Turnover Rate of Normal Blood Lymphocytes and Exchangeable Pool Size in Man, Calculated From Analysis of Chromosomal Aberrations Sustained During Extracorporeal Irradiation of the Blood

By E. O. Field, H. B. A. Sharpe, K. B. Dawson, V. Andersen, S. A. Killmann, and E. Weeke

Lymphocyte kinetics have been studied in four patients treated by extracorporeal irradiation of the blood (ECIB) for immunosuppressive purposes prior to renal transplantation. The technique involves the analysis of chromosomal aberrations sustained by lymphocytes during irradiation and demonstrable in cultures stimulated with phytohaemagglutinin (PHA). Lymphocytes containing aberrations may be regarded as labeled, and this form of labeling has the advantage that it does not require the manipulation of lymphocytes outside the body. Preliminary experiments established that about half of lymphocytes irradiated with doses of 300–400 rad fail to undergo mitosis in culture and that the aberration yield per lymphocyte is the same at the end of a course of ECIB as at the beginning. Irradiated lymphocytes were found to have a mean residence time in the blood of less than 2 min, and for the first few hours of irradiation they were replaced by lymphocytes from tissue pools at a rate such that there was no appreciable fall in the peripheral count. These findings imply that a large proportion of the vascular endothelium acts as a filter for damaged lymphocytes and that initially damaged cells were replaced at the rate of one blood pool per hour; this may represent the normal turnover time of blood lymphocytes. The size of the total exchangeable lymphocyte pool was calculated by two methods depending on a series of basic assumptions concerning the kinetics of irradiated cells. According to the most likely estimate, the total pool contains some 30 times as many cells as circulate in the blood. Forty-two per cent of lymphocytes survived an irradiation dose of 300 rad, 10%/–19%/ survived 380 rad.

That small lymphocytes recirculate between the blood and lymphoid organs has now been firmly established; the experimental background to this concept has been fully reviewed.1 Yoffey2 has applied the term “fourth circulation” to the sum total of all streams of lymphocytes migrating...

From the Radiotherapy Research Unit, Institute of Cancer Research, Royal Marsden Hospital, Sutton, Surrey, England, and the Divisions of Hematology and Nephrology, Rigshospitalet, Blegdamsvej, Copenhagen, Denmark.


E. O. Field, D.M., D.M.R.D.: Head, Radiotherapy Research Unit, Institute of Cancer Research, Royal Marsden Hospital, Sutton, Surrey, England, H. B. A. Sharpe, Ph.D., B.V.Sc., M.R.C.V.S.: Member of Scientific Staff, Radiotherapy Research Unit, Institute of Cancer Research, Royal Marsden Hospital, Sutton, Surrey, England, K. B. Dawson, M.Sc., Ph.D.: Member of Scientific Staff, Radiotherapy Research Unit, Institute of Cancer Research, Royal Marsden Hospital, Sutton, Surrey, England. V. Andersen, M.D.: Member of Medical Staff, Division of Hematology, Rigshospitalet, Blegdamsvej, Copenhagen, Denmark. S. A. Killmann, M.D., Ph.D.: Chief, Division of Hematology, Rigshospitalet, Blegdamsvej, Copenhagen, Denmark. E. Weeke, M.D., Member of Medical Staff, Division of Nephrology, Rigshospitalet, Blegdamsvej, Copenhagen, Denmark.

from the blood between the scattered members of the lympho-myeloid complex. In animals the rate of recirculation has been shown to be rapid,\textsuperscript{3-5} and the total size of the tissue pools amenable to depletion by thoracic duct drainage to be some 20 times that in the peripheral circulation.\textsuperscript{6}

There have been relatively few studies on the kinetics of normal lymphocytes in man. These have been carried out mainly on patients subjected to thoracic duct cannulation and have involved either transfusion of lymphocytes into recipients of the opposite sex, reinfusion of lymphocytes labeled isotopically in vitro into autologous recipients, or the determination of total number of lymphocytes withdrawn.

When female thoracic duct lymphocytes were infused into male patients, it was found by using the sex chromosome as a marker, that the donor cells disappeared rapidly from the circulation.\textsuperscript{7} From the published data it can be calculated that approximately one-half of the donor cells remained 1 hr after infusion; very few or none could be detected after 4 hr.

From the published data on patients undergoing thoracic duct drainage,\textsuperscript{8,9} it could be determined that, excluding newly produced, possibly short-lived, lymphocytes, the lymphocytes that could be removed by this route totaled between five and 20 times the number initially circulating in the blood. Similar values were obtained when thoracic duct lymphocytes in two patients were labeled in vitro with tritiated uridine and were reinfused into autologous recipients; the labeled cells were cleared from the blood with half times of 20–25 min.\textsuperscript{9}

The validity of conclusions drawn from these studies may be questioned on the grounds that the manipulation of lymphocytes outside the body, in particular for isotopic labeling, could affect their viability or alter their physiologic behavior on reinfusion, that allogeneic disparities could lead to their selective sequestration, or that prolonged thoracic duct drainage could disturb the normal dynamics of their recirculation.

