ANALYTICAL REVIEW

The Interpretation of Red Cell Survival Curves

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OBSERVATION OF THE BEHAVIOR of transfused red cells is now a recognized tool for investigating the mechanism of anemias. With the best technic, the method of differential agglutination yields smooth cell survival curves of various forms. When the survival is subnormal it is natural to inquire whether the form of the curve will indicate what manner of functional disturbance is present. This paper is concerned with the possibilities and limitations of such an analysis, and may be considered as an extension of the discussion given by the Oxford workers.[1, 2]

Hypotheses Concerning the Elimination of Erythrocytes

Two particularly simple notions, first clearly formulated by Schiødt[3] have proved fruitful. First we may suppose that each cell has a determinate life span, and is not subject to environmental hazards. Different cells may have differing life spans, but we can reasonably assume that these will occur with a regular and stable frequency distribution. Secondly, the cells may be supposed potentially immortal but subject to a steady risk of destruction by some external mechanism. Then the cells will be eliminated at random and without regard to their age. These notions lend themselves readily to mathematical development and to the possibility of interpreting the elements of the descriptive equations in terms of physical events. Thus a determinate life span would strongly suggest the presence within the cell of some metabolic store which eventually becomes exhausted, while, in the case of random destruction, the constant factor in the equation might be identified with the chance that a cell would, in unit time, pass through a certain organ, or be exposed to particular physiochemical conditions. Of course, as hypotheses, these notions are neither mutually exclusive nor exhaustive: clearly, a cell might have an upper limit to its existence set by internal factors and yet be subject to external hazards, or again a cell's susceptibility to destructive influences might be a function of its age, as a man becomes the more likely to fracture his femur as he grows older. The types of survival curves that follow from these and related hypotheses are described below.

Transfusion without Relevant Change of Cell Environment

When cells are transfused, their new environment may, or may not, modify their survival. The latter case will be considered first.

Without specifying the mechanism of cell elimination in the donor, we may assume it to be working in a regular way. We may define $\phi(a)$ as the probability that a cell will exceed the age a. Clearly $\phi(a)$ is a decreasing function of a having the value 1 at $a = 0$ and tending to 0. If the rate of cell production, p, is assumed

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constant, the number of cells, N_0, present in the donor's circulation at any time is given by

Equation 1.* $$N_0 = p \int_0^\infty \phi(a)\, da$$

We may define $l = \int_0^\infty \phi(a)\, da$; then l is the mean cell life since $N_0 = pl$.

Now if the cell environment in the recipient is assumed equivalent to that in the donor, the elimination of a transfused sample of N_0 will be identical with the elimination of the parent population that would occur in the donor if production were to cease suddenly. In that case, the number of cells, N_t, present at some later time, t, is given by

Equation 2. $$N_t = N_0 \int_t^\infty \phi(a)\, da$$

and this equation describes the elimination of the transfused sample.

It should be noted that when $t = 0$

Equation 3. $$d\left(\frac{N_t}{N_0}\right) \Big/ dt = -\frac{1}{l}$$

that is, the initial slope of the cell survival curve cuts the time axis at l, and that this fact is independent of the form of $\phi(a)$ and depends only on our initial assumptions, viz., that p is constant, and that $\phi(a)$ is unchanged by the transfusion.

The above statement is essentially the same as that given by Callender et al.[2] and is repeated here for convenience.

Examples

Consider the hypothesis that the individual cell life spans are determinate, and are normally distributed with mean, l, and standard deviation, s.

Figure 1 illustrates the frequency distribution of the life spans, the form of $\phi(a)$, and the cell survival curve.

It should be noted that the proportion of cells surviving at l is proportional to $\frac{s}{l'}$, in fact one may take 2.5 times the percentage survival at the mean cell life as an estimate of the coefficient of variation of the life spans.

* Explanation of symbols is as follows:

x	Life span of a cell.
df/dx	"Probability density" of life spans.
p	Rate of production of cells.
l	Actual mean cell life in subject under investigation.
l'	Potential mean cell life in absence of external hazards.
λ'	Hypothetical mean cell life of potentially immortal cells subject to steady risk of destruction.
$\phi(a)$	Probability of a cell's surviving to age, a (at least).
$\psi(\alpha,t)$	Probability of a cell's surviving for a further period, t, after transfusion at age, α.
$\theta(a)$	Probability of a cell's surviving to age, a, when transfused immediately after production, i.e. $\theta(a) \equiv \psi(o,a)$.

