Clin. exp. Immunol. (1966) **1**, 85–98.

ERYTHROCYTE SURVIVAL IN NORMAL MICE AND IN MICE WITH AUTOIMMUNE HAEMOLYTIC ANAEMIA

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(*Received* 7 *July* 1965; *accepted* 21 *July* 1965)

SUMMARY

Erythrocyte survival has been studied in a strain of mice which develop autoimmune haemolytic anaemia in adult life (NZB/Bl), and also in CBA and C57Bl mice, using one or both of two radioactive isotope labels, ^{51}Cr and ^{32}P.

Erythrocyte survival is customarily expressed as the half-life but this is unsatisfactory for purposes of statistical comparison and we have used instead a parameter 'k' which represents the slope of the line obtained when:

$$\log \frac{\text{(Counts per unit haemoglobin on day } t\text{)}}{\text{(Counts per unit haemoglobin on day 0)}}$$

is plotted against time.

Young NZB/Bl mice yield values of k intermediate between those obtained in CBA and C57Bl mice, and may reasonably be judged to show normal erythrocyte survival. On the other hand, in older NZB/Bl mice which are strongly Coombs positive and show reticulocytosis and anaemia, erythrocyte survival is greatly shortened.

The survival of erythrocytes transfused to NZB/Bl recipients from isogeneic donors was found to be similar to that of the recipient's own erythrocytes, irrespective of whether or not the donor was Coombs positive or showed other evidence of haemolytic activity.

The values of k obtained in NZB/Bl mice were essentially the same with both labels; in CBA mice, however, a significant difference was observed.

The occurrence in animals of disorders which closely resemble various forms of human disease, is important not only from the point of view of comparative pathology but also because it provides a convenient model for experimental therapeutics. One such condition

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is the haemolytic anaemia which was reported by Bielschowsky, Helyer & Howie (1959) to develop in mice of the New Zealand Black strain (NZB/Bl). Various other investigators have subsequently shown that many other features of autoimmune disease occur in the strain.

Stickel & Woodruff (unpublished), in this laboratory, treated NZB/Bl mice, either before or after the development of anaemia, with two immunosuppressive drugs, azothioprine and actinomycin C. The evolution of the disease, as assessed by Coombs testing, haematocrit and reticulocyte percentage determinations, did not appear to have been significantly influenced by therapy, but it seemed clear that more work would be required before a definite conclusion could be reached. Before embarking on further studies of this kind, therefore, it was decided to investigate another index of haemolytic activity, namely, erythrocyte survival.

EXPERIMENTAL MATERIALS AND METHODS

General plan

Prior to studying the NZB/Bl strain, erythrocyte survival was determined in standard strains. Disappearance rates of isogeneic erythrocytes labelled *in vitro* with sodium ^{51}chromate were determined in CBA and C57Bl mice; in addition, in C57Bl, the utility of *in vivo* labelling di-isopropyl-phosphoro-fluoridate (DF^{32}P) was assessed by determining survival with this label simultaneously with that of transfused ^{51}Cr-labelled erythrocytes.

Red cell survival in NZB/Bl mice was initially determined either with autologous ^{51}Cr-labelled cells alone or with the double labelling technique mentioned above. This method, a modification of one described by Hughes Jones, Mollison & Veall (1957), also lends itself to following two different cell populations simultaneously and made it possible to measure the survival of strongly Coombs positive erythrocytes in Coombs negative animals (and *vice versa*) with the ^{51}Cr label and to compare the results obtained with the survival of autologous DF^{32}P-labelled cells.

Mouse strains

NZB/Bl mice were originally sent to this laboratory from New Zealand through the courtesy of Dr Bielschowsky. When the present investigations were begun, the strain had been maintained by brother–sister mating for fifty-five generations, including fifty in New Zealand and five generations in Edinburgh. Animals used in isogeneic transfer experiments were selected on the basis of Coombs testing; age and sex, except as they are known to relate to these determinations, were not factors.

Other mice used were members of C57Bl or CBA strains maintained in this laboratory.

Serological procedures

Rabbit anti-mouse globulin antiserum was prepared by the method of Dacie & Lewis (1963). After absorption against normal mouse erythrocytes it was found to be effective in dilutions as high as 1:1024.