An alternative approach for studying lymphocyte kinetics in man is offered by the technique of extracorporeal irradiation of the blood (ECIB). During this procedure lymphocytes that are irradiated sustain chromosomal aberrations by virtue of which they may be regarded as labeled. This method of labeling, apart from being free of the objections that apply to the above-mentioned studies based on thoracic duct cannulation carries other advantages. A very large bulk of lymphocytes is labeled, as compared with in vitro isotopic labeling; thus, the proportion of labeled cells detectable in the blood is much higher. The number of aberrations contained by a cell gives a measure of the irradiation it has received (Fig. 6). Therefore, it is possible to assess whether a cell has traversed the irradiation field on more than one occasion.

The main disadvantage of ECIB as a labeling procedure is that a fraction of irradiated lymphocytes is destroyed by the irradiation. However, the fraction lost for any radiation dose received should be amenable to experimental determination (see below).

In this paper we report the results of studies, based on this approach, carried out on four nonleukemic patients with chronic renal failure who were
TURNOVER RATE OF LYMPHOCYTES

Four patients receiving ECIB for immunosuppressive purposes prior to renal transplantation were studied. The hematologic data were similar in all four: hemoglobin values ranged from 7.0 to 8.0 g/100 ml; neutrophils numbered 2,000–3,500/µl; lymphocytes, 1,000–1,500/µl; thrombocytes, 200,000–400,000/µl; and the blood urea concentrations were 80–95 mg/100 ml. Irradiation was provided by a 60Co source; the blood flowed through the arteriovenous shunt at a rate that varied from patient to patient from 60 to 100 ml/min giving transit doses of 300–600 rad.

With each patient an initial sample of blood was obtained from the venous side of the irradiation coil within a few minutes after the start of irradiation. During this procedure there was, unavoidably, a small increase in flow rate and consequently a reduction in radiation dose. The appropriate correction was therefore applied to the observed value for the proportion of lymphocytes containing aberrations to derive a value applicable during the subsequent treatment. All other blood samples were obtained from the arterial side at the times specified in Table 2.

A separated leukocyte technique was used in preparing the cultures. Seven-hundredths milliliters of phytohaemagglutinin (PHA, Wellcome) were added to 1 ml of whole blood and 1 ml of bovine serum (Flow Laboratories). The blood was centrifuged at 500 rpm, and 1 ml of supernatant containing the leukocytes was transferred to a culture bottle together with 4 ml of Eagle’s minimum essential tissue culture medium (Flow Laboratories) with added antibiotics. The cultures were incubated at 37°C for 48 hr. and Colcemid (CIBA) was added 3 hr before the cultures were fixed. The cells were treated with hypotonic potassium chloride for 10 min before they were fixed in 3:1 (v/v) ethanol:glacial acetic acid and then washed in two changes of 3:1 (v/v) methanol:glacial acetic acid. The cells were dispensed onto ice-cold slides and dried over a low Bunsen flame. The slides were stained in lactic orcein and mounted in euparal. For scoring purposes the slides were coded and randomized. Only cells with the complete chromosome complement were scored. Cells were examined for stable and unstable aberrations, but only data on dicentrics have been recorded here. Experience has shown that dicentric aberrations may be scored more accurately and consistently than other types of aberrations.

RESULTS

Preliminary Experiments

Mitotic Index of Irradiated Lymphocytes: In calculating pool size and turnover rates of lymphocytes (see Appendix) it is necessary to determine the proportion of lymphocytes, in samples of peripheral blood, that have traversed the irradiation field. The distribution of dicentric aberrations among irradiated lymphocytes is Poissonian, and, depending on the radiation dose received, a variable fraction of cells will acquire no detectable aberrations. This fraction can be found by analyzing the aberration distributions either in a blood sample obtained from the venous cannula of the ECIB coil within a few minutes of starting treatment, or in a pretreatment sample irradiated
in vitro with a dose equivalent to the ECIB transit dose. Such analysis will reveal the fraction of irradiated PHA-responsive lymphocytes that acquire no aberrations. However, if the mitotic indices of irradiated lymphocytes are lower than those of nonirradiated, relatively fewer metaphases—and therefore metaphase aberrations—will be contributed by irradiated lymphocytes to the total metaphases observed in samples of peripheral blood that comprise a mixture of irradiated and nonirradiated cells.

