Relative Labeling of Leukocytes, Erythrocytes and Platelets in Human Blood by $^{51}$Chromium

By HARMON J. EYRE, PETER J. ROSEn AND SEYMOUR PERRY

Relative labeling of peripheral blood cells by $^{51}$-chromium was determined by means of a sucrose gradient density separation technic and confirmed by radioautography. Large lymphocytes and monocytes were found to be more heavily labeled than small lymphocytes and granulocytes. Erythrocytes and platelets were relatively lightly labeled. These differences in labeling are important in evaluating data obtained when $^{51}$-chromium is utilized as a cell label for the study of leukocyte kinetics.

Radioactive chromium ($^{51}$Cr) has been widely used as a label of erythrocytes and platelets for the determination of intravascular survival and organ sequestration. $^{51}$Cr was first utilized as a leukocyte label in 1955 in leukemic patients, but it was not used again for this purpose until very recently. These studies have been concerned largely with the determination of leukocyte kinetics and organ distribution and very little information has been reported on the quantitative distribution of the label among the various cell types found in normal blood. This information is important for valid interpretation of data obtained when this label is employed to study leukocyte kinetics.

In this investigation, the relative labeling of normal human leukocytes, erythrocytes, and platelets by $^{51}$Cr is determined using a sucrose density gradient separation technic. The data obtained were confirmed by radioautography, a potentially useful technic with $^{51}$Cr recently described by Ronai.

MATERIALS AND METHODS

Fifty ml. of normal human whole blood were collected in a vial containing 10 cc. ACD-A. The leukocyte-rich fraction was obtained by sedimentation at 1 g. for 15–30 minutes with an equal amount of a solution containing three per cent dextran, 2.5 per cent dextrose and 0.45 per cent sodium chloride. The supernatant containing leukocytes and platelets was removed and centrifuged at 2000 RPM for five minutes, and the contaminating red blood cells were subjected to hypotonic lysis. The final leukocyte-platelet concentrate was then incubated with 200 pCi. of Na$_2^{51}$CrO$_4$ for 45 minutes at room temperature.

In five experiments a known aliquot of red blood cells was added prior to incubation.

From the Human Tumor Cell Biology Branch, National Cancer Institute, National Institutes of Health, Bethesda, Md.

Submitted October 27, 1969; for publication February 2, 1970.

Supported by NIH.

HARMON J. EYRE, M.D.: Clinical Associate, Human Tumor Cell Biology Branch, National Cancer Institute, NIH, Bethesda, Md.; PETER J. ROSEn, M.D.: Clinical Associate, Human Tumor Cell Biology Branch, National Cancer Institute, NIH, Bethesda, Md.; SEYMOUR PERRY, M.D.: Associate Scientific Director for Clinical Trials, Chemotherapy, National Cancer Institute, NIH, Bethesda, Md.
LABELING OF LEUKOYTES, ERYTHROCYTES AND PLATELETS

Table 1.—Relative Labeling of Leukocytes, Erythrocytes and Platelets by $^{51}$Chromium

<table>
<thead>
<tr>
<th>Cell Type</th>
<th>Range of Per Cent $^{*}$ Relative Labeling based on Lymphocyte 100 Per Cent</th>
</tr>
</thead>
<tbody>
<tr>
<td>Granulocyte</td>
<td>29–56 (9)</td>
</tr>
<tr>
<td>Monocyte</td>
<td>212–346 (4)</td>
</tr>
<tr>
<td>Erythrocyte</td>
<td>2.9–6.0 (4)</td>
</tr>
<tr>
<td>Platelet</td>
<td>1.2–1.7 (5)</td>
</tr>
</tbody>
</table>

$^{*}$ Number of determinations in parentheses.

Table 2.—Leukocyte Mean Grain Count Distribution

<table>
<thead>
<tr>
<th>Cell Type</th>
<th>Mean Grain Count $^{*}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Granulocyte</td>
<td>5.0</td>
</tr>
<tr>
<td>Small lymphocyte</td>
<td>11.1</td>
</tr>
<tr>
<td>Large lymphocyte</td>
<td>21.8</td>
</tr>
<tr>
<td>Monocyte</td>
<td>28.8</td>
</tr>
</tbody>
</table>

$^{*}$ Based on 200 cells of each type.

with $^{51}$Cr to determine relative erythrocyte labeling. The final suspension thus obtained contained approximately equal numbers of leukocytes, red cells and platelets.

At the conclusion of incubation the cells were washed twice with saline and suspended in 25 ml of Hanks Basic balanced salt solution containing 0.05 per cent sucrose and then filtered. After a small aliquot was removed for cell and differential counts, the suspension was allowed to flow rapidly into the bottom of the settling chamber devised by Peterson and Evans. A continuous gradient containing 0.3–1.75 per cent sucrose in Hanks solution was then generated in the chamber and the cells allowed to sediment for two hours. The details of this procedure are reported elsewhere.