To carry out a Coombs test one drop of a 2% suspension of thrice-washed mouse erythrocytes in 0·85% NaCl was gently mixed on a microscope slide with one drop of a 1:5 dilution of antiserum; 5–10 min later the slide was examined with the naked eye and

microscopically under low magnification (× 32). Erythrocytes from normal C57Bl or CBA mice were used as negative controls.

Results were scored as follows:

4+ = virtually every cell agglutinated; agglutination easily seen without magnification.
3+ = many three to ten cell clumps and at least one large agglutinate seen in each field; unagglutinated cells uncommon.
2+ = five to ten small clumps of cells in each field; unagglutinated cells numerous.
1+ = two to four clumps in each field; unagglutinated cells predominant.
Negative = no agglutination.

Haematological procedures

All blood samples were obtained by puncture of the retro-orbital plexus. Reticulocyte per cent counts were made on slide preparations using fresh 0·5% brilliant cresyl blue stain, lightly counterstained with 0·04% Leishman's stain. Packed cell volume (PCV) was determined with a microhaematocrit high speed centrifuge. For haemoglobin estimations, which were done in all mice at the beginning and end of each experiment and on all serial samples withdrawn for isotope counting, 0·05 ml blood was introduced into 3 ml of 0·1 N-Na_2CO_3 and the haemoglobin concentration determined with a photoelectric absorptiometer.

Radioisotope labelling

Two techniques were used; *in vitro* labelling with ^{51}Cr, as sodium chromate, and *in vivo* labelling with di-isopropylphosphoro-fluoridate (DFP) with incorporated ^{32}P.

For *in vitro* labelling blood obtained by puncture of the retro-orbital plexus was mixed in Kahn tubes with a small quantity of 0·85% NaCl containing heparin (2·5 i.u. for every 0·05 ml blood) and $Na^{51}{}_2CrO_4$ (5–10 μc for every 0·05 ml blood), and incubated at room temperature (18–20°C) for 30 min. The cells were spun down, washed twice in warm (37°C) saline and resuspended in a volume of saline equal to ten times the original volume of blood. In autologous studies 0·05 ml blood was withdrawn, labelled and replaced by injection in a tail vein; for isogeneic cell transfer sufficient blood was removed from the donor to provide, after labelling, 0·05 ml for each recipient. In preliminary experiments with CBA mice, cool saline was used for washing cells and it was noted that cell sludging was frequent; warm saline was substituted and appeared to obviate this phenomenon. Sludged cells had a half life of 10·0 days, significantly shorter than that of the 14·1 days seen when 'normal' cells were used. In all experiments reported, therefore, warm saline wash has been used.

For *in vivo* labelling $DF^{32}P$, dissolved in propylene glycol, was given by intramuscular injection in a dosage of 5 μc. The amount of $DF^{32}P$ varied from 15 to 40 μg per animal. Preliminary studies indicated that the intramuscular and intraperitoneal routes were equally effective.

In some experiments, both labels were used (see above). Autologous or isologous blood was labelled *in vitro* with ^{51}Cr as described above, then transfused to recipients which subsequently were given $DF^{32}P$.

Radioactive counting of blood samples

To obviate tedious cleansing of glassware, disposable nylon tubing was used for serial

sampling; this was cut into convenient lengths which were calibrated as 0·05 ml pipettes.

The mice were bled first on the day after the injection of labelled cells or $DF^{32}P$; thereafter they were bled at variable, but most normally 5 day intervals. Activity of samples containing cells labelled with ^{51}Cr were counted in a well-type scintillation counter after haemoglobin had been measured.

All samples containing ^{32}P were initially spun down and washed twice with 0·85% NaCl to eliminate plasma-bound isotope prior to haemolysing the cell button with Na_2CO_3 solution (final volume, 3 ml). Haemoglobin was determined, and the haemolysate was then dried on aluminium planchets under an infrared lamp and counted with an end-window Geiger-Müller tube.

If both labels were present, the cells were washed, haemolysates were prepared, the haemoglobin was determined and ^{51}Cr counting was carried out with a scintillation counter as already described. The sample was then dried and counted with the Geiger-Müller tube. This count was corrected for activity due to ^{51}Cr in order to obtain the 'true' $DF^{32}P$ count.

Radioactivity in most samples was 5–30 times background. Correction for isotope half life was seldom necessary inasmuch as all samples from one animal were usually counted on the same day. No corrections were made for self-absorption.

