KINETICS OF A BONE-MARROW STEM-CELL POPULATION

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The problem of the bone-marrow stem cells has a very extensive literature, dating back to the turn of this century, and various great haematologists, such as Maximow, Naegeli, and Ferrata, debated the morphological identity of the stem cell—or stem cells.

The problem was obvious. In the marrow there was proliferation on a very large scale, to produce something like 2–4 $\times$ 10^{11} cells per day. A large proportion of the marrow cells, however, were clearly too far differentiated to be able to "de-differentiate" and act as primitive progenitor cells. Hence the stem cell was gradually pushed into the morphological nonidentity of that 3–5 per cent of the bone-marrow cell population which is recognized by hematologists as the "poorly classifiable cells."

Although its morphological identity was unknown, the principles on which stem cells could operate was a problem which could be tackled theoretically. The oldest concept was that a differentiating cell population is maintained by the odd cell de-differentiating to a primitive form; later this was modified by postulating some cells which are stem cells proper, in the sense that they represent a self-maintaining cell population.

The Concept of Asymmetric Division

It was assumed that regulation of hemopoiesis and self-maintenance of the stem-cell population is achieved by an asymmetric division:

$$\text{Stem cell} \begin{cases} \rightarrow \text{Stem cell} \\ \searrow \text{Differentiating cell.} \end{cases}$$

This concept implies that there is either a nonself-duplicating structure which on cell division is retained in the one daughter cell which remains a stem cell, or that there is a significantly uneven division of some cellular material, and that the excess or deficiency of this causes one of the daughter cells to remain a stem cell. This way the stem-cell numbers would be maintained, and regulation of hemopoiesis would be effected by a slower or faster rate of division (shorter or longer cell cycle) of the stem cells.

The main criticism of this concept is that it cannot explain how stem cells may recover from radiation damage. After, for example, a 90-per-cent sterilizing dose of irradiation, only 10 per cent of the stem cells would remain; these would have to contract their cell cycle 10 times to re-establish normal blood-cell production. We know now that this 90-per-cent sterilizing dose is equivalent to about 200-rads whole-body irradiation[1] and we also know that a 200-rads whole-body dose can be repeated 3–5 times during the animal's lifespan. However, recovery from a second 90-per-cent sterilizing dose requires a 100-fold shortening of the cell cycle of the 1 per cent of the original stem cells remaining, a third dose would require a 1,000-fold shortening of cycle, a further dose a 10,000-fold, and a fifth

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a 100,000-fold shortening. This would only be possible if there were a very large store of stem cells with a 50,000-day maximum cell cycle, to enable them to telescope it to something like a 12-hour cycle (which is about the shortest observed in mammalian cells). The magnitude of such a store, with a cell cycle of 5×10^4 days, in order to produce 10^8 red cells (in a mouse) per day would have to be about 5×10^{12} cells, i.e., about 250 times the body weight of the mouse.

The $\alpha - \alpha$, n and $\alpha - 2\alpha$ Divisions

Fully recognizing the aforementioned difficulties, Osgood[2] devised an ingenious scheme by which the stem cells may recover their original number after an initial depopulation.[2] Osgood's concept was that the asymmetric division, which he called $\alpha - \alpha$, n (α denoting the stem cell and n the differentiating cell), can on demand, be substituted by a symmetrical cell division: $\alpha - 2\alpha$, by which one stem cell gives rise to two identical daughter stem cells. This way, instead of altering the length of the cell cycle, simply by altering the type of cell division the stem cells could produce more or less differentiating cells, and, of course, could repopulate a depleted stem-cell population at the expense of differentiation.

The disadvantage of this concept is that it is extremely difficult to imagine any stem-cell population behaving this way which is not unipotential. The available evidence[3,4] is, on the other hand, overwhelmingly in favor of a pluripotential bone-marrow stem cell.

For this purpose, and because of the difficulties in finding a suitable biochemical explanation for an asymmetric division in bone-marrow stem cells, search was made for an alternative model, which should, furthermore, offer quantitative experimental possibilities for testing it.

