The present results are in agreement with those of Capecchi and Gussin who used a similar phage assay system for in vitro suppression. It remains to be shown whether the Su-1 gene determines the structure of a serine sRNA directly or indirectly, perhaps by specifying an enzyme which modifies a pre-existent sRNA.

* Supported in part by grants from the National Science Foundation.

1 Benzer, S., and S. Champe, these PROCEEDINGS, 48, 1114 (1962).
9 Schwartz, J. H., these PROCEEDINGS, 53, 1133 (1965).

CELL TURNOVER IN MAMMALIAN TISSUES: USE OF CELL DEPLETION MEASUREMENTS TO CALCULATE X-RAY REPRODUCTIVE SURVIVAL CURVES IN VIVO*

BY THEODORE T. PUCK

DEPARTMENT OF BIOPHYSICS, UNIVERSITY OF COLORADO MEDICAL CENTER, DENVER

Communicated October 4, 1965

In previous papers it has been shown that the single cell survival curves of mammalian cells can be quantitatively applied to the interpretation of the action of ionizing radiation on mammals. In vitro studies demonstrated that cells taken from a wide variety of mammals, and originating in different tissues, exhibited a mean lethal dose, D0, of approximately 100 rads and a survival curve hit number usually lying between 1 and 3. Studies from several laboratories demonstrated that the survival curves of mammalian cells in vivo are similar to those in vitro. (The sensitivities may not be identical because of changes in tissue oxygenation and possibly other factors that could introduce differences of 20–140%.) Hence, it was proposed that reproductive death constitutes the primary action of irradiation with doses below 2,000 rads. Studies were undertaken in the bone marrow, spleen, and thymus of young adult mice quantitating the well-known cell depletion, which follows exposure to ionizing radiation. Tests were devised to determine whether the resulting cell loss was due only to the inhibition of cell reproduction, or whether cell destructive and evacuative processes were also initiated or accelerated in the affected tissues. A variety of tests gave results which appeared to indicate that reproductive inhibition alone is responsible for all or most of the observed depletion of nucleated cells, during the early period (less than 48 hr) following X irradiation. These tests included demonstration that after a limiting dose is reached, the time course of cell depletion remains constant, regardless of further increases in dose; and that agents like Colcemide and Vinblastine (Velban, Eli Lilly
Co.), whose action on cells \textit{in vitro} had been demonstrated to involve only inhibition of mitosis, yielded depletion curves similar to those obtained with maximal radiation doses. It was concluded that the limiting rate of cell depletion observed with radiation doses greater than the critical value might afford an estimate of the combined rate of all cell removal processes in the given tissues in the normal animal. If steady-state conditions prevailed initially, these should also equal the rates of normal cell reproduction.

If these considerations are valid, it follows that it should be possible to estimate reproductive survival curves for various mammalian somatic cells from analysis of the radiation-induced depletion curves of the tissues in which they occur. Such calculation is possible, at least on an approximate basis, because the limiting depletion rate in each tissue so far studied obeys a simple exponential decay curve. Therefore, since in the initial steady state, rates of cell production must equal removal by all processes, one can deduce what loss in cell reproductive capacity corresponds to any observed cell depletion, following a given dose of radiation. The present paper presents experiments from which such survival curves for cell reproduction are estimated for the bone marrow, spleen, and thymus of the young mouse, and the results are compared with single cell survival curves obtained by other methods.

\textit{Theory.---}Consider that in the organ or tissue under examination, two general types of nucleated cells exist, an \textit{A} type which is reproducing and a \textit{B} type which is not. The \textit{B}-type cells are being removed by a variety of actions. Such cell removal includes all processes which decrease the microscopic count of nucleated cells, such as enucleation of erythroblasts, or cell transfer to another body compartment. We may then consider that the over-all nucleated cell number will be determined at least approximately by the following types of processes:

\begin{align*}
A & \rightarrow 2A, \quad (1) \\
A & \rightarrow B, \quad (2) \\
B & \rightarrow \text{Removal.} \quad (3)
\end{align*}

It is important to note that these equations are only formalisms, which are being used to represent the over-all processes of nucleated cell production and removal in tissues. Each constant, \( k \), must be considered as an average, which would have to be taken in a special way, of the rate-determining parameters for the various reactions affecting the reproduction or removal of nucleated cells. Despite the lack of the detailed information about the intimate cell dynamics, it seems worthwhile to see how well such a simple model can represent the change in total cell number of mammalian tissues after X irradiation.