It has been shown that following a dose of 300 rad in vitro, the time at which PHA-stimulated lymphocytes reach mitosis is virtually unaffected, but the mitotic index is considerably reduced as compared with nonirradiated cultures. Since, some irradiated lymphocytes may be killed in interphase while others may only be prevented from entering the mitotic cycle, the direct comparison of mitotic indices (if defined as numbers of cells in metaphase expressed as fractions of total lymphocytes scored) may not give a valid estimate of the true relative mitotic index, i.e., the relative numbers of cells in metaphase expressed as fractions of total lymphocytes initially cultured. The true relative mitotic index can be derived by measuring the aberration yields obtained respectively when irradiated lymphocytes are cultured alone or mixed with an equal number of nonirradiated lymphocytes. The derivation is given in the Appendix (equation 1). Figure 1 shows the relationship obtained between relative mitotic index and radiation dose in a preliminary experiment with lymphocytes from a normal subject. At least 100 dicentrics were scored to derive each aberration yield. The data from which the calculations were made have been published elsewhere. The standard errors of the computed relative mitotic indices were calculated by an approximation of Fieller's method and are inevitably large, since they apply to ratios of pairs of measured yields. Nevertheless, it is evident that with the radiation doses used in the present studies, in the region of 300–400 rad, approximately one-half of irradiated lymphocytes fail to reach mitosis in culture.

Aberration Yield Before and After ECIB: Two classes of lymphocyte have been recognized, short- and long-lived. It could be expected that after prolonged ECIB the composition of the blood lymphocyte population would alter, showing a preponderance either of the short-lived, more actively proliferating variety, or of a younger class of long-lived lymphocyte invoked by feedback mechanisms to regenerate more rapidly. It was therefore necessary to determine whether the response of lymphocytes to irradiation, in terms of the chromosome aberration yield, was different at the end of an extended course of ECIB than at the beginning. If a difference were found to exist, the theory derived below for interpreting lymphocyte kinetics (Appendix) would not be applicable.

Table 1 summarizes the results of this type of study carried out on patient 4. The aberration yields were found to be almost identical when blood samples, obtained either before commencement of treatment or at the end of a total of 104 hr of intermittent ECIB (conditions listed in Table 2), were irradiated in vitro with doses of 300 and 500 rad, respectively. In computing the aberration yields of lymphocytes obtained at the end of treatment, the
TURN OVER RATE OF LYMPHOCYTES

Fig. 1. Irradiated human lymphocytes. Relative mitotic indices of irradiated lymphocytes, calculated as fractions retaining capacity to undergo mitosis in 48-hr PHA culture (equation (1), Appendix). Vertical bars represent standard errors.

"background" frequency of aberrations, due to the previous in vivo irradiation, was subtracted from the gross frequencies observed.

Lymphocyte Kinetics During ECIB

Peripheral Lymphocyte Count: Figure 2 shows changes in the peripheral lymphocyte count after different periods of ECIB in four patients. To allow for differences in flow rate through the irradiation field, the time scale on the abscissa has been replaced by numbers of blood volumes irradiated. The irradiation schedules differed widely from one patient to another. In one, ECIB was given continuously for 24 hr and was then given intermittently after a break of 4 days. In another, ECIB was given continuously for 8 hr and then intermittently three times a week after a break of 4 days; In the

Table 1. Yield of Chromosomal Aberrations in Lymphocytes Irradiated In Vitro at Beginning and at End of 104 Hr of ECIB

<table>
<thead>
<tr>
<th>Sample</th>
<th>Dose In Vitro (rad)</th>
<th>No. of Cells Exam.</th>
<th>Aberrations</th>
<th>Experimental Findings</th>
<th>Increment $f$ Calculated per 100 Cells</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>All*</td>
<td>Dicentric</td>
<td>Cells With Dicentrics</td>
</tr>
<tr>
<td>Pre-ECIB</td>
<td>0</td>
<td>100</td>
<td>1</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>300</td>
<td>112</td>
<td>177</td>
<td>127</td>
<td>75</td>
</tr>
<tr>
<td></td>
<td>500</td>
<td>95</td>
<td>315</td>
<td>213</td>
<td>87</td>
</tr>
<tr>
<td>Post-ECIB</td>
<td>0</td>
<td>280</td>
<td>165</td>
<td>111</td>
<td>49</td>
</tr>
<tr>
<td></td>
<td>300</td>
<td>65</td>
<td>142</td>
<td>102</td>
<td>47</td>
</tr>
<tr>
<td></td>
<td>500</td>
<td>49</td>
<td>191</td>
<td>119</td>
<td>44</td>
</tr>
</tbody>
</table>

*Including centric and acentric rings and fragments.
†Comparing values obtained without irradiation and those obtained after 300 or 500 rad in vitro.
‡Calculated by the expression $\frac{y - fx}{x - fx}$ where $x$ and $y$ are the numbers of cells examined and containing dicentrics, respectively; $f = 49/280$, the proportion expected to contain dicentrics before in vitro irradiation.
Fig. 2. Changes in peripheral lymphocyte count in four patients treated by repeated courses of ECIB. Different curves relating to individual patients correspond with those in Fig. 4. patient 1, ——-.; patient 2, ———; patient 3, . . . . ; patient 4, ——-.

Fig. 3. Observed aberration yields in blood samples from patient 4, during 8-hr continuous ECIB and for subsequent 2½ treatment-free days. Vertical bars represent standard errors.

remaining two patients, treatment was given intermittently for 6-10-hr periods twice and three times weekly, respectively (Table 2).