Principles of the New Stem-Cell Model

The model to be discussed here has been described in detail elsewhere.[5,6] Only the principles and main salient features will be mentioned here.

The first principle used was that in the vast majority of instances in nature a cell division produces two identical daughter cells. Although asymmetric divisions do occur in nature, these are highly specialized exceptions to the rule that mitotic division primarily serves the maintenance and growth of a cell line.

The second principle was that differentiation is unconnected with cell division. Cells may differentiate, irrespective of whether they are or are not capable of cell division. The initiation of differentiation is probably a form of enzyme induction for a particular purpose. Whether this enzyme induction involves alterations in the genome of the cell or not, it does not depend upon and is not connected with the mitotic processes.

The third principle was that, as in the case of any organ which is capable of regeneration, there is a feedback control mechanism which regulates the *size* of the stem-cell population. This feedback control mechanism maintains the normal size (normal steady-state value) of the population, in spite of and independent of the mechanisms which "remove" cells from the stem-cell population for differentiation. The term "remove" is used in the sense that once a stem cell begins to differentiate (i.e., a specific enzyme induction has taken place in it), it is by definition a differentiating, rather than a stem cell.

Finally, the bone-marrow stem-cell population is envisaged as being one of the Type IIb cell populations described previously.[7] The main characteristic feature of such populations is that although all their cells are capable of growth, at any

given time only a random proportion of their cells are in a state of growth. This concept implies that "state of growth" is a cell cycle (G_1–S–G_2 periods) followed by a cell division,[8] and that the cell cycle is of a relatively constant length in a cell population. The statement, that a proportion of cells in a population are not in a state of growth, means that normally mitosis is followed by a state of "G_0," that is, a gap at true "dormancy" or "resting stage" in respect of cell cycle. Cell cycle is then initiated in the G_0 population by triggering a proportion of them into G_1 stage, from which they inevitably enter the S and G_2 stages and mitosis (unless specifically inhibited from doing so).

Qualitative Kinetics of the Model

The bone-marrow stem cells are envisaged as principally a "resting" cell population, i.e., not in any stage of the growth cycle ("resting" in G_0). In normal steady state, however, there is a continuous "removal" of cells from the population by continually applied stimuli for differentiation (e.g., the physiological concentration of erythropoietin may remove x proportion of these stem cells per unit time).

This "removal" results in a steady production of differentiating cells, but also in a steady depletion of the stem-cell population. The depletion, however, is counteracted by the population-size feedback control mechanism which "notices" the deficiency in number of cells (or total cell mass), and—after some finite time delay—triggers off stem cells into cell cycle. Such triggered cells have to progress through G_1–S–G_2 periods and mitosis, and the daughter cells then rest again in G_0 unless triggered again. The sequence of events is shown in FIGURE 1.

It should be emphasized that the cell compartments in FIGURE 1 do not represent anatomically defined compartments: the "feed" from G_0 state to cell cycle and from cell cycle to G_0, or from G_0 to differentiating cells, may not necessarily mean any bodily movement of the cell in question. The differentiating cells are, by definition, removed from the stem-cell population and its size-regulating feedback control. If this feedback control acts by inhibition (i.e., the G_0 cells are held in G_0 by inhibition of growth), then the differentiating cells, freed from this inhibition, should enter into exponential growth. This, in fact, they appear to be