There is now evidence from various sources that figure 1 approximates to the state of affairs in the normal. If in a pathologic condition the mean value of the life span is decreased, one would not expect the standard deviation to decrease proportionally, that is, the coefficient of variation would probably increase. The effect of this on the shape of the survival curve is, broadly, to expand the curved "tail" at the expense of the linear part. When the coefficient of variation becomes larger than, say, 33 per cent, the assumption that the life spans are normally

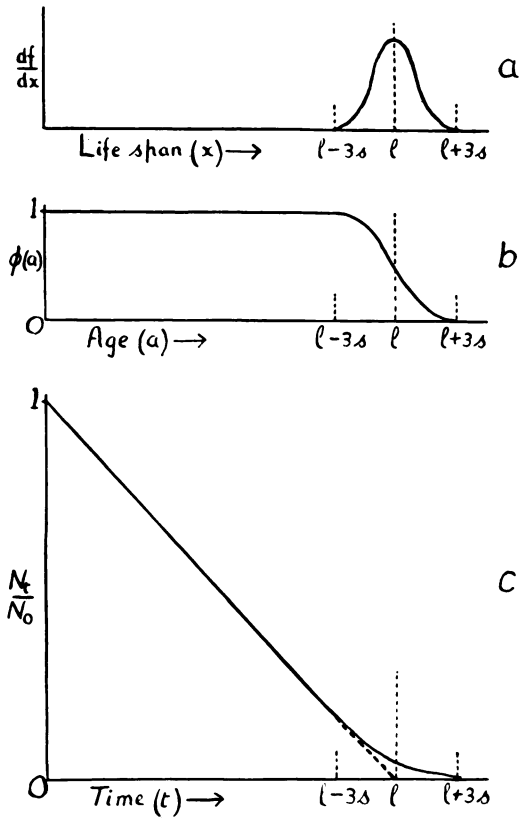

FIG. 1

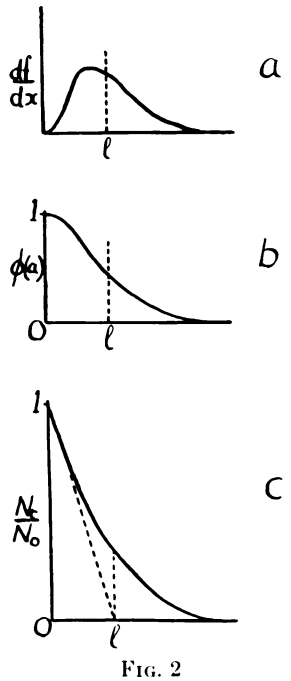

FIG. 2

FIG. 1.—The derivation of the normal cell survival curve. The symbols are defined in the text.

FIG. 2.—The effect of increased variability in cell life spans.

distributed becomes unrealistic; some form of skew distribution such as is sketched in figure 2a becomes probable. Figures 2b and c indicate the corresponding forms of $\phi(a)$ and the survival curve. The superficial resemblance of the latter to curves described below on the hypothesis of random destruction should be noted.

The assumption of random destruction of potentially immortal cells yields a simple exponential equation $N_t = N_0 e^{-t/\lambda}$ where λ is the mean cell life; the time at which the percentage survival is about 37. If we accept the view that the normal cell has an upper limit to its life, it is clearly unreasonable to assume

that this limit is removed in a pathologic state where random destruction is operating. We are thus more concerned with the hypothesis that cells whose potential life span is determinate are subject to random attenuation. If this attenuation is such as to reduce the chance of the cell's completing its life span to, say, one-half, little error is introduced by ignoring the variance of the life spans, i.e., by assuming that all cells have the same potential life span l'. Now