Calculation of erythrocyte survival

In determinations of erythrocyte survival in mice, Van Putten (1958) expressed his results as counts per unit of haemoglobin nitrogen; Edmondson & Wyburn (1963) simplified this by relating sample counts to the haematocrit. Inasmuch as it was essential to follow NZB/Bl mice individually, the necessity to minimize serial blood loss led us to measure haemoglobin and radioactivity in the same sample; activity could then be expressed as counts per unit of haemoglobin. 'Zero day' was that of first bleeding and for any subsequent day, 't', there was recorded the corresponding fraction

$$Y_t = \frac{\text{Counts per unit of haemoglobin on day } t}{\text{Counts per unit of haemoglobin on day 0}}$$

Individual elimination curves were initially plotted on plain graph paper and their curvilinear form was interpreted as reflecting either random destruction, elution of label or a combination of the two. Plots of '$\log_{10} Y_t$' against 't' were essentially linear; following Garby's (1962) recommendation, early sample values were ignored if they departed too much from a 'best-fitted' line. If 'k' denotes the slope of the curve on day 't', then $k \doteq (\log_{10} Y_t)t$, and the antilog of k (i.e. 10^k) represents the fraction of the previous days' activity remaining. Suppose, for example, that $k = -0{\cdot}2158$, then $10^k = 0{\cdot}61$ and erythrocyte elimination, hereafter expressed as the erythrocyte destruction rate (EDR), is estimated to be occurring at the rate of 39% per day. If the rate remains constant over the experimental period then the plot of the $\log_{10}$ of the group mean counts (i.e. $\overline{Y}_t$) will give a linear plot against 't'. This is indeed what was observed, to a sufficient approximation for the present purposes.

It is customary to state erythrocyte survival in terms of half life, but, as Bergner (1965) has observed, half life 'has hitherto not been given any general physicobiological significance and its commonly accepted clinical significance is merely phenomenological and is due to the parameter having been presented together with clinical data for such a long time'. The

half life is, moreover, unsatisfactory for purposes of statistical analyses because of the way in which its scatter depends on the erythrocyte destruction rate. For these reasons, and the fact that the slope is a direct measure of erythrocyte elimination, we have preferred to use the parameter 'k' for statistical comparison. However, to conform with accepted usage, the parameter designated by Bergner (1965) as the t_{50}, which is equivalent to what is commonly called the half life, has been calculated and used in the tables. Mathematically, t_{50} is determined to be the value of 't' at the point where $\underline{Y}_t = 0{\cdot}5$, and hence $t_{50} = \log_{10}(0{\cdot}5)/k$, i.e. $-0{\cdot}3010/k$. For group mean t_{50}, the mean slope ($\bar{k}$) is used in the formula.

TABLE 1. Erythrocyte survival in standard mice strains (C57Bl and CBA) determined with ^{51}Cr label

Survival Values*	Strain	
	C57Bl	CBA
t_{50}	10·0 days	14·1 days
k	$-0{\cdot}0300 \pm 0{\cdot}0021$	$-0{\cdot}0213 \pm 0{\cdot}0035$
EDR	6·7% per day	4·8% per day
n	8	7

The difference between the two means is significant ($t = 3{\cdot}69$, $P < 0{\cdot}01$).

In C57Bl the mean t_{50} given by DF^{32}P is 6·5 days (consult Table 3).

*For this table and those which follow: t_{50} = half life; k = slope of isotope disappearance curve (see Methods); EDR = erythrocyte destruction rate; n = number of animals in experiment.

Determination of residual organ activity

Spleens and livers were removed from CBA mice and one group of NZB/Bl following completion of erythrocyte survival studies. Residual radioactivity was determined using a well-type scintillation counter. Organ specific activities were determined by the quotient:

$$\frac{\text{Absolute count of radioactivity}}{\text{Organ weight in grams}}$$

The spleen/liver activity ratio is found from:

$$\frac{\text{Spleen specific activity}}{\text{Liver specific activity}}$$

RESULTS

Table 1 shows the results of erythrocyte survival studies in C57Bl and CBA mice obtained with *in vitro* ^{51}Cr-labelled cells.