Despite the different treatment schedules, the response, in relation to the blood volumes irradiated, was remarkably uniform. It is difficult to assess the early response owing to wide fluctuations in the peripheral lymphocyte count induced by heparinization and by stress. However, only after 15-20 blood volumes had been irradiated did the count begin to fall below 1000/μl, and after 40 blood volumes had been irradiated the count stabilized at just under 500/μl.

Chromosome Aberration Yields During Short-Term ECIB: Figure 3 shows the pattern of accumulation of aberrations in blood lymphocytes at various intervals during an 8-hr course of continuous ECIB and for the next 57 hr following the termination of this course in patient 4. During ECIB there was a progressive accumulation of cells containing aberrations, reaching a value of 4.3% after 8 hr. Immediately on cessation of treatment there was a rapid fall to a lower level, which was then maintained for 2½ treatment-free days at a mean value of 1.1% ± 0.4% (standard error) for five consecutive samples.

This pattern of accumulation was repeated in the other three patients. In Figs. 4 and 5 the fractions of irradiated lymphocytes in the blood throughout a prolonged course of treatment (calculated by equation (3), Appendix), are plotted against the number of blood volumes irradiated. Comparing the slopes
Blood Volumes

TURNOVER RATE OF LYMPHOCYTES

Fig. 4. Proportions of irradiated lymphocytes in blood (calculated by equation (3), Appendix) during initial period of continuous ECIB. Different curves relating to individual patients correspond with those in Fig. 2.

obtained during the early stages of ECIB (Fig. 4), when blood samples were drawn while treatment was in progress, with the slopes given when samples were drawn some hours after the terminations of treatment periods (Fig. 5), it is evident that a substantial proportion of aberrations that accumulated in the blood during irradiation were eliminated after irradiation ended.

Chromosome Aberration Yields During Long-Term ECIB: Table 2 lists the yields of dicentric aberrations recorded in the four patients at different stages of their courses of treatment. The total numbers of blood volumes irradiated, at the times that the last measurements were made, were in the range 64–87, yet less than one-quarter of circulating lymphocytes contained aberrations. In patient 1, aberrations were scored 2½ days after the end of his course and again 11 days later, with no further treatment given meanwhile. The small reduction in percentage of lymphocytes bearing aberrations (from 24.8% to 20.3%) that took place in this interval was not significant (p = 0.5 by the $x^2$ test).

Figure 5 shows the distribution of dicentrics among lymphocytes bearing these aberrations at different times during treatment in the four patients, as well as distributions obtained for normal lymphocytes (pooled data from three donors) with different radiation doses given in vitro. In patient 2 a transit dose of 360 rad was employed during the irradiation of the first 24.2 blood volumes, and of 600 rad for the remainder of the treatment course (Table 2). This was reflected in the differences in distributions of dicentrics observed between the earlier blood samples and the last sample, obtained after 64.8 blood volumes had been irradiated. In the remaining three patients there was very little shift toward higher numbers of aberrations per metaphase with time of treatment, suggesting that the dose rate had been essentially uniform and that almost all lymphocytes irradiated more than once had been eliminated.

DISCUSSION

For the purpose of the discussion that follows, the kinetics of lymphocyte exchange between the blood, extravascular pools, and ECIB shunt, the
Fig. 5. Proportions of irradiated lymphocytes in blood (calculated by equation (3), Appendix) during intermittent course of ECIB, with blood samples being drawn at least 24 hr after a treatment period. (Higher transit dose was employed for second portion of course of patient 2, see Table 2).

elimination of damaged cells, and the generation of new ones, are depicted schematically in Fig. 7.

**Turnover of Blood Lymphocytes**

*Removal of Irradiated Cells:* It can be calculated from the data plotted in Fig. 4, where allowance has been made (by applying equation (3) Appendix) for lymphocytes that fail to undergo mitosis in culture, that only 3%-5% of irradiated lymphocytes remained in the blood after the first blood volume had flowed through the irradiation coil in 41-87 min. If no exchange of lymphocytes with extravascular pools had taken place, the proportion of irradiated cells would have been 63%. This finding points to a rapid rate of replacement of the irradiated cells.

An estimate of the rate of removal of irradiated lymphocytes, $k_2$ in equation (4) (Appendix), might be obtained by substituting the values for the irradiated fractions of lymphocytes, $q$, observed at the time that one blood volume had been irradiated, i.e., when $t = 1/k_1$, $k_1$ being the known rate of irradiation of lymphocytes. (These values of $q$ can be gauged from curves drawn through the origin and the experimental points in Fig. 4, with a slight initial upward convexity, as expected from a consideration of equation (4)). These data are listed in Table 3, the calculated values of $k_2$ ranging, for the four patients, from 22.3 to 27.6 blood pools removed per hour. The corresponding mean residence times of irradiated lymphocytes in the blood, given by $1/k_2$, were 2.2–2.7 min.

