STUDIES ON QUANTITATION OF THE ^{51}Cr-ERYTHROCYTE SPLEEN-TO-LIVER RATIO

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After one tags hemoglobin (within erythrocytes) with ^{51}Cr, the labeled cells distribute themselves throughout the vascular tree (*1*). With the passage of time, the spleen and other sites remove aged cells. Therefore radioactivity found in the spleen may change from the initial value (*2*). In cases of hypersplenism, splenic radioactivity often increases markedly, forming the basis for calculating the spleen-to-liver ratio of radioactivity as a function of time. Studies of this ratio have remained empirical, and there have apparently been no reports of attempts to explain the kinetics of the process. As an initial step in quantitating the phenomenon as well as its variation in disease states, we report here a model of the ^{51}Cr-erythrocyte spleen-to-liver ratio and discuss procedures for evaluating the relevant quantities. Use of a new parameter of splenic activity, the splenic exit rate constant, is proposed. The latter part of the paper gives experimental data bearing on the model as well as analog-computer solutions of the equations.

THEORY

Following ^{51}Cr tagging of hemoglobin, the measured activity in erythrocytes within the vascular tree decreases with time. Our first assumption in developing a mathematical model describing this decrease is that two processes account for it (we will neglect radio-decay by assuming that all counts can be appropriately corrected). The first process is red-cell destruction, which we assume takes place in the spleen, the liver and possibly in one or more additional sites. The second process is elution of ^{51}Cr. We assume that the eluted radiolabel no longer attaches to other erythrocytes, is quickly cleared and does not accumulate in the spleen, liver or other organs. On the basis of present data, this appears to be a reasonable assumption (*3*). (The models can be corrected if this proves not to be the case.)

These statements are summarized by the equation

$$\frac{dB_t}{dt} = -(\lambda_{d,spleen} + \lambda_{d,liver} + \lambda_{d,other} + \lambda_e)B_t \quad (1)$$

in which B_t is the activity in erythrocytes in the vascular tree, λ_e is the rate constant for ^{51}Cr elution and $\lambda_{d,spleen}$, $\lambda_{d,liver}$ and $\lambda_{d,other}$ are the rate constants associated with red-cell destruction (sequestration) by the spleen, liver and other sites, respectively. Note that we are considering a decrease due to removal of radiochromium only (and not physical decay). Defining an "effective rate constant," λ, as follows:

$$\lambda = \lambda_{d,spleen} + \lambda_{d,liver} + \lambda_{d,other} + \lambda_e \quad (2)$$

we have as the solution for Eq. 1:

$$B_t = B_o e^{-\lambda t} \quad (3)$$

in which B_o is the activity at $t = 0$.

Radioactivity in the spleen increases as the spleen sequesters erythrocytes. Our assumption is that this entry is a first-order process described by:

$$\text{Spleen uptake rate} = \lambda_{d,spleen} B_t = \lambda_{d,spleen} B_o e^{-\lambda t} \quad (4)$$

The rate constant $\lambda_{d,spleen}$ probably depends on the rate of blood flow to the spleen, the size of the spleen and whether or not it is diseased. We will assume that $\lambda_{d,spleen}$ is constant with time; in more sophisticated treatments (if the data justify such an extension) $\lambda_{d,spleen}$ can be made an explicit function of flow, size, time or degree of splenic saturation with erythrocytes.

In this model we would like to take into account the *exit* of ^{51}Cr from the spleen. In the simplest possible mechanism for exit, the exit term would be proportional to the amount of ^{51}Cr in the spleen.

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Letting this proportionality constant be r, we have

$$\text{Spleen exit rate} = rS \quad (5)$$

in which S is the activity in the spleen due to red-cell destruction. Combining Eqs. 4 and 5 (recalling the previous assumption that the exiting radiolabel is immediately removed from the blood), we have

$$dS/dt = \text{uptake} - \text{exit}$$
$$\text{or} \quad dS/dt = \lambda_{d,\text{spleen}} B_o e^{-\lambda t} - rS \quad (6)$$

This differential equation has the two solutions

$$S = \left(\frac{B_o\lambda_{d,\text{spleen}}}{r-\lambda}\right)e^{-\lambda t} + \left(S_o - \frac{B_o\lambda_{d,\text{spleen}}}{r-\lambda}\right)e^{-rt} \quad (\text{if } \lambda \neq r) \quad (7)$$

$$\text{and} \quad S = (B_o\lambda_{d,\text{spleen}}t + S_o)e^{-\lambda t} \quad (\text{if } \lambda = r) \quad (8)$$

in which S_o is the radioactivity at time zero due to initial sequestration (in simplest model S_o is zero).