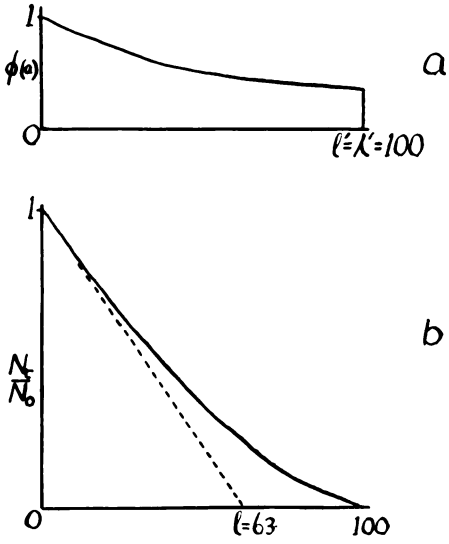

FIG. 3

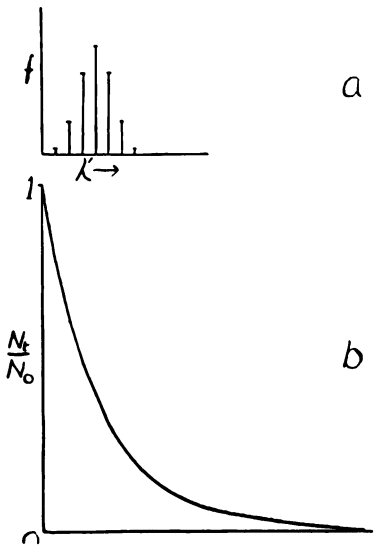

FIG. 4

FIG. 3.—The effect of a mild random destruction acting together with normal age loss. The random destruction is assumed to have operated on the donor cells before as well as after transfusion.

FIG. 4.—The effect of varying susceptibility in the transfused cells. The abnormal destruction is assumed to be rather intense and normal age loss has been ignored. There is a fatter "tail" than would be expected with a homogeneous population having the same mean survival.

if the random destruction would involve a mean cell life of λ' were the cells potentially immortal, we shall have

Equation 4.
$$\begin{cases} \phi(a) = e^{-a/\lambda'}, & (a < l') \\ \phi(a) = 0, & (a > l') \end{cases}$$

The cell survival curve is then given by

Equation 5.
$$N_t = N_0 \frac{e^{-t/\lambda'} - e^{-l'/\lambda'}}{1 - e^{-l'/\lambda'}}$$

and the actual mean cell life by

Equation 6.
$$l = \lambda'(1 - e^{-l'/\lambda'})$$

The curve is an exponential with the base line raised so that the function vanishes at $t = l'$. When $\lambda' \gg l'$ the curve approaches a straight line, and when $\lambda' \ll l'$ it approaches a complete exponential. Figure 3 gives $\phi(a)$ and the survival curve for the case $l' = \lambda' = 100$ days, $l = 63$ days approximately.

In practice the susceptibility to the destructive influence might well vary from cell to cell: that is λ' would not be constant but would be distributed around a mean value, presumably in some regular fashion. Figure 4 shows a symmetrical distribution of λ' and the resultant survival curve. In this example a normal distribution has, for ease of calculation, been approximated by a binomial. The normal case is of interest, both on account of its physical plausibility and because curves of the type of figure 4 are not uncommon in practice.[4, 5]

The following example is instructive: consider the case of cells having an indefinitely long potential life span but whose vulnerability to some hazard is proportional to age. This yields

Equation 7. $$\phi(a) = e^{-a^2/k}, \qquad (k = \text{constant})$$

Now figure 2 can be used to illustrate this case as well as that previously considered of determinate life spans of large coefficient of variation. The form of $\phi(a)$ and of the survival curve may well be identical and so the hypotheses, although conceptually quite distinct, cannot be distinguished on such evidence. The curve $\frac{df}{dx}$ of figure 2a has to be interpreted differently in the two cases: in one as a distribution of cell characters, and in the other as a distribution of cell experiences.

Transfusion with Relevant Change of Cell Environment

Where the transfusion alters the cell's environment in such a way that their survival is modified, the situation is much more complicated. Defining $\phi(a)$ and l' as before for the donor cells in their original environment, and the function $\psi(\alpha, t)$ as the probability that a cell transfused at age, α, will survive for a further period, t, we can write:

Equation 8. $$N_t = N_0 \frac{1}{l'} \int_0^\infty \phi(\alpha)\psi(\alpha, t)\, d\alpha$$

but this expression is of very limited utility because of the difficulty of suggesting realistic forms for $\psi(\alpha, t)$, a function which has to express any delayed effects of the first environment as well as the effects of the second, together with the intrinsic limitation of life span. Perhaps the most plausible restriction to impose is that the environments should have no delayed effects. In that case, the probability of a cell of age, a_1, surviving to a later age, a_2, is independent of α so long as $\alpha < a_1$, and $\psi(\alpha, t) = \frac{\theta(\alpha + t)}{(\theta\alpha)}$ where $\theta(a)$ is the probability that a cell will exceed the age, a, when $\alpha = 0$. Even with this rather drastic restriction, the resultant expression for the survival curve

Equation 9. $$N_t = N_0 \frac{1}{l'} \int_0^\infty \frac{\phi(\alpha)}{\theta(\alpha)}\, \theta(\alpha + t)\, d\alpha$$

will yield explicit equations for only the simplest forms of ϕ and θ.