However, if these values calculated for $k_2$ are substituted in equation (4), it becomes evident that the irradiated fraction should reach 99% of its steady-state value, when the loss of irradiated cells from the blood is balanced by their rate of generation, in 10–12 min. Nevertheless, as shown in Fig. 4 the proportions of irradiated cells continue to increase for several hours during ECIB. This divergence between theory and practice could be explained by the reentry into the blood of irradiated cells from the tissue pools or by saturation of the clearance mechanism. The extent of this process cannot
### Table 2. Treatment Schedules and Yields of Dicentric Aberrations in Four Patients Given Prolonged Courses of ECIB

<table>
<thead>
<tr>
<th>Patient, Sex and Age</th>
<th>Treatment Schedule</th>
<th>Mean Flow Rate (Blood/vis/hr)</th>
<th>Transit Dose (rad)</th>
<th>Sample Examined</th>
<th>Total Metaphases Scored</th>
<th>Metaphases With Dicentrics (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. NCN Male 50</td>
<td>6-10-Hr treatment periods, 2 days/wk</td>
<td>1.45</td>
<td>300</td>
<td>First transit, from venous cannula (270 rad). 75 min continuous ECIB. 150 min continuous ECIB. 14 hr after end of first 10-hr period. 60 hr after end of intermittent course totaling 60 hr. 11 days later (no treatment meanwhile).</td>
<td>113</td>
<td>53.0</td>
</tr>
<tr>
<td>2. NR Male 33</td>
<td>7.5-9-Hr treatment periods, 3 days/wk</td>
<td>0.69</td>
<td>360</td>
<td>First transit, from venous cannula (420 rad). 75 min continuous ECIB. 150 min continuous ECIB. 24 hr after end of first 4 periods, totaling 35 hr. 24 hr after end of 11 periods, totaling 96 hr.</td>
<td>65</td>
<td>76.0</td>
</tr>
<tr>
<td>3. EP Female 32</td>
<td>24 Hr continuous, then 4-8-hr periods, 3 days /wk, starting on 5th day</td>
<td>1.18</td>
<td>380</td>
<td>First transit, from venous cannula (405 rad). 90 min continuous ECIB. 180 min continuous ECIB. 24 hr after end of first continuous 24-hr period. 4 1/2 days after end of intermittent course totaling 54 hr.</td>
<td>72</td>
<td>72.2</td>
</tr>
<tr>
<td>4. GL Female 42</td>
<td>8 Hr continuous, then 5.5-8.3-hr periods, 4 days/wk, starting on 4th day</td>
<td>0.82</td>
<td>380</td>
<td>First transit, from venous cannula (350 rad). 4 hr continuous ECIB. 6 hr continuous ECIB. 8 hr continuous ECIB. Average of 5 samples during 2 1/2 treatment-free days. 2 1/2 days after end of intermittent course totaling 104 hr.</td>
<td>67</td>
<td>59.7</td>
</tr>
</tbody>
</table>

**Turnover Rate of Lymphocytes**
be assessed, but if it did not take place the values of \( q \) would have been even lower than those actually found after the irradiation of one blood pool. The calculated mean residence times (Table 3) therefore represent upper limits; the actual mean residence time could well be shorter than 2 min.

This observation could have important physiologic implications. Since the cardiac output is of the order of one blood volume per minute at rest, a mean residence time of 2 min implies that at least half of the irradiated lymphocytes in the blood were removed during one circulation time. It follows that virtually the whole of the vascular endothelium, either of the pulmonary or of the systemic circulation, or of both, must have acted as a "filter bed" for irradiated lymphocytes. If this were so, then the view held hitherto that lymphocytes are filtered out of the blood predominantly in lymphoid organs would no longer be tenable, at least in regard to cells damaged by irradiation.

**Replacement of Irradiated Cells:** Since no appreciable fall in the peripheral lymphocyte count was observed during the extracorporeal irradiation of the
TURNOVER RATE OF LYMPHOCYTES

Table 3. Mean Residence Times of Irradiated Lymphocytes in Blood (Upper Limits)

<table>
<thead>
<tr>
<th>Patient</th>
<th>Irradiated Fraction</th>
<th>Blood Pools Irradiated 'Hr</th>
<th>Blood Pools Replaced/Hr</th>
<th>Mean Residence Time (min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>5.0</td>
<td>1.45</td>
<td>27.6</td>
<td>2.2</td>
</tr>
<tr>
<td>2</td>
<td>3.0</td>
<td>0.69</td>
<td>22.3</td>
<td>2.7</td>
</tr>
<tr>
<td>3</td>
<td>5.0</td>
<td>1.18</td>
<td>22.4</td>
<td>2.7</td>
</tr>
<tr>
<td>4</td>
<td>3.0</td>
<td>0.82</td>
<td>26.6</td>
<td>2.3</td>
</tr>
</tbody>
</table>

*Derived by interpolation as explained in text. (Fig. 4).

first few blood volumes, despite the very rapid rate of removal from the blood of irradiated cells, lymphocytes from tissue pools must have replaced the irradiated cells virtually as rapidly as these were "generated" in the extracorporeal shunt, i.e., at the rate of approximately one blood pool per hour. The crucial question is whether the lymphocytes that replaced the irradiated cells reentered the blood at their normal rate (K in Fig. 7), or were mobilized from tissue depots at an accelerated rate in response to homeostatic mechanisms operating to maintain a constant circulating pool.