Survival of autologous ^{51}Cr-labelled cells in unselected NZB/Bl gave a wide range of t_{50}, as might be expected. We have followed the practice of others (Bielschowsky *et al.*, 1959; Long, Holmes & Burnet, 1963) in estimating the haemolytic activity in NZB/Bl mice by means of Coombs testing and reticulocyte per cent counts, and Fig. 1 shows that there is good correlation between these values and individual t_{50}s. The 'breakaway' point on this graph appears to be at a reticulocyte count of about 10%. With the exception of one animal, those with high reticulocyte levels had strongly positive (3+ or 4+) Coombs test. These retrospective observations led us to formulate the following classification of activity based on haematologic and serologic values:

Highly active: 3+ or 4+ Coombs occurring during the term of the experiment. Reticulocyte count of 10% or more.

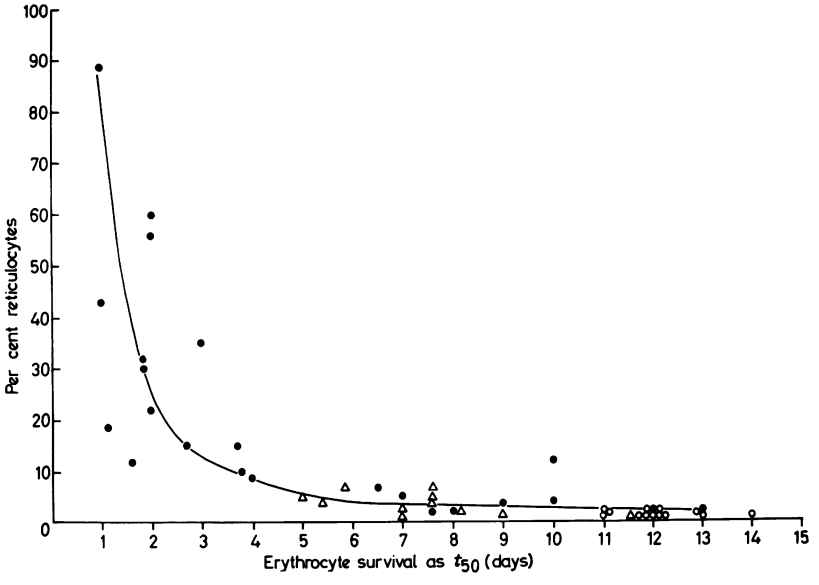

FIG. 1. Relationship of erythrocyte survival to reticulocyte count in NZB/Bl mice. ●, Coombs positive throughout experiment; △, converted to positive; ○, Coombs negative throughout.

Inactive: Without regard to reticulocyte count, this group was constituted of animals which failed to give a positive Coombs reaction at any testing. It follows, not surprisingly, that none of these had a reticulocyte count greater than 2%.

Intermediate: These are the animals which either (a) converted from negative to positive Coombs over the experimental period, (b) were weakly positive (1+ and 2+) throughout, or (c) the exceptional case, combined a strong Coombs with a reticulocyte percent count of less than 10%.

Table 2, in which NZB/Bl mice have been classified according to the above criteria, shows the pooled results of autologous ^{51}Cr studies; it seemed, on the basis of the significant differences between the groups, that the use of this tentative grading system was justified in further studies.

To evaluate the utility of the technique for subsequent use in isogeneic erythrocyte

transfer experiments in NZB/Bl, simultaneous autologous double labelling was done in this strain, without regard to Coombs testing, and also in C57Bl mice. Results appear in Table 3; for each animal, the difference (δ) between the slopes determined by the two methods was calculated as k_{Cr}–k_{DFP}. It will be noted that, in the NZB/Bl, the differences

TABLE 2. Survival of ^{51}Cr-labelled autologous erythrocytes in NZB/Bl mice, grouped with respect to disease activity

Survival values	Activity groups		
	Highly active	Intermediate	Inactive
t_{50}	2·1 days	8·2 days	13·3 days
k	—0·1448 ± 0·0274	—0·0368 ± 0·0108	—0·0227 ± 0·0052
EDR	28·4% per day	8·2% per day	5·1% per day
n	9	8	8

The difference between any two groups is significant ($P < 0{\cdot}01$).