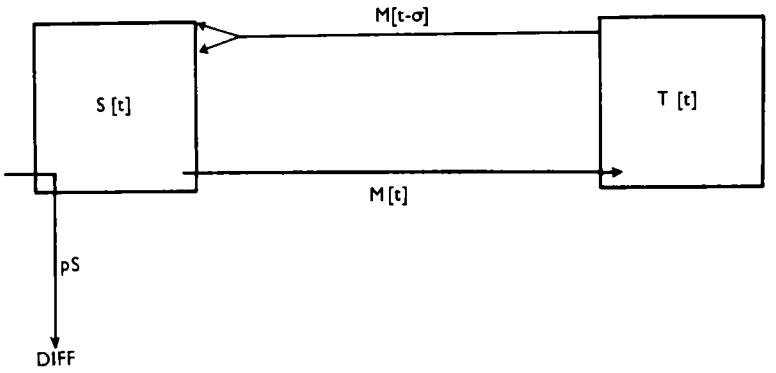

FIGURE 1. Kinetics of bone-marrow stem cells: $S(t)$ = stem cells resting in G_o ("available"); $T(t)$ = stem cells in state of cell cycle ("triggered"); $M(t)$ = rate of triggering into cell cycle (result of population-size feedback control); $M(t\text{-}\sigma)$ = replenishment of available stem cells from divisions of previously ($t\text{-}\sigma$) triggered cells, and Diff = cells removed from the available stem-cell population for differentiation (e.g., by erythropoietin).

doing, and the limit of this growth is set by the fact that the cells differentiate faster than their growth rate and therefore after 3–5 cell cycles they finish up as fully differentiated cells unable to grow any more (e.g., metamyelocytes or late polychromatic normoblasts).

Quantitative Kinetics of the Model

The qualitative picture described above may be put into quantitative form:

$$S_n = S_{n-1} - d_{n-1} - t_{n-1} + X_{n-1} ,$$

where S_n is the number of "available" stem cells (e.g., in state of G_0) at any given time, $n-1$ is the immediately preceding time unit; d is the proportion (pS) differentiating; t is the number of cells triggered; and X is the number of daughter cells coming from divisions of previously (one cell-cycle earlier) triggered cells.

Steady state values would be given by

$$S = \frac{100}{(1 + 2\sigma p)},$$

where σ is units of time per cell cycle and p is the proportion of stem cells differentiating per unit time.

The feedback control may be signified by

$$S + 2T = 100$$

or

$$100 - S - 2T = 0$$

where T is the number of cells in triggered stock, or, in continuous form,

$$\frac{dS(t)}{dt} = -pS(t) - M(t) + 2M(t-\sigma),$$

where M is the number of cells triggered per unit time (t) and

$$\frac{dT}{dt} = M(t) - M(t-\sigma).$$

The feedback control would be given by

$$M(t) = f(S_1 T),$$

where f is some function of S and T.

The above information was treated by computer analysis, and it was found that the system does reach a good steady state and will respond to various degrees of stimulation in an elastic manner with very small well-damped oscillations (FIGURE 2).

It should be noted that as the formulae are set up, differentiation occurs *only* from the G_0 cells ("available" stem cells) and not from stem cells in state of cycle ("triggered cells). This is not an essential criterion of the principles involved but is due to the observation that even prolonged intensive stimulation of the rate of differentiation cannot "run down" the stem-cell population. The computer analysis of the two alternatives—(*a*), triggered cells also can differentiate or (*b*), triggered cells cannot differentiate — predicted the situations shown in FIGURE 3, i.e., an increasing response to stimulation and "run down" in case of (*a*) (due to removal of triggered cells at a higher rate than the cells can reproduce) and an increasing response with a plateau of diminishing values in case of (*b*). As the experiments confirmed curve (*b*) it was concluded that