Elution of $^{51}$Cr during the sedimentation procedure was not significant as determined by measurement of specific radioactivities of the various cellular elements before and after separation. At the conclusion of the sedimentation, cell fractions of 50 ml. were collected in 0.2 ml of 10 per cent bovine albumin in 0.9 per cent NaCl. Ten ml of each fraction were used for cell counts. Slides were made by means of a cytocentrifuge and stained with Wright's stain. In four experiments, radioautographs were prepared using Kodak AR 10 stripping film in the standard fashion. The remaining cells were concentrated and dissolved in 1 ml of NCS reagent. Liquifluor-toluene was then added and the EC decay of chromium was counted in a liquid scintillation spectrometer. Quenching was monitored by an external standard and was not significant. Platelet counts were done by phase microscopy. Red and white blood cell counts were determined in an electronic cell counter; red blood cells were also counted by a direct method.

RESULTS

At the conclusion of sedimentation in the settling chamber, three or more discrete bands were visible consisting of platelets, erythrocytes and leukocytes. (The system's capability in separating the cellular elements of the blood is described in detail elsewhere.) It is consistently possible to obtain fractions containing 95 per cent lymphocytes or granulocytes (including eosinophils and basophils), respectively. The monocyte fraction is less pure, but in several experiments aliquots containing 60–80 per cent monocytes were obtained.

The results of relative labeling with $^{51}$Cr as determined by liquid scintillation counting are shown in Table 1. The most variation was observed in the labeling of erythrocytes. There were several reasons for this including the basic inaccuracy of red blood cell counting at high dilutions, and a small but...
variable overlap of lymphocytes in the erythrocyte fraction. Nevertheless, it was established with confidence that the erythrocyte labeled on the order of 3–6 per cent compared to the lymphocyte. In contradistinction to the data of Dresch and Najean, McMillan and Scott, who reported much higher labeling of granulocytes than lymphocytes, in the present study it was found that lymphocytes consistently labeled more heavily than granulocytes. In these previous studies relative granulocyte labeling was estimated following the separation of lymphocytes by nylon fiber or glass wool column technics. These methods would lead to an overestimate of granulocyte labeling since it is known that the monocyte and large lymphocyte are retained along with the granulocyte in the column.

Because the present data conflicted with the results of previous investigators, radioautographs of the separated cells were made in order to validate the data from liquid scintillation counting. Mean grain counts for the various classes of leukocytes are listed in Table 2 and a representative radioautograph illustrating the heavier labeling of the large lymphocyte and monocyte is shown in Fig. 1. The radioautographs revealed that the large lymphocytes and the monocytes were the most-heavily labeled cell types and had similar mean grain counts. The small lymphocyte, while more-heavily labeled than the granulocyte, was much-less-heavily labeled than the large lymphocyte.

**DISCUSSION**

The significantly higher labeling of lymphocytes and monocytes compared to granulocytes with $^{51}$Cr is of importance in the interpretation of leukocyte kinetic data, especially when the differential count reveals appreciable numbers of these cells as, for example, in the neutropenic patient. The high labeling of lymphocytes with $^{51}$Cr combined with improved methods of cell separation, such as the one outlined here, should make reliable intravascular lymphocyte
LABELING OF LEUKOCYTES, ERYTHROCYTES AND PLATELETS

kinetic studies possible in normal patients. Previously, this type of information could only be obtained in patients with chronic lymphocytic leukemia or in other clinical situations with high lymphocyte counts.

The finding of low, although significant, levels of labeling of erythrocytes and platelets confirms the necessity of obtaining relatively pure preparations of leukocytes for kinetic studies. From the data presented here, 10–20 erythrocytes would contribute the same radioactivity as one granulocyte.

The technic of \(^{51}\text{Cr}\) radioautography is not generally appreciated, but Ronai\(^7\) recently demonstrated the feasibility of this procedure. High-resolution radioautography is possible because 70 per cent of the disintegrations of \(^{51}\text{Cr}\) resulting from electron capture give rise to Auger electrons with an energy of 4.5 keV, which is similar to that of \(^{3}\text{H}\) and other soft beta emitters. The quality of the radioautographs processed in the usual manner with stripping films is excellent, and because of the short half-life of \(^{51}\text{Cr}\) developing time is relatively short. Analysis of the radioautographs confirmed the heavy labeling of lymphocytes and monocytes. It also served to distinguish the more-heavily labeled large lymphocyte from the relatively lightly labeled small lymphocyte.

While not as quantitative as liquid scintillation counting, radioautography may be of further value in elucidating the relative labeling of leukemic cells by \(^{51}\text{Cr}\).

ACKNOWLEDGMENT

We wish to acknowledge with appreciation the technical assistance of Miss Ada Brooks.

REFERENCES


Relative Labeling of Leukocytes, Erythrocytes and Platelets in Human Blood by $^{51}$Chromium

HARMON J. EYRE, PETER J. ROSEN and SEYMOUR PERRY