If homeostatic mechanisms were involved, the response could have been evoked either by a fall in the peripheral lymphocyte count or by chemicals released from damaged cells. It has been observed that the peripheral lymphocyte count does not fall appreciably after the removal by leukapheresis, in a period of 6 hr, of six times the number of lymphocytes initially present in the blood. Hence, damaged cells were probably not implicated in the response. Unless homeostatic mechanisms are extremely sensitive to changes in the peripheral count, the possibility must be considered that the observed rapid replacement of irradiated cells by lymphocytes from tissue pools reflected the normal turnover of blood lymphocytes.

Calculation of Pool Size

Except in the case of patient 2, distributions of dicentrics among irradiated lymphocytes throughout a prolonged course of treatment were such as would have been expected with a uniform transit dose and a virtual elimination of lymphocytes irradiated more than once. Under these conditions it is valid to apply equation (7) (Appendix) to the calculation of the total body pool of lymphocytes. The equation cannot be applied in the case of patient 2, where \( k_i \) was varied during the course of treatment.

In calculating pool sizes, two sources of uncertainty arise. One derives from the finding that during a treatment period irradiated lymphocytes accumulate in the blood for a time owing to a delay in their elimination. Thus, the average fraction of irradiated lymphocytes pertaining during a treatment period may be somewhat higher than would be suggested by a curve drawn through experimental points (Fig. 5) measured when sufficient time has elapsed since the previous period of treatment for the accumulated damaged cells to be eliminated. A more valid estimate is given by equation (8).

The other derives from the fact that a proportion of irradiated lymphocytes may be present, but their presence is not revealed if they fail to enter mitosis.
Fig. 7. Schematic diagram to illustrate exchange of lymphocytes between the blood and extravascular pools, their irradiation in extracorporeal shunt, elimination of lethally damaged cells, and generation of new lymphocytes. \( k_1 \) and \( k_2 \) are rate constants for generation and removal of irradiated blood lymphocytes, respectively; \( k_3 \) rate constant for exchange with extravascular poor under normal steady-state conditions.

If these lymphocytes are eliminated selectively from the blood, they should not be taken into account when estimating the fraction of irradiated lymphocytes present in the blood, and equation (2) applies. If, however, irradiated lymphocytes that respond to PHA are eliminated as rapidly as those that do not, then equation (3) applies. Two separate series of calculations were therefore made based on the respective assumptions that: (i) PHA-unresponsive cells are selectively eliminated, and (ii) PHA-unresponsive cells are not selectively eliminated.

The results of these calculations are listed in Table 4. The second assumption (ii) gives the lowest estimate for the ratio of total/circulating pool size, at values ranging from 25 to 30. Estimates by the first assumption (i) give ratios of 31–34. These estimates are a little higher than the values derived from studies on thoracic duct drainage,\(^8\)^,\(^9\) possibly because some types of lymphocytes in the blood do not circulate through the thoracic duct.

In these computations no account has been taken of the contribution made by newly produced lymphocytes. It has been estimated that about \( 0.6 \times 10^9 \) lymphocytes are produced per day during prolonged thoracic duct drainage.\(^8\) Since the total exchangeable pool contained at least \( 100–200 \times 10^9 \) lymphocytes (Table 4) and since the periods of study spanned only 20–30 days, the contribution made by the newly produced lymphocytes is comparatively small.
Table 4. Total Exchangeable Body Pool and Radiosensitivity of Lymphocytes, Computed by Two Methods*  

<table>
<thead>
<tr>
<th>Patient</th>
<th>Transit Dose (rad)</th>
<th>Blood Pool (× 10⁶)</th>
<th>Total/Blood</th>
<th>S(%)*</th>
<th>Total/Blood</th>
<th>S(%)*</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>300</td>
<td>4.3</td>
<td>33.1</td>
<td>42</td>
<td>25.6</td>
<td>77</td>
</tr>
<tr>
<td>3</td>
<td>380</td>
<td>3.6</td>
<td>34.1</td>
<td>19</td>
<td>28.5</td>
<td>34</td>
</tr>
<tr>
<td>4</td>
<td>380</td>
<td>6.5</td>
<td>31.4</td>
<td>15</td>
<td>29.7</td>
<td>26</td>
</tr>
<tr>
<td>Mean</td>
<td></td>
<td></td>
<td>32.9</td>
<td>27.9</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*PHA-unresponsive cells are assumed to have been selectively eliminated in method 1, but are included in calculations in method 2. For full explanation, see text.