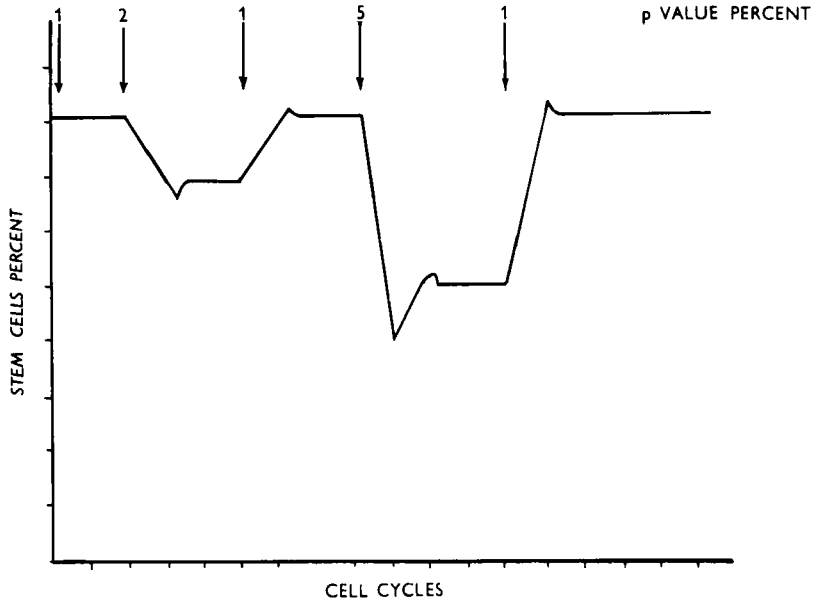

FIGURE 2. Response of stem cells to various degrees of stimulation for differentiation. Percentage figures refer to per cent cells differentiating per unit time: abscissa = available stem cells; ordinate = time in cell cycles.

triggered stem cells (i.e., cells in state of cell cycle—either in the whole cycle or in some part of it) are not available for differentiation.

Initial Experimental Check of the Model

The experimental system in which the computer predictions were checked was the response of the polycythemic mouse to a standard test dose of erythropoietin.[6] In this system a 70–90-per-cent sterilizing dose of X-irradiation gave remarkably good agreement with the predicted time sequence of events during the first five to seven days after irradiation (FIGURE 4).* The existence of the postirradiation "dip" in a polycythaemic mouse furthermore indicated the possibility that the bone marrow stem cells are pluripotential and that the "dip" and the suboptimal plateau are caused by the turnover of the stem cell population, e. g., granulopoiesis (first at normal and later at increased rate, as the peripheral granulocyte level demands it).

FURTHER CONSIDERATIONS

The model behaved as a bone-marrow stem-cell population was expected to behave,[5,6] i.e., it maintained a remarkable steady state; it was able to respond to various degrees of differentiation in the same way as was indicated by the erythropoietin dose-response relationship; it could withstand a chronic radiation damage and maintain normal erythrocyte production; and it could predict the qualitative and quantitative responses to a single dose of radiation remarkably well.

* For detailed explanation of the computer calculation, see ref. 5.

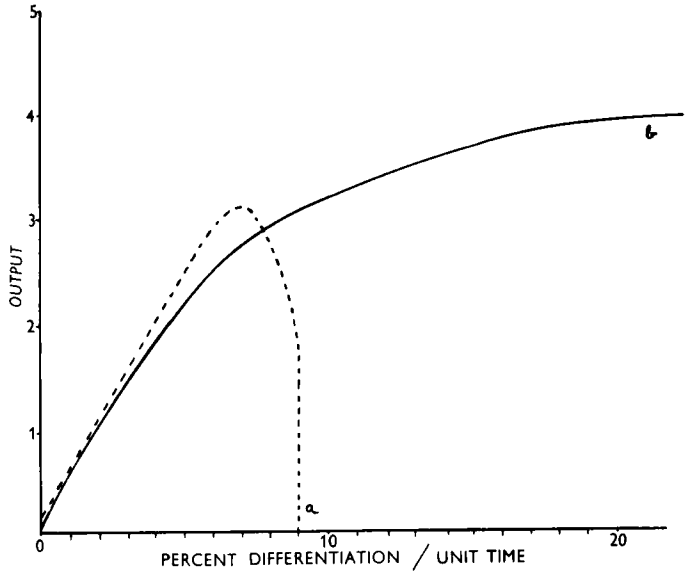

FIGURE 3. Response of stem cells to continuously increasing stimulation for differentiation (*a*) if triggered cells can also differentiate; (*b*) if triggered cells cannot differentiate.