In addition to the ^{51}Cr present in the spleen due to red-cell destruction, some is present in viable cells that happen to be in the splenic vasculature. This latter quantity represents a fraction f of the tagged cells in the blood. We assume that the labeled cells mix instantaneously and completely throughout the vascular system. Therefore, the amount of activity in the spleen at time zero (before sequestration) is fB_o. Assuming that the fraction of blood in the spleen remains constant, the total radioactivity of the spleen will be given (for $\lambda \neq r$) by

$$S_{\text{total}} = S + f(B_o e^{-\lambda t}) \quad (9)$$

$$S_{\text{total}} = \left(f + \frac{\lambda_{d,\text{spleen}}}{r-\lambda}\right)B_o e^{-\lambda t} + \left(S_o - \frac{B_o\lambda_{d,\text{spleen}}}{r-\lambda}\right)e^{-rt} \quad (10)$$

DISCUSSION OF THE PARAMETERS

To quantitate the model, we must have a means of evaluating the relevant parameters.

With the assumption that the decay constants for chromium elution (λ_e) and chromium loss due to red-cell destruction (λ_d) are additive, an effective decay constant for ^{51}Cr-tagged erythrocytes was defined in Eq. 2. The effective half times for ^{51}Cr red cells vary between the extremes of about 30 days (normal) to 1 day (greatly accelerated destruction). Therefore because $\lambda = \ln 2/t_{1/2}$, the decay constant will vary from 0.0231/day to 0.693/day. The term λ may be determined experimentally as the slope of $\ln B_t$ as a function of time; this follows from Eq. 3.

By means of the Ashby technique, it has been well recognized that normal erythrocytes have a life span of about 120 days. Hence, 1/120 or 0.83% of the erythrocyte population is destroyed daily (λ_d = 0.0083/day). Since

$$\lambda = \lambda_d + \lambda_e \quad (11)$$

we can calculate that for a $t_{1/2}$ of 30 days (λ = 0.023/day) using λ_d above, the rate constant for ^{51}Cr elution is about λ_e = 0.0148/day. There are no accurate data for the value of λ_e in disease states. As a first approximation we assume λ_e is invariant.

The discussion up to this point has assumed a single erythrocyte population. Following ^{51}Cr tagging of red cells, there may functionally be two or more populations. For example, some of the cells may be traumatized during the labeling process. The simplest approach is to assume that the total activity in the blood is the sum of the activity of the two components and that the kinetics of each component can be handled separately. Thus, if B_I and B_{II} represent the activity of undamaged and damaged cells,

$$B = B_I + B_{II} \quad (12)$$

$$\text{with} \quad dB_I/dt = -\lambda_I B_I \quad (13)$$

$$\text{and} \quad dB_{II}/dt = -\lambda_{II} B_{II} \quad (14)$$

If the damaged cells disappear very rapidly (i.e. λ_{II} is very much larger than λ_I), then we can still use the simpler model described by Eq. 10 by adjusting the initial conditions (i.e. S_o and B_o) in that equation. Thus, S_o in Eq. 10 will be the amount of radioactivity in the spleen that appears very rapidly due to the sequestration of damaged cells, and B_o will be correspondingly reduced.

A technique for estimating f is as follows. Splenic dimensions can be obtained by using x-rays of the abdomen or radioisotope scans of the spleen. From these dimensions, splenic weight can be estimated (*4,5*). We can then employ the relationship

$$sm/V = f \quad (15)$$

in which s is the splenic weight, m is the quantity of blood per unit splenic weight and V is the blood volume. The contribution of f to the over-all model is fairly small. For example, if it were as much as one-half blood, a spleen of 200 gm would contain 100 ml blood. This is 100/5,000 = 2% of the total blood volume. Hence, even a considerable error in estimating f will probably not grossly influence the curve of the splenic content of tagged red blood cells.