TABLE 3. Comparison of survival measured by two methods (^{51}Cr and DF^{32}P) simultaneously in individual animals of NZB/Bl and C57/Bl strains

Animal No.	Strain					
	NZB/Bl			C57Bl		
	k_{Cr} all negative	k_{DFP} all negative	$\delta = k_{Cr}$–k_{DFP}	k_{Cr} all negative	k_{DFP} all negative	$\delta = k_{Cr}$–k_{DFP}
1	0·0569	0·0553	—0·0016	0·0301	0·0509	+0·0208
2	0·0357	0·0319	—0·0038	0·0387	0·0620	+0·0233
3	0·0319	0·0252	—0·0067	0·0301	0·0585	+0·0284
4	0·0268	0·0292	+0·0024	0·0284	0·0398	+0·0114
5	0·0469	0·0432	+0·0037	0·0319	0·0402	+0·0083
6	0·0796	0·0796	0	0·0347	0·0437	+0·0090
7	0·0237	0·0276	+0·0039	0·0252	0·0328	+0·0076
8	—	—	—	0·0282	0·0398	+0·0190
	Mean difference $\delta = -0{\cdot}0013$. This is *not* significantly different from zero ($n = 7$, $t = 1{\cdot}08$, $P > 0{\cdot}05$)			Mean difference = $\delta = +0{\cdot}0160$. This significantly differs from zero ($n = 8$, $t = 6{\cdot}35$, $P < 0{\cdot}01$)		

between slopes were not great in spite of the fact that the group includes animals with widely disparate haemolytic activities. This agreement can be seen more clearly in Fig. 2, which shows the results (t_{50}) of all the double labelling experiments in NZB/Bl; the points representing the seven animals which received autologous ^{51}Cr labelled cells are shown by $\triangle$ symbols. On the other hand, in C57Bl mice the DF^{32}P slopes were steeper than that

observed with ^{51}Cr; that is, t_{50} indicated by $DF^{32}P$ was shorter than that measured with ^{51}Cr. The mean discrepancy between the two methods observed in C57Bl ($\bar{\delta} = +0{\cdot}0160$) is significantly greater ($t = 4{\cdot}8$; $P < 0{\cdot}01$) than that seen with NZB/Bl ($\bar{\delta} = -0{\cdot}0013$).

Having established that $DF^{32}P$ studies in individual NZB/Bl mice were reliable measures of haemolytic activity, correlating very well with results of ^{51}Cr-labelled autologous erythrocyte disappearance, the next step was to examine the behaviour of Coombs positive

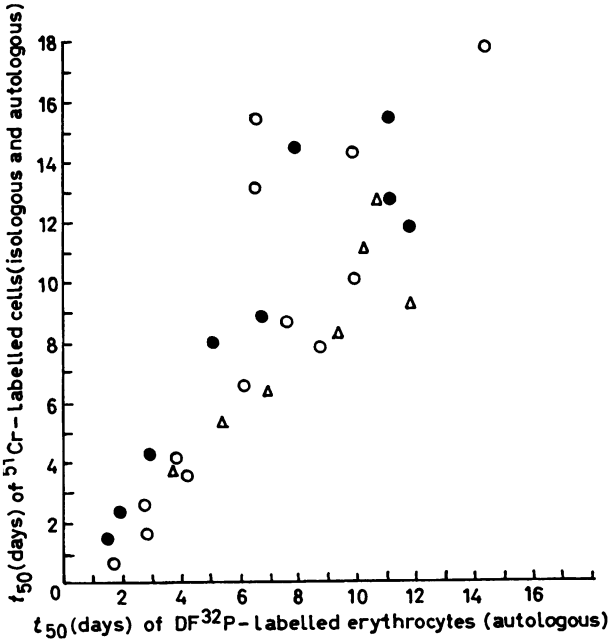

FIG. 2. Relationship between the survival, as t_{50}, of *in vitro* ^{51}Cr-labelled autologous and isologous cells and the survivals of *in vivo* $DF^{32}P$-labelled autologous cells. ○, Coombs negative donor; △, autologous cells; ●, Coombs positive donor.