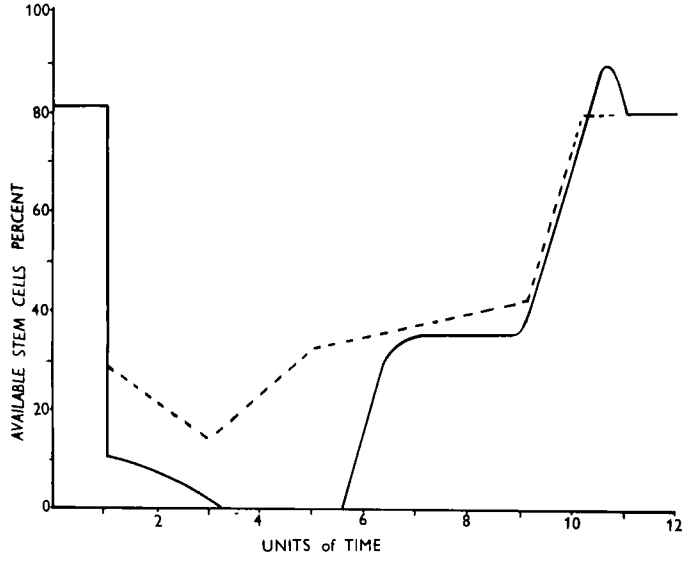

FIGURE 4. Predicted (—) and experimentally found (- - - -) response of stem cells to irradiation. Abscissa = available stem cells; ordinate = time in cell cycles.

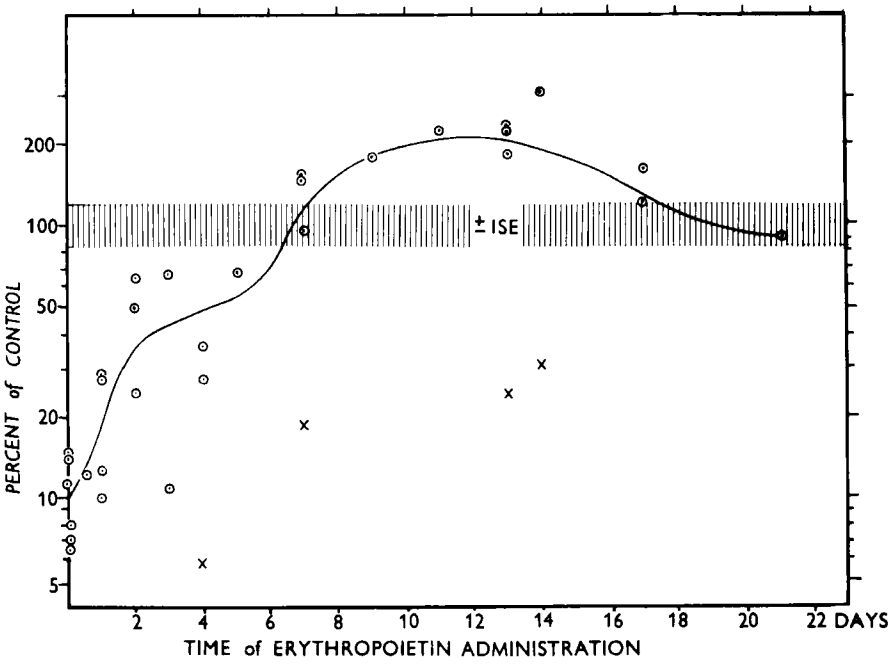

FIGURE 5. Recovery and overshoot of stem-cell response to erythropoietin after 150 rads. ⊙ = 150 rads on 0 day; × = 150 rads on 0 day & indicated day.

Two points, nevertheless, demanded some further consideration.

First, the feedback control, as proposed in its original form, $(S + 2T = 100)$, or in perhaps better form, $(100 - S + 2T = 0)$, was instantaneous. Although this served for first approximations, it was obvious that some time delay is unavoidable in any feedback system. To ensure a quick and efficient regulation and to prevent coarse oscillations, such time delays would have to be short, but nevertheless they are bound to exist. Further experiments were required to investigate whether some time delay is demonstrable in the system.