The following equations may be written:

\begin{align*}
\frac{dA}{dt} &= (k_1 - k_2)A, \quad (4) \\
\frac{dB}{dt} &= k_2A - k_4B. \quad (5)
\end{align*}
In the normal steady state of a mature animal, each component of the system is presumably constant, so

\[
\frac{dA}{dt} = \frac{dB}{dt} = 0. \tag{6}
\]

Therefore,

\[ k_1 = k_2 \tag{7} \]

and

\[ k_2 A^0 = k_4 B^0, \tag{8} \]

where \( A^0 \) and \( B^0 \) represent the normal values for the respective cell types.

These equations assume that nucleated cells from other tissues do not enter the region under study, and so consideration is limited to those tissues all of whose cells originate by self-reproduction; and to depletion periods sufficiently short so that cell recolonization in the affected tissue from other body regions does not occur.

Now, consider administration of a dose of whole-body irradiation to the animal such that the fraction of cells in which reproductive capacity has survived is represented by \( S \). Hence, immediately after irradiation,

\[ A = A^0 S. \tag{9} \]

We shall assume that the \( A \) cells which have been killed reproductively now behave like \( B \) cells with respect to removal. Then, immediately after irradiation,

\[ B = B^0 + A^0(1 - S). \tag{10} \]

Our principal hypothesis that radiation acts only to decrease the number of reproductive cells implies that \( k_1, k_2, \) and \( k_4 \) are unchanged by the exposure to radiation. The existence of radiation-induced reversible lag in the reproduction of the surviving cells, a situation which temporarily decreases \( k_1 \) among the viable survivors, would appear to contradict this proposal. However, it can be calculated from preliminary data on mouse cells \textit{in vivo}, as well as from more extensive studies on other mammalian cells \textit{in vitro}, that the reversible lag produced by the doses here considered would result in an effect less than that due to the uncertainty of the cell counting procedures, and hence may be ignored at this stage of these studies.

Therefore, after irradiation, from equations (6) and (9),

\[ A = A^0 S \tag{11} \]

and is constant.

Equation (5) can be integrated, since \( A \) is constant, and the initial value of \( B \) is \( B^0 + A^0(1 - S) \). Therefore,

\[
B = \frac{k_2}{k_4} A^0 S + \left[ B^0 + A^0(1 - S) - \frac{k_2}{k_4} A^0 S \right] e^{-k_4 t} \tag{12}
\]

but

\[
\frac{k_2}{k_4} A^0 = B^0 \text{ from (8).}
\]

Therefore,

\[ B = B^0 S + (A^0 + B^0)(1 - S) e^{-k_4 t}. \tag{13} \]
The technique which we have previously described measures only the total nucleated cells in each organ without differentiating between $A$ and $B$ cell types. Hence, if we define $n$ as the total nucleated cell number, at any time, and $n_0$ as the value of $n$ before irradiation,

$$n = A + B = (A^0 + B^0)S + (A^0 + B^0)(1 - S)e^{-kt} \text{ from } (11) \text{ and } (13)$$

or

$$\frac{n}{n_0} = S + (1 - S)e^{-kt}. \quad (15)$$

From this equation it follows that: (a) for very high doses of radiation, $S \rightarrow 0$ and the value of $n/n_0$ becomes a simple exponential function, from which the value of $k_4$, the normal removal rate constant of nondividing cells for the tissue in question, can be calculated. This behavior has been demonstrated for the bone marrow\(^3\) and for the spleen and thymus.\(^4\) (b) For values of $1 > S > 0$, the value of $n/n_0$ will at first fall after irradiation and eventually become constant, so long as the recolonization time is not exceeded. Such behavior was observed for the bone marrow.\(^5\) (c) Finally, equation (15) provides a means for estimating $S$, the fraction of cells surviving reproductively after a given dose of radiation, and so constructing a survival curve.

