to random destruction are transfused from a normal donor to an equivalent recipient, the survival of the transfused cells in the recipient is given by

$$n_t = n_0 \frac{e^{-kt} - e^{-kT}}{1 - e^{-kT}};$$

where n_t = number of transfused cells surviving at time t after transfusion,
n_0 = number of transfused cells present at time 0,
k = coefficient of random destruction, and
T = potential life span.

This equation assumes that the survival of the transfused cell sample in the recipient animal is identical with that of the parent cell population in the donor, and also ignores the variation in potential life span. The latter assumption has little consequence in the situation under discussion, but in applying the method of analysis to ^{51}Cr survival data account must be taken of the elution of ^{51}Cr from the labelled cells in the recipient's circulation. If the latter process obeys an exponential law, the ^{51}Cr content of the circulating blood will fall according to the relationship

$$N_t = N_0 \frac{(e^{-kt} - e^{-kT}) . e^{-k_e t}}{(1 - e^{-kT})};$$

where N_t = total amount of ^{51}Cr in circulating red cells at time t after transfusion,
N_0 = total amount of ^{51}Cr in circulating red cells at time 0,
k = coefficient of random destruction,
k_e = coefficient of ^{51}Cr elution, and
T = potential life span.

The results of the studies with ^{59}Fe indicate values of 59 days and 0·0049 days^{-1} for T and k, respectively. Difficulty in the accurate analysis of the ^{51}Cr data arises, however, because of lack of knowledge concerning the coefficient of

elution k_e. Ebaugh, Emerson & Ross (1953), Necheles, Weinstein & LeRoy (1953) and Read, Wilson & Gardner (1954), report a value of 77 days for the ^{51}Cr half-clearance time for ^{51}Cr elution from human red cells, corresponding to a value of 0·0090 days^{-1} for k_e. If a similar value applies for the red cells of the rat, the expected form of the ^{51}Cr survival curve may be predicted, and is seen to fall well within the observed range (Fig. 8). The ^{59}Fe data suggest that the mean potential life span is relatively constant, but that the extent of random destruction may vary considerably between individual animals. The rather wide variation observed in ^{51}Cr survival can be in part attributed to such variation in random destruction rate.

DISCUSSION

The results of the studies with ^{59}Fe may reasonably be interpreted in terms of a hypothetical random destruction mechanism operating on a cell population whose potential life spans are symmetrically distributed, although the data do not exclude the possibility of a skew distribution of potential life span. The

TABLE 3. Summary of results with ^{59}Fe

Condition of animal	Method of investigation	Number of animals	Iron-dextran dosage interval (days)	Mean potential life span (days)	Coefficient of random destruction (% per day)	Mean life span (days)
Normal	Assay of ^{59}Fe in blood	2	2	59	0·48	51·5
	Autoradiography	2	—	61	0·50	53
	Assay of ^{59}Fe in faeces	12	—	59±2·1	—	—
Splenectomized	Assay of ^{59}Fe in blood	2	2	65	0·48	55·5
	Autoradiography	2	—	68·5	0·41	60
	Assay of ^{59}Fe in faeces	8	—	68±4·1	—	—

results of the various studies with ^{59}Fe are summarized in Table 3. The values of mean life span quoted in this table represent the mean survival times of all cells, including both those cells undergoing random destruction and those removed as a result of senescence; these values have been derived from the observed values of mean potential life span and random destruction rate according to the relationship

$$\bar{T} = \frac{1}{N_0}\int_0^\infty N_t . dt \ ;$$

where $\bar{T}$ = mean life span,

N_0 = number of cells initially labelled with ^{59}Fe, and

N_t = number of labelled cells surviving at time t after emergence into circulation.

The combined results of the ^{59}Fe studies in normal animals by all three methods described indicate a mean potential life span of 59 days with a standard deviation of ± 2 days. However, because of the contribution of random destruction, the mean life span of all red cells is less than this value by a variable amount depending on the degree of random destruction and may be estimated at 49–55 days.

These results are in general agreement with those of other recently reported studies of red cell survival in the rat with ^{14}C-labelled cells (Van Dyke, Asling, Berlin & Harrison, 1955) and with ^{59}Fe-labelled cells (Burwell *et al.* 1953; Davis *et al.* 1955). However, a direct comparison with the values quoted by these workers is not possible, since they have based their estimates of mean life span on the time at which the circulating radioactivity fell to the 50% level, and have made no attempt to assess the relative importance of random destruction and senescence. Burwell *et al.* (1953) remark that destruction appears predominantly random in nature in the rat and evidence of initial random loss also appears in the experimental data of Berlin *et al.* (1953) and Davis *et al.* (1955). On the other hand, no random destruction can be observed in the results obtained by Berlin & Lotz (1951), Berlin, Meyer & Lazarus (1951), Fryers & Berlin (1952) or Van Dyke *et al.* (1955) on normal rats.

The finding of a significant degree of random destruction in the rat is of interest in view of the similar observations by Neuberger & Niven (1951) with ^{15}N-labelled cells in rabbits, by Brown & Eadie (1953) with ^{59}Fe-labelled cells in cats and rabbits and by Bush, Berlin, Jensen, Brill, Cartwright & Wintrobe (1955) with ^{59}Fe-labelled cells in swine. On the other hand, no evidence of random destruction in the cat was found by Valentine, Pearce, Riley, Richter & Lawrence (1951). Brown & Eadie (1953) found only very slight random destruction in dogs, and the results of Shemin & Rittenberg (1946) do not suggest a significant degree of random destruction in normal humans.