Second, the model as described did not predict—in fact it excluded—a "residual damage" following a single dose of radiation. However, Baum and Alpen,[9] investigating the recovery from repeated doses of radiation, concluded that the erythropoietic system carries an "exponentially fading" residual damage after irradiation.

In view of these two considerations, further experiments were designed to test the stem-cell response *in vivo*.

The Stem-Cell "Overshoot"

When the response of the polycythemic mouse to a standard test dose of erythropoietin was followed in time after whole-body irradiation, it was found that recovery to normal levels was reached by the 6th–8th day, but the response "overshoots" the normal values between 10–15 days, returning to normal again by the 18th–20th day. The degree of this overshoot was about twice normal level at its peak (FIGURE 5).

As our current assumption was that a certain dose (concentration) of erythropoietin differentiation in a certain *proportion* of stem cells, the data in FIGURE 5 indicated that at the time of overshoot there is an increased number of stem cells available. However, as this conclusion was based on the assumption that the same dose of erythropoietin removes the same proportion of stem cells for differentiation, it had to be tested more directly. FIGURE 6 shows the blood-reticulocyte response to a similar dose of radiation, and it can be seen that, similarly to polycythemic mice, the reticulocyte count in normal mice also "overshoots" on days 10–14 following irradiation. This was also confirmed with the appearance of a tracer dose of Fe^{59} in normal mice and in mice 14 days after 150 rads (FIGURE 7). Both these experiments indicated a temporary elevation of erythropoiesis in normal mice to about twice normal levels at the time of the overshoot following irradiation.

Both the elevation of the available stem cells (FIGURE 5) and the increased erythropoiesis (FIGURES 6, 7) indicated that the "overshoot" is due to an increased stem-cell population. To confirm this more directly, a series of whole-body X-ray doses were given to groups of 16 mice each to test whether the "overshoot" time confers radioprotection as would be expected with an increased supply of stem cells.

The results are shown in TABLE 1 and they confirm that the "overshoot" mice survive larger doses of whole-body irradiation, in conformity with the concept of a true stem-cell overshoot resulting on the twelfth to fourteenth day after 150 rads whole-body X-ray dose.

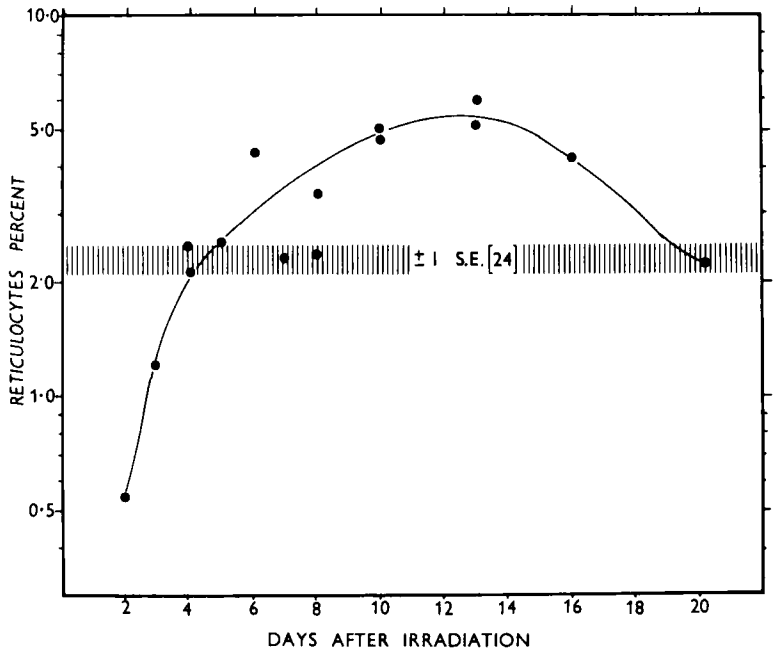

FIGURE 6. Blood reticulocytes in normal mice following 150 rads.