TABLE 4. Survival of isogeneic ^{51}Cr-labelled erythrocytes in NZB/Bl mice, grouped with respect to disease activity

A. Positive donor

Survival values	Recipients	
	Inactive	Intermediate
t_{50}	13·5 days (13·3)	8·4 days (8·2)
k	$-0{\cdot}0223 \pm 0{\cdot}0026$	$-0{\cdot}0357 \pm 0{\cdot}0020$
EDR	5·2% per day	15·3% per day
n	4	2

B. Negative donor

Survival values	Recipients	
	Intermediate	Highly active
t_{50}	6·8 days (8·2)	0·9 days (2·1)
k	$-0{\cdot}0443 \pm 0{\cdot}0210$	$-0{\cdot}3263 \pm 0{\cdot}3021$
EDR	9·0% per day	52·8% per day
n	8	16

The figures in parentheses are t_{50} values observed with autologous ^{51}Cr-labelled cells (see Table 2).

cells in 'inactive' mice and, conversely, of Coombs negative erythrocytes in mice classified as 'highly active'.

The results are summarized in Table 4. There is no significant difference between the destruction rate of autologous ^{51}Cr-labelled erythrocytes and that of transfused isogeneic erythrocytes in any group, irrespective of the presence or absence of a Coombs test discrepancy between donor and recipient, nor is there any significant difference between the results obtained with cells from a Coombs positive donor transfused to either an 'inactive' or 'intermediate' recipient (Table 4A). On the other hand, cells from Coombs negative donors (Table 4B) were destroyed at a significantly greater rate in 'highly active' recipients than in those classed as 'intermediate' ($t=2{\cdot}36$; $0{\cdot}05>P>0{\cdot}02$). In some animals the survival of autologous erythrocytes (labelled with DF^{32}P) and transfused isogeneic erythrocytes (labelled with ^{51}Cr) were determined simultaneously. The correlation between the t_{50}s determined by the two labels, reflecting the behaviour of different erythrocyte populations, can be seen in Fig. 2; the mean difference ($\bar{\delta}$) between results observed in individuals (k_{Cr}–k_{DFP}) was not significantly different from zero. It thus appears clear that the

TABLE 5. Erythrocyte survival measured by DF^{32}P in NZB/Bl mice, grouped with respect to disease activity

Survival values	Inactive	Intermediate	Highly active
t_{50}	12·0 days	7·4 days	2·4 days
k	—0·0251 ± 0·0028	—0·0406 ± 0·0109	—0·1248 ± 0·0412
EDR	5·7% per day	8·3% per day	25% per day
n	5	19	11

The difference between 'inactive' and 'intermediate' is significant ($t = 2{\cdot}30$, $0{\cdot}05>P>0{\cdot}02$) as is the difference between 'intermediate' and 'strong positive' ($t = 8{\cdot}49$; $P<0{\cdot}01$).

disappearance rate of transfused isogeneic erythrocytes is essentially the same as that of autologous erythrocytes in NZB mice, irrespective of any difference in Coombs testing or haemolytic activity between donor and recipient.

Table 5 shows the results of erythrocyte survival measured with DF^{32}P in NZB/Bl mice grouped with respect to haemolytic activity; the difference between each group is significant. As might be expected from observations noted above, however, there is not significant difference within a particular activity group between the group mean survival determined by ^{51}Cr (Table 2) and that measured by DF^{32}P.

Post-mortem residual activities in the liver and spleen of 'inactive' NZB/Bl were not significantly different from those observed in CBA mice. In general, spleen weights were greater in 'highly active' mice than in 'inactive' mice but there was no clear difference between the spleen residual activities found in the two groups. Liver residual activity (absolute counts) and specific activity were higher in 'highly active' mice than in 'inactive' animals. Fig. 3 illustrates the finding of very low spleen/liver activity ratios in several 'highly active' mice showing rapid erythrocyte elimination.

DISCUSSION

Table 6 summarizes the available information on erythrocyte survival in mice, determined by ^{51}chromium-labelling. It is conceivable that the variations represent true interstrain differences but the possibility that these are methodological in origin must be considered. It does appear from our results, however, that there are genuine differences, at least between the three strains studied. This wide range may, in some way, be related to interstrain