Both the results of the ^{59}Fe studies and the ^{51}Cr studies presented above suggest that whereas the mean potential life span in the normal rat is relatively constant, the rate of random destruction may vary considerably from one individual to another. Similar conclusions were reached by Bush *et al.* (1955) and by Neuberger & Niven (1951) in their respective studies on swine and rabbits. The exact nature of the random destruction process remains obscure. The results of the autoradiographic studies of variation in grain count exclude the possibility that it could be due to catabolism of haemoglobin by the circulating red cells. It must therefore represent a true loss of cells from the circulation either by extravasation, phagocytosis or intravascular destruction. There is good evidence that this random loss may be accelerated in infection, inflammation, thermal injury and other abnormal conditions.

The combined results of the ^{59}Fe studies in splenectomized animals indicate a mean potential life span of 67 ± 4 days and a mean life span of 54–63 days.

These results indicate a significant increase ($P < 0{\cdot}001$) in the mean potential life span of the rat after splenectomy, although there is no evidence that the rate of random destruction is affected by removal of the spleen. Ponder (1948) has emphasized that sequestration of blood in the spleen under conditions of partial anoxia and haemoconcentration may accelerate the mechanisms of senescence leading to the eventual destruction of the red cells. Experimental data relating to the effects of splenectomy on red cell survival in experimental animals are scanty, although the finding of accelerated red cell destruction in experimentally produced splenomegaly is well documented. Giblett *et al.* (1956) using the ^{51}Cr technique observed very marked shortening of ^{51}Cr survival in rats with 'hypersplenism' induced by methylcellulose injection. They also found a slight lengthening of ^{51}Cr survival in splenectomized rats as compared with normal rats, but could not attribute statistical significance to this finding. Because of the variation in random destruction rate between different individuals, the ^{51}Cr technique cannot, however, be regarded as a very sensitive index of small changes in potential life span.

The ^{51}Cr half-clearance time of $20{\cdot}7 \pm 2{\cdot}5$ days reported above is slightly but significantly greater than that of $18{\cdot}4 \pm 1{\cdot}5$ days quoted by Giblett *et al.* (1956). The slight discrepancy can be explained by the fact that these workers have not corrected their data for changes in blood volume with growth during the course of their experiments. The lower values of 10 days quoted by Donohue *et al.* (1955) and 8 days by Hall, Nash & Hall (1957) may be attributed to the use of low specific activity ^{51}Cr (1–3 $\mu c/\mu g$ Cr) with the consequent incorporation into the labelled blood of amounts of metallic chromium sufficient to cause irreversible damage to the red cells. Giblett *et al.* (1956) observed abnormally shortened survival at chromium concentrations of 20–25 μg/ml. of red cells.

Precise analysis of the ^{51}Cr data in terms of red cell survival is not possible because of the asymmetrical distribution of red cell life span and because of lack of knowledge concerning the elution rate of ^{51}Cr from the rat red cell. Giblett *et al.* (1956) assumed that red cell destruction in the rat is a purely exponential process, and on this basis arrived at a figure of 39 days for the half-life of the elution process. The ^{59}Fe data reported above show, however, that the assumption of exponential destruction is invalid, and that the elution rate is probably thereby overestimated. From a comparison of the results of the studies with ^{59}Fe and ^{51}Cr it appears that the half-life for ^{51}Cr elution from rat red cells is close to the value of 77 days reported for human red cells by Ebaugh *et al.* (1953), Necheles *et al.* (1953) and Read, Wilson & Gardner (1954). If such a value is assumed, good agreement is obtained between the results of studies with these two isotopes.

SUMMARY

1. Red cell life span was studied in male rats of the 'August' strain by radioactive tracer techniques with ^{59}Fe and ^{51}Cr. Studies with ^{59}Fe were based on radioactive assay of circulating blood, on autoradiographic measurements of the percentage of labelled cells in circulating blood and on radioactive assay of faeces after labelling the red cells *in vivo* by intravenous injection of ^{59}Fe as iron-citrate complex. Studies with ^{51}Cr were based on radioactive assay of circulating blood after intravenous injection of red cells labelled with ^{51}Cr *in vitro*.

2. Studies with ^{59}Fe on normal rats indicated a mean potential life span of 59 ± 2 days. Because of a loss of ^{59}Fe from the circulation throughout the potential life span attributable to random destruction, the mean life span was found to be less than this value and had a range of 49–55 days. It was shown that this loss of activity was not due to turnover of iron by the circulating cells but represented a real loss of cells from the circulation by extravasation or intravascular destruction.

3. Studies with ^{59}Fe in splenectomized rats indicated a mean potential life span of 67 ± 4 days and a mean life span of 54–63 days. The mean potential life span in splenectomized animals was significantly greater than the corresponding value in normal animals, but there was no evidence that the rate of random destruction was affected by removal of the spleen.

4. Studies with ^{51}Cr in normal rats indicated a ^{51}Cr half-clearance time of $20{\cdot}7 \pm 2{\cdot}5$ days, in good agreement with the results obtained with ^{59}Fe.

5. The significance of these results is discussed in regard to the mechanisms of red cell destruction.

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