Fate of Irradiated Lymphocytes

By virtue of the rapid exchange found to take place between lymphocytes in the blood and in tissue pools, these combined pools may be regarded as a single physiologic compartment. The fall in the proportion of irradiated lymphocytes in the blood, which follows the end of a period of extracorporeal irradiation, is then due to a slower process of redistribution in which irradiated lymphocytes pass out of the rapidly exchanging compartment either into pools that exchange more slowly with this compartment or, if they were lethally damaged, to be eliminated by the reticuloendothelial system. The observation that irradiated lymphocytes accumulated in the rapidly exchanging compartment to a greater extent when the rate of irradiation was high (1.2–1.4 blood volumes/hr) than when the rate of irradiation was low (0.7–0.8 blood volumes/hr) (Fig. 4) supports this conclusion.

Table 4 also lists the values calculated for the fraction of lymphocytes that survived various transit doses. Calculations based on the assumption that PHA-unresponsive lymphocytes are selectively eliminated gave the lowest estimates: 42% of lymphocytes survived a transit dose of 300 rad, and 15%–19% survived 380 rad. These values for surviving fraction appear high when compared with the radiosensitivity parameters derived for various lymphoid tissues in animals, but lymphocytes in the blood have been shown to be considerably more radioresistant. The data presented in the present communication are in agreement with this latter observation.

APPENDIX: ABERRATION ANALYSIS—THEORY

Mitotic Index of Irradiated Lymphocytes

The mitotic index of irradiated, relative to that of nonirradiated, lymphocytes can be derived by measuring the aberration yields obtained when irradiated cells are cultured either alone or mixed with an equal number of nonirradiated cells.

Let \( A_i \) and \( A_r \) be the proportions of mitoses bearing chromosomal aberrations in cultures of irradiated cells alone and in mixed cultures, respectively, \( m_i \) and \( m_r \) be the respective mitotic indices (i.e., the proportions seen in mitosis
in PHA-culture) of irradiated and of nonirradiated lymphocytes. If \( y \) cells of each type are cultured together, the total mitoses produced will be \( y \left( m_i + m_o \right) \), and the number bearing aberrations will be \( A_i y m_i \). Hence,

\[
A_2 = \frac{A_i y m_i}{y \left( m_i + m_o \right)}
\]

which on rearranging gives the relative mitotic index

\[
\frac{m_i}{m_o} = \frac{A_2}{A_i - A_2}
\] (1)

Irradiated Fraction of Lymphocytes

If, in a sample of blood, the mitotic index of irradiated lymphocytes were the same as that of non-irradiated, and if no lymphocytes have been irradiated more than once, the fraction that has been irradiated is given by

\[
q = \frac{a}{A}
\] (2)

where \( a \) is the aberration yield found for the blood sample and \( A \) the yield produced by one transit dose, or the equivalent dose given in vitro. (Aberration yield could be defined either as mean number of aberrations per mitosis, or as proportion of mitoses carrying aberrations, in PHA-stimulated cultures. In this paper, the second definition is applied, since the number of aberrations per mitosis varies as a power, greater than one, of the radiation dose, and hence any cells surviving two transit doses contribute a disproportionately large number of aberrations.)

If, however, lymphocytes unresponsive to PHA are also present in the blood sample, then the mitotic index of irradiated lymphocytes relative to that of nonirradiated, \( m/m_o \), is given by equation (1). The irradiated fraction is then computed as follows:

Let \( p \) be the total number of lymphocytes and \( x \) be the number of irradiated lymphocytes in the blood at any time. Then, \( (p - x) m_o + x m \) cells would enter mitosis in culture, and \( x m A \) cells would contain aberrations. Hence,

\[
a = \frac{x m A}{(p - x) m_o + x m}
\]

Rearranging and substituting \( q = x/p \), gives

\[
\frac{1}{q} = \frac{m}{m_o} \left[ \frac{A}{a} - 1 \right] + 1
\] (3)

Turnover Rate of Blood Lymphocytes

It was found experimentally that during the irradiation of the first few blood volumes the rate of emigration of all classes of lymphocytes, irradiated as well as non-irradiated, is balanced by their rate of replacement. An esti-
mate of the turnover rate of blood lymphocytes can be derived from an analysis of chromosomal aberrations acquired during the first period of continuous ECIB, as follows (Fig. 7):

Let \( x \) be the number of irradiated lymphocytes in the blood at any time and \( p \) the total number of lymphocytes, assumed to be constant. \( k_1 \) and \( k_2 \) are fractional rates of irradiation and of removal of irradiated blood lymphocytes, respectively. If reentry of irradiated lymphocytes from tissue pools was neglected, then

\[
\frac{dx}{dt} = k_1 (p - x) - k_2 x
\]

Integrating gives

\[
t \frac{k_1 p}{k_1 p - x (k_1 + k_2)} = \ln \frac{x}{p} \left( \frac{k_1 + k_2}{k_1} \right) = 1 - e^{-(k_1 + k_2) t} \quad (4)
\]

This can be approximated to

\[
\frac{x}{p} = q = \frac{k_1}{k_1 + k_2} \quad (5)
\]

that is, the irradiated fraction becomes constant. In this analysis, \( q \), the irradiated fraction, is defined in terms of all lymphocytes that have traversed the irradiation field. Therefore their mitotic index, relative to that of non-irradiated lymphocytes, is given by equation (1), and \( q \) is computed from the observed aberration yield, \( a \), by equation (3). The mean residence time of irradiated lymphocytes in the blood is then \( 1/k_2 \).