An alternate approach to finding f is as follows. If the splenic weight s in the normal individual were related to the body weight W by an allometric relationship (*6*)

$$s = aW^x \quad (16)$$

then we can combine Eqs. 15 and 16 to obtain

$$f = maW^x/V \quad (17)$$

We will return to these considerations later.

There are several approaches to estimating the splenic exit rate constant r.

1. The splenic content of radioactivity can be followed as a function of time. Since the other constants are known or can be closely approximated, an analog-computer solution of Eq. 9 can be set up and the value of r varied until the curve matches the known data. For this purpose, the analog computer can be set into the repetitive-operation mode so that the potentiometer representing r can be rapidly varied. It is also possible to use a digital computer to perform this "fitting" procedure.

2. The most definitive experimental method for calculating the splenic exit rate constant is as follows. The subject's erythrocytes are tagged with ^{51}Cr, and several days are allowed to pass after reinjection. At that point, blood is replaced by an exchange transfusion. The fraction F of original blood remaining in the experimental subject is calculable from (*7*)

$$F = (1 - T/c)^n \qquad (18)$$

Here c is the number of endogenous cells, T cells are removed at each cycle of transfusion and n cycles are carried out. F is reduced to a negligible value by repeated transfusions. At that point there is no longer any entry (or negligible entry) of radioactivity into the spleen, and there is essentially no radioactivity in the circulation (i.e., B is nearly zero). Equation 6 reduces to

$$dS/dt = -rS \qquad (19)$$

In terms of Eq. 10 this is of course

$$S = S_o e^{-rt} \qquad (20)$$

By following the subsequent decrease in splenic radioactivity with time, r can be calculated. Results using this approach are reported in a later section.

3. If heat-denatured tagged cells, which quickly leave the blood stream and are taken up by the spleen (*8*), are handled identically to non-heated cells or bear some constant relationship to non-denatured cells as far as their exit or disappearance from the spleen is concerned, they can be used to calculate r. This approximates the above situation in which the entry term was absent. A literature value for r determined this way is about r = 0.099/day, or a half time of 7 days (*9*) for removal of ^{51}Cr from the spleen when there is no additional entry.

4. If tagged erythrocytes could be directly injected into the spleen and if they were trapped in that organ, there would again be the above situation of negligible activity in the blood and a measurable egress.

5. A useful clinical approach to the splenic exit rate constant is as follows. Splenic counts are corrected for circulating tagged erythrocytes (the term $fB_oe^{-\lambda t}$). The corrected counts are described by Eq.

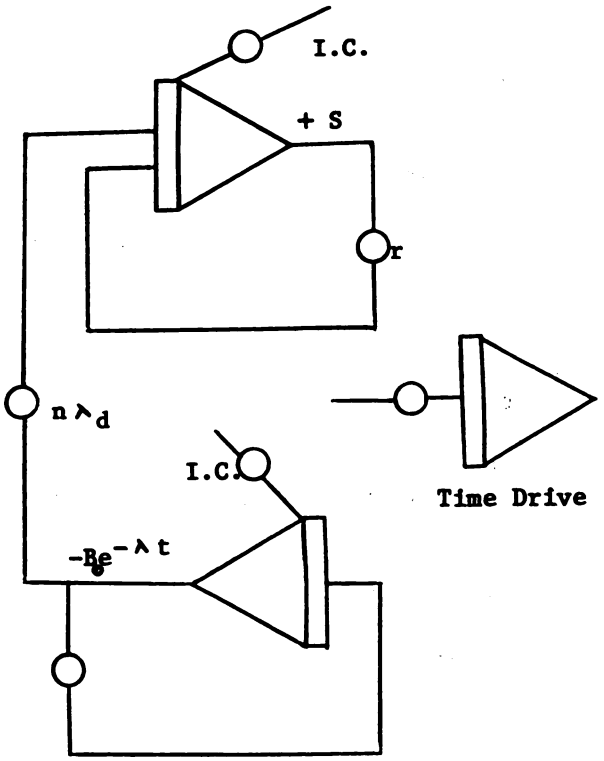


FIG. 1. Analog-computer patching diagram for solving Eq. 6 has triangle representing operational amplifier with feedback, triangle with extra back line indicating integrator and circle depicting a potentiometer. I.C. stands for initial condition. $\lambda_{d,spleen}$ is indicated as $n\lambda_d$ where n is number between 0 and 1; this serves as reminder that $\lambda_{d,spleen}$ is fractional value of λ_d. In Fig. 3, for example, n = 0.43.