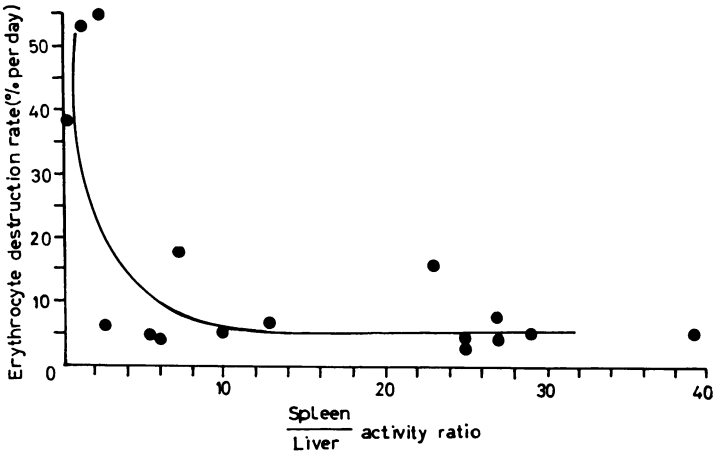

FIG. 3. The relationship between erythrocyte destruction rate and spleen/liver activity ratio.

TABLE 6. Erythrocyte survival in various mice strains, determined by ^{51}Cr-labelling

Strain	Erythrocyte half-life (days)	Reference
(101 × C3H)F_1	20·0	Smith & Tohá (1958)
LAF	14·0	Oliner, Schwartz & Dameshek (1961)
Wild	8·8	Anderson, Heustis & Motulsky (1960)
A	8·6	Möller (1965)
C57Bl	6·3	Schneiberg & Watras (1961)
C57Bl	10·0	This study
CBA	14·1	This study
NZB/Bl 'inactive'	13·3	This study

differences in basal metabolic rate, as suggested by Rodnan, Ebaugh & Fox (1957). Suffice it to say that the young New Zealand Black mouse is probably 'normal' with respect to erythrocyte elimination.

Eadie, Smith & Brown (1960) have suggested that *in vivo* labelling with DF^{32}P tags all cells, but it is pertinent to ask if individual cells can carry both ^{51}Cr and ^{32}P labels. If,

indeed, they do, then the discrepancies observed between the t_{50} measured by ^{51}Cr and that of $DF^{32}P$, particularly apparent in the C57Bl, suggests that either (a) the elution of DFP is more rapid than chromate, or (b) the cells which survive *in vitro* chromium labelling may be more durable. If erythrocyte double-tagging does not occur, then the early fall-off in $DF^{32}P$ activity seen in the C57Bl may be due to cell damage by the label (Cline & Berlin, 1962) or, again, to an elution differential. It is, perhaps, surprising that in a population such as the NZB/Bl, which is non-uniform as regards haemolytic activity, the erythrocyte survivals shown by two methods agree so much better than they do in a standard strain. Lacking information regarding label elution, such as might be obtained by differential agglutination techniques (Ashby, 1919), we have not attempted to formulate a hypothesis to explain these findings.

Accurate prediction of haemolytic activity in NZB/Bl mice, on the basis of Coombs test and reticulocyte count alone, is not always possible. Persistent Coombs positivity may, for example, be too stringent an index of activity; according to Dacie (1962), patients with autoimmune haemolytic anaemia 'can undergo clinical and haematologic cures without showing concomitant serologic improvement'. In reflecting on some of our data, it seemed that most of the 'outliers' could have been better classified if account had also been taken of haemoglobin values. For example, several mice placed in the 'highly active' group on the basis of the Coombs test and reticulocyte counts alone, did not have significant anaemia and were found to have erythrocyte survival which fell more properly in the 'intermediate' category. If all three of the more simply determined haematologic and serologic values are to be utilized in predicting the activity of a haemolytic process, a formula becomes necessary if complicated sub-classifications are to be avoided and this must take into account the fact that considerable changes in these parameters, particularly the reticulocyte count, can occur during the course of a few weeks. An empirical 'activity coefficient' (AC) has been used, in which

$$AC = \left[\left(\frac{C_s + 1}{H_s - R_s + 100}\right) + \left(\frac{C_f + 1}{H_f - R_f + 100}\right)\right] \times 100$$

where C_s = Coombs test at start of test period;
H_s = haemoglobin at start of test period;
R_s = reticulocyte per cent count at start of test period;
C_f, H_f and R_f are corresponding values at the finish of the test period.

With AC calculated for each animal, it was found that the resultant values plotted against the corresponding slopes (k) give acceptable linearity. Activity coefficients have varied from 1 to 10, with low values for 'inactive animals' and high for 'highly active'.