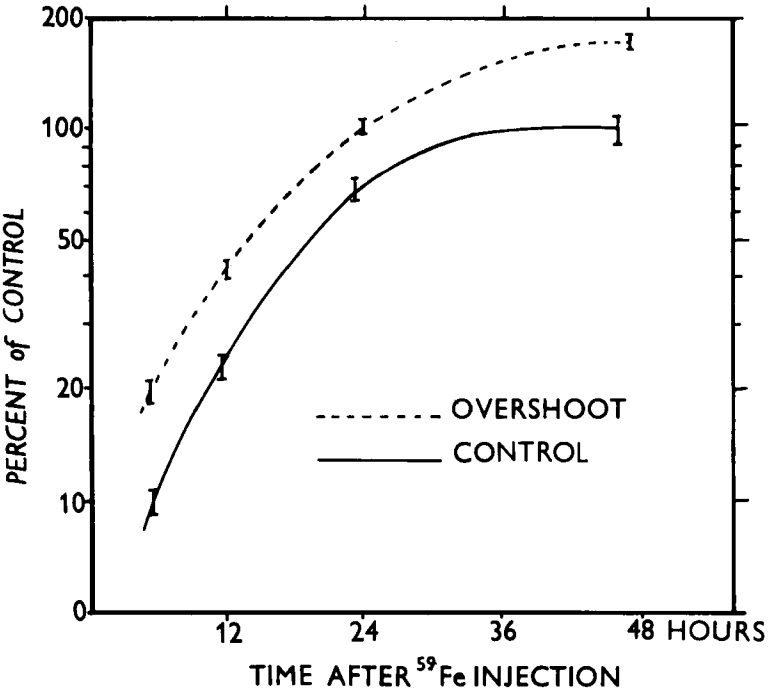

FIGURE 7. Rate of appearance of Fe^{59} in control and overshoot mice [± ISE].

Absence of Residual Damage

FIGURE 5 indicates that a second dose of 150 rads given on day 4, 7, 13, or 14 following the first 150-rad dose gives the same 90-per cent depression of stem-cell response as the first 150 rads. The absolute values obtained after the second 150 rads will depend on the time which has elapsed between the first and second irradiation. On day 4, the recovery is still subnormal, on day 13, it is supranormal —hence the same 90-per-cent depression of a subnormal or a supranormal response will give a lower or higher absolute response. The observation that a second dose of 150 rads whole-body radiation gives the same 90-per-cent depression as the first 150 rads, irrespective of when during the subnormal or supranormal recovery it is given, indicates the absence, in mice, of a residual damage after 150 rads.

This observation appears to contradict Baum and Alpen's report on a residual damage following 300 rads in rats.[9] However, the contradiction may be only

TABLE 1

SURVIVIAL OF NORMAL AND "OVERSHOOT" MICE FOLLOWING WHOLE-BODY IRRADIATION*

X-ray dose (rads)	600	635	670	705	740
Control	0/16	2/16	12/16	13/16	15/16
"Overshoot"	2/16	3/16	1/16	3/16	6/16

* Figures indicate number of deaths in groups of 16 mice.

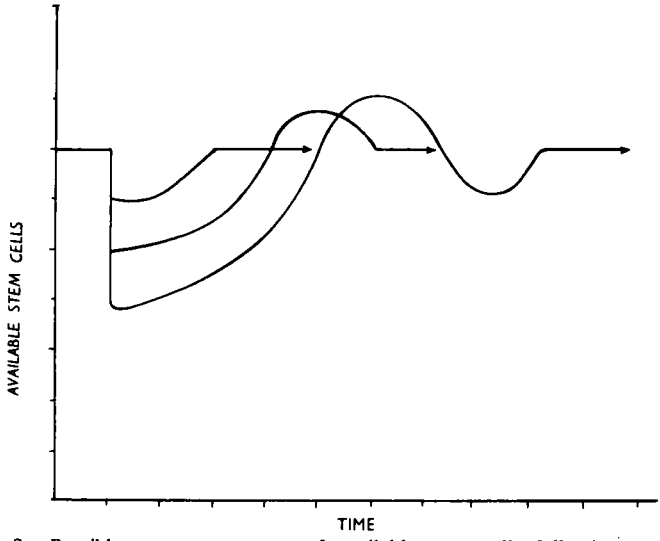

FIGURE 8. Possible recovery patterns of available stem cells following various degrees of initial depopulation.