6. When the curve of sequestered activity in the spleen reaches a peak, dS/dt = 0 and Eq. 6 reduces to

$$r = (1/S)\,\lambda_{d,spleen}\,B_oe^{-\lambda t} \qquad (21)$$

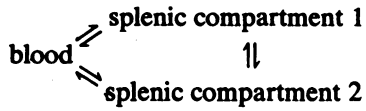
Since the remaining terms are known or can be closely approximated, a means is at hand for calculating r.

The final parameter in the model is $\lambda_{d,spleen}$. The term $\lambda_{d,spleen}/\lambda_d$ is the fraction of destroyed cells going to the spleen. We can approximate $\lambda_{d,spleen}$ as follows. Initial counts over the spleen (before sequestration) define $fB_oe^{-\lambda t}$. Counting a short time later (so that some sequestration has occurred but before there is appreciable exit from the spleen) gives: sequestered cells + $fB_oe^{-\lambda t}$. That is, Eqs. 9 and 10 have been solved at an early time before exit has become appreciable. In Fig. 3 a solution is shown that matches data on ^{51}Cr-erythrocyte uptake by the spleen of the rat. The value of $\lambda_{d,spleen}/\lambda_d$ is 0.43. The solution leads us to believe that the model is a reasonable one and worth investigating with more sophisticated techniques. We plan to use a digital computer to fit $\lambda_{d,spleen}$ and r simultaneously.

REFINEMENT OF THE MODEL

The model discussed up to this point can be extended in several directions. The limiting factor will be the availability of reliable data to test more complicated assumptions. We have already mentioned that $\lambda_{d,spleen}$ may not be constant but depends on whether or not the spleen is "preloaded." There might be a limited ability of the spleen to remove cells; this ability may be related to the size of the organ and in part to whether the reticuloendothelial system is competent or affected by disease processes.

A second possible extension is that the spleen actually consists of more than one compartment. Jandl and Aster (*10*) have recently summarized much data on hypersplenism. They conclude that in hypersplenism two distinct splenic compartments are present. The normal mixing compartment (50–80% of splenic radioactivity) is accompanied by a second compartment that has a slower turnover. It is as yet uncertain whether the true relationship is as simple as: blood $\rightleftharpoons$ splenic compartment 1 $\rightleftharpoons$ splenic compartment 2 or is more complex such as:



Appropriate equations can be written for each case and analog-computer models constructed.

The rate constants for red cells going from splenic compartments to blood might be important in two cases: (1) when a muscular spleen contracts (as in the dog) and forces cells back into the circulation (*11*), and (2) during exchange transfusions when some of the tagged cells might be leached into the circulation. There is also the possibility that the exit rate constant is not invariant but depends on other factors.

Experiments are underway to assess the validity of the present model and determine the need for possible refinements.

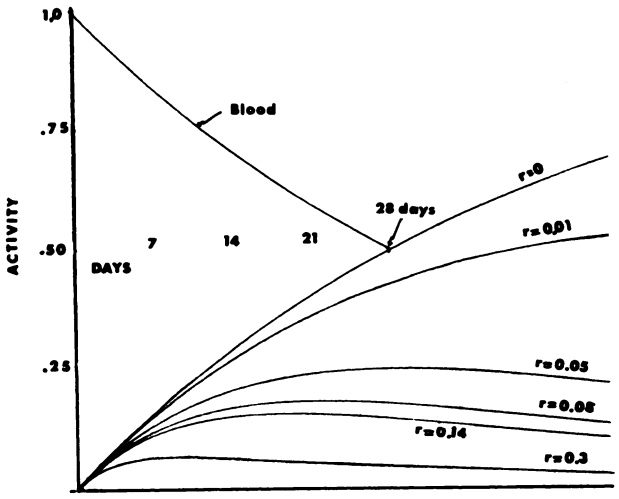


FIG. 2. Solutions of Eq. 6 obtained with analog computer and recorder. With ^{51}Cr-erythrocyte half-life in bloodstream of 28 days and all radioactivity removed from blood assumed to go to spleen ($\lambda_{d,spleen} = \lambda$), different solutions were generated by varying splenic exit rate constant r.