Methods and Materials.—Female Swiss mice, 3–5 weeks of age and 20–25 gm in weight, were irradiated with Co\(^{60}\) $\gamma$ rays delivered to the whole body at a rate of 30.5 rads per min. After periods ranging from 16.5 to 31.0 hr, animals were sacrificed and $n$, the total nucleated cell count in the bone marrow, spleen, and thymus, was measured by methods previously described.\(^3\), \(^6\) The fraction of nucleated cells relative to uniradiated controls, $n/n_0$, was recorded for each of the test animals. In each experimental set, a range of doses between 50 and 250 rads was employed, and the values of $n/n_0$ were determined. Determinations were limited to situations where both $n/n_0$ and $e^{-kt}$ were not too small, since otherwise the numerator of equation (16) becomes the difference between two small numbers and hence the uncertainty mounts greatly. The precision of such an experiment involves an uncertainty of $\pm 20$ per cent in each measurement as previously described.\(^3\) For each dose and time, $S$ was computed as

$$S = \frac{n}{n_0} - e^{-kt}$$

$k_4$ was determined for each tissue from the limiting slope of the depletion curve, obtained with radiation doses greater than 250 rads, as explained earlier.\(^5\) For the bone marrow of the mice studied, $k_4$ was 0.065 hr\(^{-1}\), while for the spleen and thymus the corresponding values were 0.106 hr\(^{-1}\) and 0.14 hr\(^{-1}\), respectively.\(^3\), \(^5\)

Experimental Results.—(1) Reproductive survival curve for bone marrow cells in vivo: In Table 1 and Figure 1 are presented typical data and a curve showing $S$, the fraction of cells surviving reproductively as a function of the radiation dose delivered, as calculated from equation (16). As expected, the points show a somewhat greater scatter than those obtained from in vitro cell-plating experiments. However,
### TABLE 1

**NUCLEATED CELL CONTENT OF TIBIAL BONE MARROW OF IRRADIATED MICE, FOR DIFFERENT DOSES, MEASURED AT DIFFERENT TIMES OF SACRIFICE**

<table>
<thead>
<tr>
<th>Dose (rads)</th>
<th>Time in hr between irradiation and sacrifice</th>
<th>n = Nucleated cell count $\times 10^7$</th>
<th>$S = \frac{n}{n_e - e^{-k_d}}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0</td>
<td>$1.00 \pm 0.20$</td>
<td>1.00</td>
</tr>
<tr>
<td>50</td>
<td>24.0</td>
<td>0.88</td>
<td>0.85</td>
</tr>
<tr>
<td>100</td>
<td>20.0</td>
<td>0.59</td>
<td>0.44</td>
</tr>
<tr>
<td>150</td>
<td>24.0</td>
<td>0.52</td>
<td>0.39</td>
</tr>
<tr>
<td>200</td>
<td>20.0</td>
<td>0.64</td>
<td>0.60</td>
</tr>
<tr>
<td>250</td>
<td>24.0</td>
<td>0.38</td>
<td>0.22</td>
</tr>
<tr>
<td>31.0</td>
<td>0.82</td>
<td>0.53</td>
<td>0.46</td>
</tr>
<tr>
<td>100</td>
<td>24.0</td>
<td>0.38</td>
<td>0.22</td>
</tr>
<tr>
<td>150</td>
<td>20.0</td>
<td>0.40</td>
<td>0.18</td>
</tr>
<tr>
<td>200</td>
<td>24.0</td>
<td>0.28</td>
<td>0.09</td>
</tr>
<tr>
<td>250</td>
<td>24.0</td>
<td>0.31</td>
<td>0.23</td>
</tr>
<tr>
<td>31.0</td>
<td>0.30</td>
<td>0.30</td>
<td>0.22</td>
</tr>
</tbody>
</table>

*S is the fraction of survival of reproductive cells as calculated by equation (16); $k_d$ is 0.065 hr$^{-1}$ for this tissue in these animals.*

A survival curve appears to be defined. The average value and standard deviation of the mean lethal dose, $D_0$, obtained in a series performed on 37 animals was $121 \pm 18$ rads. The extrapolation number was $1.24 \pm 0.25$. The means and standard deviations were determined by least-squares analysis of all the points beyond 50 rads.