**Pool Size Determination**

ECIB is given in repeated periods, during each of which \( l \) is the mean number of lymphocytes per ml of blood, \( f \) the mean blood flow rate (ml/min), and \( T \) the duration of the treatment period (min). Then the total number of lymphocytes that traverse the irradiation field

\[
n = l f T \quad (6)
\]

If, however, the blood contains a fraction, \( q \), of lymphocytes that have been irradiated previously, then the total number of newly irradiated lymphocytes,

\[
N = n (1 - q)
\]

Let \( S \) be the fraction of lymphocytes surviving one transit dose in vivo. Then the total number of newly irradiated lymphocytes that survive a period of ECIB is \( S N \), and the corresponding yield of potential aberrations is \( AmSN \). However, if it is assumed that negligible numbers of lymphocytes irradiated more than once survive, then \( nq \) lymphocytes are destroyed during this period. The net gain in lymphocytes irradiated once therefore becomes \( SN - qn \), and the net gain in potential aberrations \( Am(SN - qn) \).
Thus, the total yield of lymphocytes irradiated once, after a course of several periods of ECIB is

\[ \sum [SN - qn] = \sum n[S(1 - q) - q] \]

and the total yields of potential mitoses and aberrations are, respectively,

\[ mnS[(1 - q) - q] \quad \text{and} \quad AmE[S(1 - q) - q] \]

Let \( P \) be the total number of lymphocytes initially present in exchangeable body pools. Then \((P - \Sigma N)\) lymphocytes remain nonirradiated at the end of the course. Of these, \( m_0 \) \((P - \Sigma N)\) would be seen in mitosis in culture. Therefore, the total number of potential mitoses, irradiated and nonirradiated, remaining in the pool is

\[ m \sum n[S(1 - q)] + m_0(P - \Sigma N) \]

and the aberration yield observed at the end of the course of treatments,

\[ A_t = \frac{Am \sum n[S(1 - q) - q]}{m \sum n[S(1 - q) - q] + m_0[P - \Sigma n(1 - q)]} \]

Rearranging gives

\[ P = \sum n(1 - q) + \frac{m}{m_0} \left[ \frac{A}{A_t} - 1 \right] [S \cdot n(1 - q) - \Sigma nq] \]

The values of all the terms in equation (7) are determined experimentally, with the exception of \( P \) and \( S \), the size of the initial lymphocyte pool in the body, and the fraction surviving one transit dose of irradiation, respectively. These two parameters can be calculated by solving the simultaneous equations obtained by substituting values for \( A_t \) and \( nq \) determined at two separate stages during the course of treatment.

The value of \( n \) is that given in equation (6). The derivation of \( q \), the fraction of cells previously irradiated, presents certain difficulties. It is not known whether irradiated lymphocytes that fail to undergo mitosis in culture have suffered lethal damage such that they are eliminated from the body and never reenter the circulation, or whether these cells, although they fail to progress to mitosis, persist and behave kinetically in the same way as do the PHA-responsive lymphocytes. If it is assumed that they are eliminated then the mitotic index of the remaining irradiated lymphocytes can be taken to be identical with that of nonirradiated, and \( q \) is given by equation (2). If, however, PHA-unresponsive lymphocytes persist, then \( q \) has the value given in equation (3).

It was found that \( q \), determined by either of these two relationships, increased almost linearly with treatment time (Fig. 5), when blood samples were examined at least 24 hr after the end of any treatment period. Thus the value of \( q \) at the start of each treatment period during a course of ECIB
TURNOVER RATE OF LYMPHOCYTES

could be estimated by interpolation, the actual measurements having been made at the end of the course and at some intermediate stage. During any treatment period $q$ rises more rapidly than indicated by the curves in Fig. 5, owing to a delay in the elimination of irradiated cells from the blood. The true rate is suggested by the curves obtained during the first treatment periods (Fig. 4). A more accurate estimate of the mean value of $g$ for a treatment period would therefore be given by

$$q = q_o + s(1 - q_o) \frac{T}{2}$$  \hspace{1cm} (8)

where $q_o$ is the irradiated fraction at the start of a treatment period of duration $T$, and $s$ the slope of the corresponding initial curve. (Fig. 4).

REFERENCES

17. Ottesen, J.: On the age of human


Turnover Rate of Normal Blood Lymphocytes and Exchangeable Pool Size in Man, Calculated From Analysis of Chromosomal Aberrations Sustained During Extracorporeal Irradiation of the Blood

E. O. Field, H. B. A. Sharpe, K. B. Dawson, V. Andersen, S. A. Killmann and E. Weeke