ANALOG-COMPUTER SOLUTIONS OF THE MODEL

The model proposed for the splenic content of ^{51}Cr-erythrocytes is a simple one that depends on entry of the cells from the bloodstream and their exit in proportion to the splenic content. This model is strongly dependent upon the splenic exit rate constant. To illustrate this, an analog computer was wired to provide a solution to Eq. 6 (Fig. 1). Note that the contribution due to the circulating erythrocytes is ignored because their contribution to the shape of the curve is minimal (this is true until r approaches λ). Typical solutions using a PACE TR 20 analog computer are shown in Fig. 2. If the splenic exit rate constant is zero, then in the simple case of all of the tagged cells going to the spleen and no elution (i.e., $\lambda_{d,spleen} = \lambda$), the splenic content will be a mirror image of the blood content of ^{51}Cr. This is shown in Fig. 2 as the curve for $r = 0$. As the splenic exit rate constant is increased, the resultant curves of splenic content not only are depressed downward, but also have their peak shifted toward the origin (until $r = \lambda$).

A case in which r is zero, or quite small, is provided by the uptake of ^{51}Cr-erythrocytes by the spleen of the rat (*3,12*). The solution for this case is shown in Fig. 3.

Another way of looking at the curve of the splenic content of ^{51}Cr is to recognize that, depending on the value chosen for r after the other constants are known, the curves can be made to rise, to reach a peak or to fall at the time the $t_{1/2}$ of circulating erythrocytes is reached. Goldberg, *et al* (*13*) have pointed out that the slope of the spleen-to-liver ratio of ^{51}Cr can be rising, stable or falling during the period from time zero to the half time of the circulating tagged erythrocytes. Looking at the slope of the splenic content of ^{51}Cr rather than that of the spleen-to-liver ratio may prove to be of clinical use (when coupled with the determination of r and ^{51}Cr-erythrocyte half time).

As an initial condition in the model, the spleen can be made to contain a fraction of the total tagged erythrocytes (due to rapid trapping of cells damaged during the labeling process). An analog-computer simulation of such a situation is shown in Fig. 4. The converse situation—of rapid elution of chromium from cells (*14*)—is also known to exist and

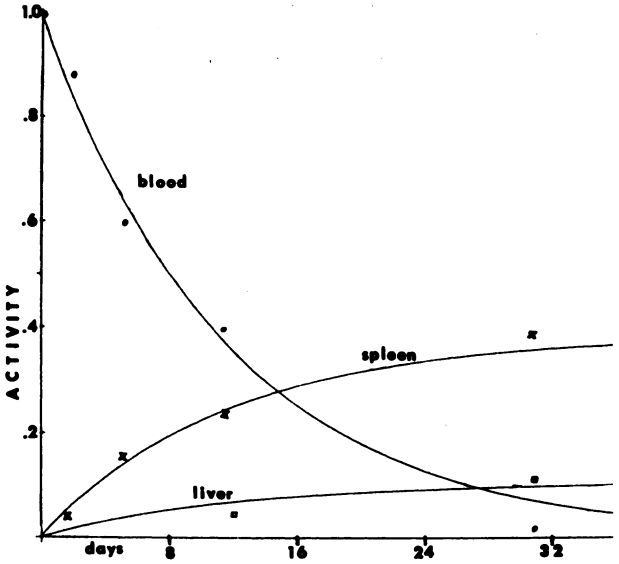


FIG. 3. Handling of ^{51}Cr-erythrocytes by rat. Points are data from Donohue *et al*, (12). Curves represent analog-computer solution of Eq. 6 using constants $\lambda_{d,spleen}/\lambda_d = 0.43$; $\lambda = 0.087$ (half-life in blood of 8 days). $\lambda_d = 0.077$, $\lambda_e = 0.01$ (elution = 0.01/day as shown by Owen and Orvis, ref. 16) and $r = 0$. Since spleen and liver contents alone do not equal loss from blood, there must be at least a third site of ^{51}Cr storage. Owen *et al* (3) have shown that bone marrow is active in storage. Rat is unusual because spleen avidly holds on to ^{51}Cr from sequestered tagged cells (3,12) (and hence r is some value close to zero). Reasonable value for r, from Owen's studies, is about 0.01/day.