Examples

Assume that in the donor the cells have uniform life span and are not subject to environmental hazard, i.e., $\phi(a) = 1$, $(a < l')$ and $\phi(a) = 0$, $(a > l')$ and

that recipient environment exerts an exponential attenuation, $\frac{1}{\lambda'}$, so that $\theta(a) = e^{\frac{-a}{\lambda'}}$, $(a < l')$ and $\theta(a) = 0$, $(a > l')$. Then the survival curve equation becomes:

Equation 10. $$N_t = N_0 e^{-t/\lambda'}\left(1 - \frac{t}{l'}\right)$$

This is plotted in figure 5 for $l' = \lambda' = 100$ days. Rather more generally, if the

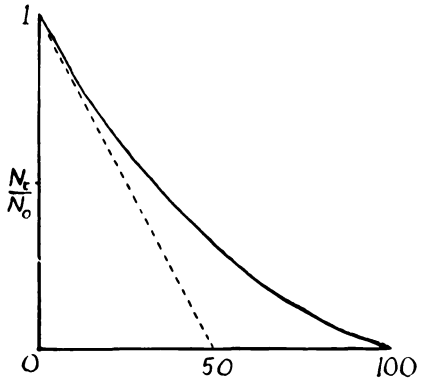

FIG. 5.—The effect of mild random destruction acting on normal donor cells only after transfusion. Contrast with figure 3: the two curves must be analyzed in different ways.

donor cells are destroyed independently of their age, though not completely at random, e.g., if the rate of destruction were proportional to cell diameter,

Equation 11. $$N_t = N_0\theta(t)\left(1 - \frac{t}{l'}\right)$$

When the cell environment has been changed by the transfusion, the initial slope of the survival curve no longer cuts the base line at l. For example, in figure 5 the initial slope cuts the base line at 50 days, whereas the value of the mean cell life is $\int_0^\infty \theta(a)\,da = 63$ days approximately.

Variations in the Rate of Cell Production

Hitherto we have assumed that p is constant over the material time; that is for a time preceding the transfusion long enough to include the maximum life span, say four or five months. This assumption is probably correct where the donor is a normal subject, questionable where he is an anemic subject, and almost certainly incorrect in the case of an infant donor. If variations of p must be considered, further complication ensues.

Example

Suppose that in the donor the cells have a uniform life span l but that production has decreased linearly with time from a value, p_l, l days ago; so that x days ago the production was $p_x = p_l\left(1 - \frac{l - x}{c}\right)$ if $(l - x) < c$ or $p_x = 0$ if

$(l - x) \geqslant c$. Such a situation is by no means inconceivable in, for example, relapsing Addisonian anemia. If the donor cells are now transfused without change of environment, the survival curve becomes a parabolic arc:
See figure 6.

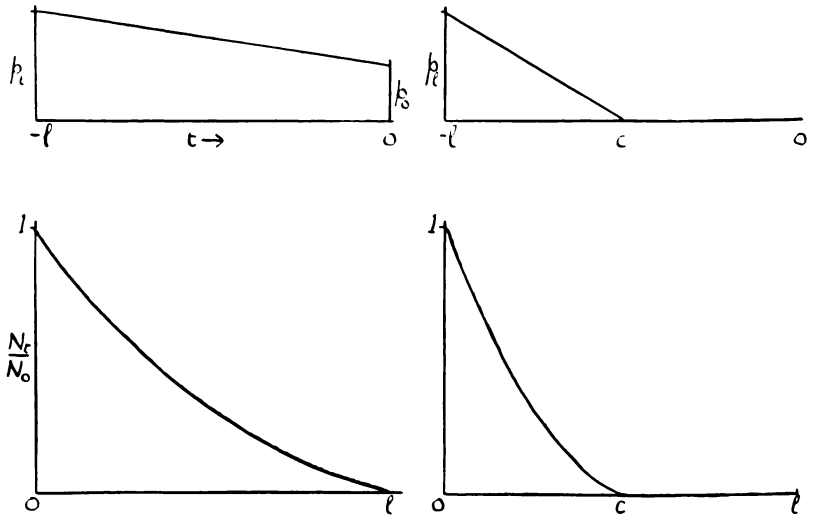

FIG. 6.—The effect of decreasing production in the donor before transfusion. The individual cells are assumed to survive normally.

Equation 12.

$$\begin{cases} N_t = N_0 \left[\dfrac{\left(1 - \frac{t}{c}\right)^2 - \left(1 - \frac{l}{c}\right)^2}{1 - \left(1 - \frac{l}{c}\right)^2} \right], & c > l \\ N_t = N_0 \left(1 - \frac{t}{c}\right)^2, & c \leqslant l \end{cases}$$

Such findings might in practice be erroneously interpreted as evidence of reduced mean cell life in the donor.