It has been reported (Loutit, 1946; Mollison & Patterson, 1949; Selwyn & Hackett, 1949; Culp & Chaplin, 1960) that Coombs positive erythrocytes from patients with autoimmune immune haemolytic anaemia can survive normally in normal human patients, whereas normal cells are destroyed rapidly in patients with autoimmune haemolytic anaemia. The present findings, and similar observations made independently by Long, Pye & Holmes (personal communication), demonstrate that the same phenomena occur in mice, and confirm the conclusion that the presence of globulin or incomplete antibody on the surface

of the red cell is not a sufficient condition for shortened cell survival. Whether, in the present system, it is a necessary condition remains undetermined because the shortened survival of normal cells in Coombs positive animals might conceivably depend, in part, on these cells becoming coated with antibody after being transferred to their new host.

The finding that Coombs positive cells survive normally in Coombs negative (inactive) recipients raises several interesting questions. Is the fact that the cell surface is coated with globulin simply irrelevant, or does it mediate an effect which is more or less counter balanced by some other property of the transferred cell population? In the latter event, what might this property be?

There would seem to be at least two ways in which the transferred cell population is atypical, in addition to the fact that many of its members are Coombs positive. In the first place it almost certainly contains a high proportion of young cells, and secondly it contains a high proportion of cells which may be described as 'premature' in the sense that they have entered the circulation when they were less mature than is normally the case. Reticulocytes certainly fall in the category of premature cells (Wasserman & Rubin, 1964), and as has been seen they were present in relatively large numbers in the cell populations transferred from Coombs positive donors. It seems reasonable to expect that cells which were merely young would have a relatively long life expectancy. In contra-distinction, those cells which have entered the circulation prematurely might well be subject to a high 'infant mortality', much as has been proposed by Wasserman & Rubin (1964) with respect to thalassaemia. This is consistent with the observation of Sutherland, Eisentraut & McCall (1963) that, in dogs, reticulocytes transfused from blood-compatible donors have a shorter survival than more mature erythrocytes and may also account for the observations that, following the induction of acute anaemia in rats (Berlin & Lotz, 1951; Stohlman, 1961) and dogs (Cline & Berlin, 1962), erythrocyte survival is shortened. It seems worthy of consideration that, in NZB/Bl mice, the acquisition of a coating of globulin, though possibly disadvantageous from an immunological point of view, might protect reticulocytes and other immature cells from the non-immunological consequence of entering the circulation before they should normally have done so. The notion that a coating of globulin may be protective in various contexts is by no means new; the concepts in this regard have recently been reviewed by Thomas (1964).

It would be of interest in this connection to compare the uptake and elution of ^{51}Cr and ^{32}P in cells differing in respect of age or state of maturation, but the available data is fragmentary and perhaps not entirely consistent. It would seem, for example, from the first report of the use of ^{51}Cr to determine erythrocyte survival in man by Ebaugh, Emerson & Ross (1953) that selective tagging of old cells occurs, since the only other possible explanation of their finding that untagged cells survive longer than tagged cells would seem to be that the label damaged the cells, and this seems unlikely in the light of the work of Necheles, Weinstein & LeRoy (1963) and Giblett *et al.* (1956). On the other hand it has been reported more recently that the uptake of chromium by erythrocytes is independent of cell age in both humans (Donohue *et al.*, 1955) and dogs (Stohlman & Schneiderman, 1956).

It is hoped that it may be possible to resolve some of these questions by making use in NZB/Bl mice of the technique described by Sutherland *et al.* (1963) for obtaining reticulocyte rich suspensions.

Splenectomy has been found by Holmes & Burnet (1963) to aggravate the nephritic

component of the autoimmune syndrome that occurs in NZB/Bl females while ameliorating the haemolytic anaemia in males, but the remission was only temporary. This short-lived improvement may be explained by the results of organ counting which suggest that the liver also plays an important part in red cell destruction.

ACKNOWLEDGMENTS

Part of the cost of this investigation has been defrayed by a generous grant from the Distillers Company to the University of Edinburgh for the study of congenital disorders. One author (E.L.) is in receipt of a U.S. Public Health Service Special Fellowship (1-F3-HE-23, 384-01) from the National Heart Institute; another (G.W.K.D.) is Wilkie Research Fellow of the University of Edinburgh.

Our thanks are due to Dr Donald Michie for helpful advice in matters statistical and to Miss Marjory McArthur and Mr George Brooks for their excellent technical assistance.

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