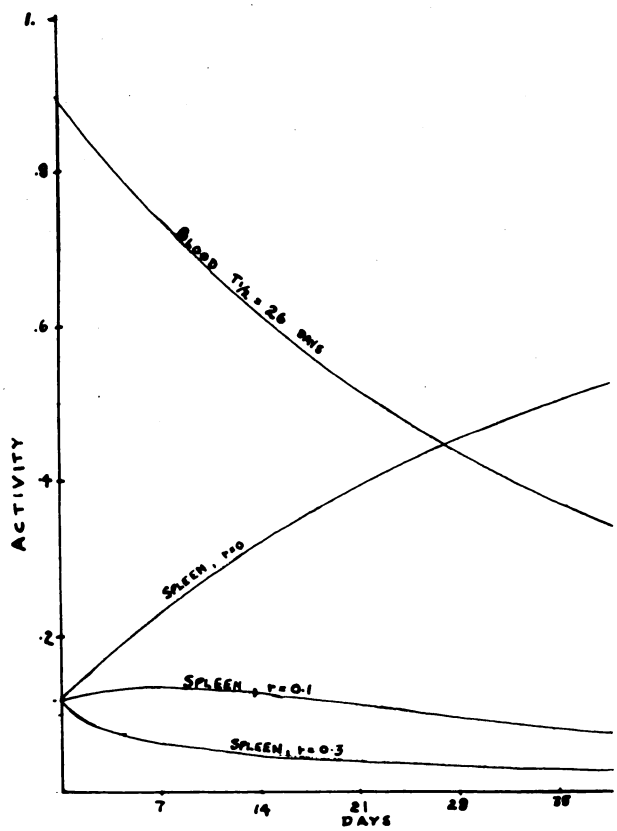


FIG. 4. Analog-computer simulation of splenic radioactivity content after initial condition of 10% tagged cells trapped in spleen. Computer has solved Eq. 6 with $\lambda_{d,spleen} = \lambda$. In this case blood half-life was assumed to be 26 days. Solutions are given with splenic exit rate constant r set equal to 0, 0.1 and 0.3/day. A value of $r = 0.1$–0.2 approximates situation of normal spleen illustrated by Weinstein *et al* (17). Hence value of r in human is about 10 times that in rat ($r_{rat} = 0.01$/day).

could be accounted for by appropriate correction of the factor λ_e.

The analog computer can also be used to solve more complex models of the splenic uptake of ^{51}Cr-erythrocytes such as those discussed under "refinements." A set-up similar to that in Fig. 1 is used (with adjustment of the constants) for hepatic uptake of ^{51}Cr. The spleen and liver contents are then divided to yield the spleen-to-liver ratio. It should be recognized that if the entire splenic and hepatic areas are counted, the resultant S/L ratio will largely reflect the relative sizes of the organs and the uptake per gram of tissue. The weight of spleen-to-liver remains close to the value 0.098 throughout most of life (Fig. 5).

EXPERIMENTAL ESTIMATE OF EXIT RATE CONSTANT

Several possible procedures for estimating the splenic exit rate constant r were discussed in a preceding section. It was pointed out (Eq. 20) that a host having tagged erythrocytes could have them replaced by exchange transfusion so that the blood level would be reduced to a negligible amount. This experiment was performed in two mongrel dogs (each weighing about 16 kg). Three donor dogs were used in each case. After tagging an aliquot of the