apparent and not real. Firstly, there is a difference in the species of the animals used and in the dose of radiation used. Secondly, Baum and Alpen measured not the *immediate* depression caused by radiation, but the recovery on the fifth day following irradiation. Obviously, the greater the *absolute* depopulation of the stem cells, the longer it takes to recover to normal levels, and it is possible that the stem-cell response pattern in respect of "available" stem cells follows the pattern shown in FIGURE 8. According to this pattern, a small initial depopulation results in a smooth recovery to normal level without any overshoot (as found by Gurney following a 79-per-cent depopulation).[10] A larger depopulation will result in an overshoot, as observed by us. A still larger depopulation may cause greater oscillation and an overshoot may be followed by an "undershoot."

It follows then that a second dose of radiation—although it may cause the same proportionate depopulation—will cause a smaller or greater *absolute* depopulation, depending on when during the recovery from the first dose it is given. The recovery from this second dose of radiation will then depend on the degree of absolute depopulation, hence Baum and Alpen may find a longer recovery if the second dose of radiation is given at the time of an "undershoot."

CONCLUSION

The information presented above illustrates that there are numerous methods by which the kinetics of the bone-marrow stem cells can be studied. As the experimental data accumulate, the need arises to present a coherent interpretation. An attempt has been made to construct a quantitative kinetic model of the bone-marrow stem-cell population. The model as presented is still preliminary—it can explain and predict certain characteristics of the stem cells. The feedback control as presented is too rigid to allow for the observed overshoot following a certain radiation damage. Further calculations are in progress to examine the possibility of a more realistic model of the feedback control and to design ex-

periments for further critical testing. It is hoped that a better understanding of the bone-marrow stem-cell kinetics will be of help in the analysis of changes occurring in such pathological conditions as aplastic anemia and, more particularly, in leukemias.

References

1. McCulloch, E. A. & J. E. Till. 1962. The sensitivity of cells from normal mouse bone marrow to gamma radiation *in vitro* and *in vivo*. Radiation Research. **16**: 822.
2. Osgood, E. S. 1957. A unifying concept of the aetiology of leukaemias, lymphomas and cancers. J. Natl. Cancer Inst. **18**: 155.
3. Ford, C. E., J. L. Hamerton, D. W. H. Barnes & J. F. Loutit. 1956. Cytological identification of radiation-chimaeras. Nature. **177**: 452.
4. Becker, A. J., E. A. McCulloch & J. E. Till. 1963. Cytological demonstration of the clonal nature of spleen colonies derived from transplanted mouse marrow cells. Nature (London). **197**: 452.
5. Lajtha, L. G., R. Oliver & C. W. Gurney. 1962. Kinetic model of a bone-marrow stem-cell population. Brit. J. Haematol. **8**: 442.
6. Gurney, C. W., L. G. Lajtha & R. Oliver. 1962. A method of investigation of stem-cell kinetics. Brit. J. Haematol. **8**: 461.
7. Lajtha, L. G. & R. Oliver. 1962. Cell population kinetics following different regimes of irradiation. Brit. J. Radiol. **35**: 131.
8. Howald, A. & S. R. Pelc. 1953. Synthesis of desoxyribonucleic acid in normal and irradiated cells and its relation to chromosome breakage. Heredity (Suppl.) **6**: 261.
9. Baum, S. J. & E. L. Alpen. 1959. Residual injury induced in the erythropoietic system of the rat by periodic exposures to X-radiation. Radiation Research. **11**: 844.
10 Gurney, C. W. 1963. Effect of radiation on the mouse stem cell compartment *in vivo*. Perspect. Biol. and Med. **6**: 233.