(2) Reproductive survival curves for spleen and thymus cells by means of depletion curve analysis: Similar experiments were carried out for the spleen and thymus. The resulting curves are of interest because both these tissues have different turn-
over constants from that of the bone marrow, and the thymus cells also have a different physiological function from those of the bone marrow. Hence, such curves would offer a further test of the postulate that somatic cells of different differentiation characteristics would still exhibit survival curves with closely similar $D^0$ values. The resulting survival curves obtained by the same technique as used for the bone marrow are shown in Figure 2. The $D^0$ values and extrapolation numbers may be considered to be identical with those of the bone marrow cells, within the limits of experimental accuracy.

Discussion.—The value for $D^0$ here obtained may be compared with the value 128 rads for the S3 HeLa cell which has been used as a reasonably representative curve for a variety of mammalian cells;\(^2\) and with the values 122, 114 ± 4, 115 ± 8, 105 ± 13, 110, and 95 ± 9 rads which have previously been reported for a variety of normal and malignant mouse cells, in experiments conducted \textit{in vivo} and \textit{in vitro}.\(^2,\)\(^8\) The extrapolation numbers of all these survival curves have lain in the region between 1 and 2.5 in further agreement with the current findings. It may be concluded that cell depletion in the tissues studied follows the course predicted by the theory of cell reproductive inhibition as the major causal process in the tissues studied for the ranges of time and dosage here employed.

The method as here employed is simple and rapid, but in its present form suffers from the relatively large degree of experimental error which increases as the turnover time of the tissue involved increases. If the validity of this approach is confirmed and the experimental accuracy can be increased, depletion analysis of tissue cells would appear to lend itself to a variety of possible applications: (1) It permits testing the reproductive survival curves of cells in a variety of tissues, and so permits search to be made to determine whether cell populations do exist with survival curves widely different from the standard pattern which has been found so often for mammalian cells. (2) It makes possible rapid determination of over-all rates of removal of cells in a variety of tissues and so furnishes important data on cell turnover. (3) By carrying out such measurements on the various subpopulations of each organ, it promises to aid in determining details about the intimate cellular dynamics of the system. (4) It makes possible comparison of cell depletion of normal with pathological tissues, and of adult tissues with those in different developmental states. (5) Finally, comparison of the depletion effects induced by drugs with the maximum rate obtainable by the use of ionizing radiation makes possible a simple means of study of some of the effects of various drugs on cell turnover in specific tissues.

Summary.—Further test of the theory that the principal action of ionizing radiation is to inhibit reproduction of somatic mammalian cells \textit{in vivo} has been carried out, by analysis of depletion curves of nucleated cells of the bone marrow, thymus, and spleen of mice exposed to $\gamma$-irradiation. A simplified, formalistic model based on this theory permits establishment of a single cell survival curve for the reproductive function. Survival curves so calculated for the cells of bone marrow, the spleen, and thymus of young mice agree well with the curves obtained by direct measurement for mammalian cells generally and mouse cells specifically. Other possible applications of depletion curve analysis have been indicated.

\(^1\) It is a pleasure for the author to acknowledge the highly competent technical assistance of Mr. Paul Wuthier.
ERRATA

In the article entitled "Alterations in Cellular Metabolism Associated with Cell Death Induced by Uracil Mustard and 6-Thioguanine," by Barbara A. Booth, William A. Creasy, and Alan C. Sartorelli, which appeared in the December 1964 issue of these Proceedings [Vol. 52, 1396–1402 (1964)], the following corrections should be made: ordinate of Figure 2B and heading of column 2, Table 3, should read "μmoles RNA ribose/5 × 10⁶ cells."

ERRATUM

In the paper entitled "Theory of the Flow of Action Currents in Isolated Myelinated Nerve Fibers, VI," by R. Lorente de Nó and V. Honrubia, which appeared in the October issue of these Proceedings [Vol. 54, 1061–1069 (1965)], on page 1063 the reference number cited at the end of the second paragraph should be 2 instead of 3. Also on page 1063, in the third line of the last paragraph the words "central pool" should be replaced with "first gap."