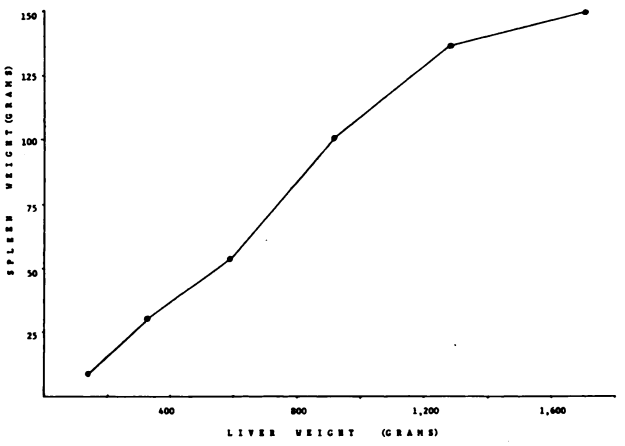


FIG. 5. Plot of spleen weight as function of liver weight. Data are from Seltzer *et al* (18). Points represent newborn, 1-year old, 5 years, 10 years, 15 years and "standard man."

dog's blood with ^{51}Cr and washing to remove unbound ^{51}Cr, the blood was reinjected. A week later, surface counts were taken over the spleen. Blood of each dog was replaced by that from donor dogs. This was done by means of 60 cycles of removal of 50 ml and replacement by an equal quantity from the donors (calcium was added to prevent tetany). Counts were then taken over the spleen area daily. Results were as follows:

1. Removal of radioactive cells from the circulation by exchange transfusion closely followed the prediction of Eq. 18. In the first dog 92% of the tagged erythrocytes were removed from the circulation. In the second dog 84% of the tagged cells were removed. The data for one dog are illustrated in Fig. 6.

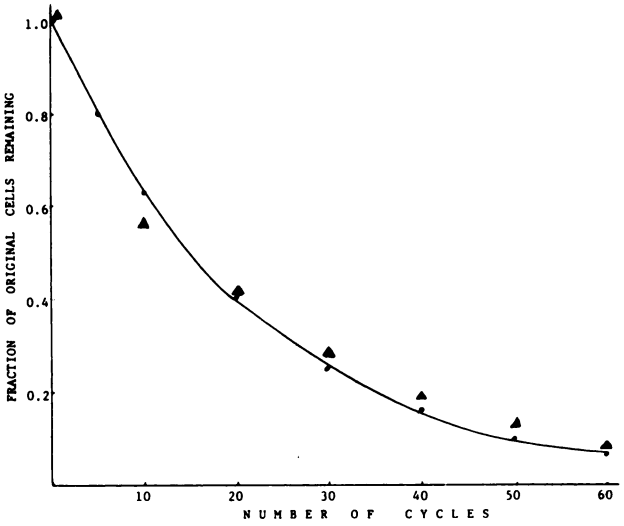


FIG. 6. Fraction of original ^{51}Cr-tagged erythrocytes remaining in 16-kg dog as function of number of cycles of 50-ml blood removed and replaced by donor (nonlabeled) blood. Points and smooth curve are prediction of Eq. 18. Triangles are values found by comparing blood samples with pre-transfusion sample.

2. A day after the exchange transfusion was completed, and at 1–2-day intervals thereafter, counts were taken over the splenic area. These counts per unit time decreased each day with an effective half time of about 7 days (in the dog with 92% of the tagged erythrocytes removed) and 13 days (in the dog with 84% of the tagged erythrocytes removed). The first value agrees with the results in ref. *9*. The fit to a first-order decay process was good (Fig. 7). Hence, the initial assumption that r is a first-order decay constant may be justified (at least for the first part of the exit in the dog).

DISCUSSION

The model proposed for splenic activity of ^{51}Cr following injection of tagged red cells is but a first approach and multiple improvements are possible. However, it does make clear two points.