The Problem of Analysis

So far we have proceeded entirely synthetically, constructing survival curves from certain initial hypotheses. There is in principle always a unique solution to any such problem. In practice, the converse problem presents itself: an array of points is given, and it is required to suggest hypotheses which would generate it. To this problem there is in general no unique solution. Some choice or restriction of hypotheses must be made on grounds external to the data at hand. The fact that a curve of certain form can be made to fit the data tolerably gives in itself scant assurance that the hypothesis from which the curve is derived is in fact the most appropriate. A glance at the figures should persuade the reader of the general similarity in shape of curves derived from quite distinct assumptions.

The following suggestions may be of some help in analysis: in a hemolytic

syndrome one wishes firstly to know the mean life in the patient of his own cells, since the reduction in mean life is the most appropriate way of characterizing the severity of the hemolysis. Secondly, one would like to determine whether cell elimination is random and, if not, whether it is dependent on cell age.

Where it can be assumed that the transfusion has not caused a relevant change in cell environment, one may, having fitted a curve freehand to the survival data, find the mean cell life by noting the point at which the initial slope cuts the time axis. One may then obtain a new curve by plotting the steepness, i.e., the negative derivative of the first curve against time. This new curve is an estimate of $\phi(a)$, and if it has at first increasing steepness (as in fig. 2b) it is likely that cells are eliminated as a function of their age. In the contrary case, e.g., in figure 3a, where elimination is probably independent of age, one may replot $\phi(a)$ on semi-logarithmic paper. If the new plot is linear, elimination is completely random; if not, then variation in cell susceptibility exists.

When normal blood has been transfused to a patient with an abnormal hemoclastic mechanism, one may use the relations given in Equation 11 by taking $l' = 120$ days and plotting $\frac{N_t}{N_0\left(1 - \frac{t}{120}\right)}$ as an estimate of $\theta(a)$. The mean cell life is now found by dividing the area under the curve between $t = 0$ and $t = 120$ by the initial height, and measuring the resultant length along the time axis from $t = 0$. When elimination is rapid, say, complete within 30 days, the correction for ageing of the donor cells becomes unimportant, and the mean cell life may be estimated by applying this area measuring process directly to the survival curve.

It should be remembered that even when in a hemolytic state there is good reason to believe that the primary abnormality can be localized in cells or environment as the case may be, it is not always safe to assume that the other component is normal. Thus, in nocturnal hemoglobinuria, the primary fault is thought to lie in the cells, but a transfusion from an affected to a normal subject would introduce a relevant change of environment, since it appears that the patient's plasma usually contains subnormal amounts of some substance involved in the hemolytic process.[6] Such a transfusion would, therefore, be expected to yield an apparent value for l lower than that appropriate to conditions in the patient.

Conclusion

The method of differential agglutinations rapidly gave important and clear-cut results when used to distinguish among frank hemolytic states those with primary cellular anomalies from those with abnormal cytoclastic mechanisms. In studies of that kind the question to be settled was merely whether the transfused cells survived normally or not. Now that these major questions have been answered it seems probable that attention will be directed towards less dramatic deviations from normal survival and towards a greater refinement of analysis (cf. the discussion by Callender et al.[4] of the survival curves of sickle cells). These probable developments furnish the justification of the rather extensive treatment offered here.

REFERENCES

[1] Brown, G. M., Hayward, O. C., Powell, E. O. and Witts, L. J.: The destruction of transfused erythrocytes in anemia. J. Path. Bact. *56:* 81, 1944.

[2] Callender, S. T., Powell, E. O. and Witts, L. J.: The life span of the red cell in man. J. Path. Bact. *57:* 129, 1945.

[3] Schiødt, E.: On the duration of life of the red blood corpuscles. Acta Med. Scandinav. *95:* 49, 1938.

[4] Callender, S. E., Nickel, J. F., Moore, C. V. and Powell, E. O.: Sickle cell disease: studied by measuring the survival cf transfused red blood cells. J. Lab. & Clin. Med. *34:* 90, 1949.

[5] Dacie, J. V. and Mollison, P. L.: Survival of transfused erythrocytes from a donor with nocturnal haemoglobinuria. Lancet *1:* 390, 1949.

[6] —: Transfusion of saline washed red cells in nocturnal hemoglobinuria. Clin. Sc. *7:* 65, 1948.