1. Activity over the spleen should be plotted separately and analyzed. That is, combining the data in

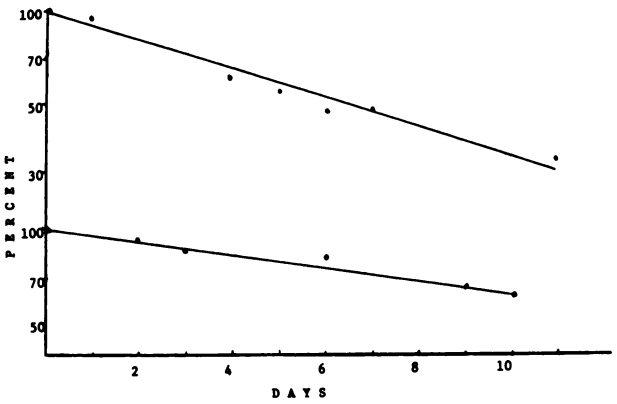


FIG. 7. Fit to first-order decay process of ^{51}Cr in spleen of two dogs following exchange transfusion to remove 92% and 84% of circulating tagged erythrocytes. Half-times are about 7 and 13 days and corresponding exit rates are $0.693/7 = 0.099$ and $0.693/13 = 0.053$. Value of r in dog may therefore be somewhere between rat and human values.

a spleen-to-liver ratio may actually be confusing rather than clarifying the role of the spleen.

2. The model is highly dependent upon the splenic exit rate constant r. Techniques for estimating this constant were pointed out.

Only with considerable additional experimentation can the relative reliability of each of the parameters be determined. Each disease entity might possibly be characterized by—and ranges determined for—λ, $\lambda_{d,spleen}$, $\lambda_{d,liver}$, f and the splenic exit rate constant r.

There is an additional aspect of this problem that deserves mention. It is desirable to have an estimate not only of function but of organ size or weight as well. In the case of the spleen, linear dimensions can be obtained by using abdominal roentgenograms as well as spleen scans. The formula of Whitley *et al*, (*4*) can then be used to calculate the splenic weight (within certain limits) from these dimensions. It should also be recognized that information about spleen size is also available from the apparent surface area of the spleen obtained by spleen scans. For example, using the points of the table of Holzbach *et al* (*5*) relating spleen weight to surface area on the lateral scan one obtains an equation of the form:

$$s = nA^z \qquad (22)$$

in which the spleen weight s is in grams, A is the surface area in cm^2 and n and z are constants. Scanning from more than one direction may give data that are useful in refining these estimates. Similarly, on

the basis of liver scans, the technique of Yagan *et al* (*15*) can be used to yield a reasonable estimate of hepatic weight. Then

$$\frac{\text{Spleen weight (scan)}}{\text{Liver weight (scan)}} = \text{Weight ratio (scan)} \quad (23)$$

The spleen-to-liver ratio of weights (from the scan) can be compared with the spleen-to-liver ratio of ^{51}Cr activity.

In the "average" adult primate the allometric relationship describing spleen weight s in terms of body weight W in grams (*6*) is

$$s = 0.0042W^{0.85} \quad (24)$$

For the liver the relationship is

$$L = 0.052W^{0.93} \quad (25)$$

The theoretical S/L weight ratio is thus approximately of the magnitude

$$S/L = 0.081W^{-0.08} \quad (26)$$

The prediction of Eq. 26 can be compared with that of Eq. 23. We could also estimate the weight of the organ on the basis of the scan data and on the basis of the allometric relationship. Thus

$$\frac{\text{Spleen weight (scan)}}{\text{Spleen weight (allometric)}} = S_{ratio} \quad (27)$$

And similarly for the liver

$$\frac{\text{Liver weight (scan)}}{\text{Liver weight (allometric)}} = L_{ratio} \quad (28)$$

Although the techniques are still in the earliest stages of development, it may be possible eventually to calculate activity as a function of organ weight. Such functional indices (activity/weight = a functional index) would be a significant advance beyond present methods of interpreting results.

SUMMARY

A reasonable first conceptual model of the splenic content of ^{51}Cr following injection of labeled erythrocytes is presented. Analog-computer solutions of the equations are shown, and the model is found to be highly sensitive to the splenic exit rate constant. This rate constant is suggested as an additional indicator of splenic activity. Procedures are proposed for clinical estimation of the splenic exit rate constant (such as computer matching of the known data and solution of the equations at the point at which splenic radioactivity has peaked). Experiments on dogs indicated that the exit of splenic radioactivity is approximately first order with the quantity present when additional entry from the blood is negligible. It is suggested that such functional indications of activity be combined with estimates of organ weight obtained by scanning and by the allometric equations to yield indices of splenic and hepatic activity.

ACKNOWLEDGMENTS

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