Eosinophil Kinetics in Two Patients with Eosinophilia

By J. C. Herion, R. M. Glasser, R. I. Walker and J. C. Palmer

Leukocyte kinetics were studied in two individuals with marked eosinophilia. Blood leukocytes, labeled in vitro with Na₂⁵¹CrO₄, disappeared from the circulation at different rates in the two subjects during the first eight hours after infusion. In one, label disappeared exponentially with a T/2 of 4.5–5.0 hours; in the other, the level of radioactivity remained constant. In both, however, a significant rise in radioactivity appeared during the second 24 hours, suggesting recirculation of the eosinophils. Blood leukocyte DNA-³²P labeling was similar in both subjects and closely resembled neutrophil DNA-³²P labeling observed in normal individuals. Hydrocortisone infusion produced an exponential decrease in circulating eosinophils in one subject and, for reasons unknown, a linear decrease in the other; the drug exhibited no effect on eosinophils in vitro.

Cell labeling with inorganic radiophosphorus, ³H-thymidine, DF²³²P, and sodium radiochromate has yielded much information about the kinetics of granulocytes. As a consequence of the usual distribution of these cells in blood, studies in humans have provided information chiefly about neutrophils. Studies of eosinophils have been done mostly in rodents using radioautography. In mice and rats given ³H-thymidine, eosinophil labeling index exceeds that of neutrophils. In mice, but not rats, eosinophils enter and exhibit maximum labeling in the blood about 24 hours earlier than neutrophils. Normally, eosinophils, like neutrophils, are thought to leave the blood in random manner and not recirculate.

A single study in the dog showed transfused Pelger-Huet eosinophils to disappear randomly from blood but more rapidly (T/2, 30 minutes) than neutrophils (T/2 4.8 hours). In humans, however, adrenal steroids produce an arithmetic decrease in the number of circulating eosinophils.

Two patients referred to the North Carolina Memorial Hospital because...
of marked eosinophilia provided an opportunity to study the kinetics of eosinophils in humans. This paper comprises results of these studies.

**Materials and Methods**

**Subjects Studied**

R. H. (NCMH No. 22-69-15), an asymptomatic 16-year-old Negro student had eosinophilia (W.B.C. 45,700/cu. mm. with 90 per cent eosinophils). Hematocrit and platelet count were normal. No specific cause for the eosinophilia was found, although transient hilar adenopathy and splenomegaly, plus a few cells seen in the anterior chamber of the eye suggested that he had sarcoidosis. After 10 months, he remains well (W.B.C. 7800/cu. mm.; 15 per cent eosinophils).

E. W. (NCMH No. 23-19-07), a 31-year-old Negro mechanic had resolving pneumonitis and a vasospastic disorder associated with ischemia of the fingertips. His hematocrit was 52 per cent, the W.B.C. 31,500/cu. mm. with 78 per cent eosinophils and the platelet count varied between 81,000/cu. mm. and 150,000/cu mm. He continues asymptomatic after one year, although the ischemic changes have disappeared on treatment with isoxsuprine hydrochloride. His W.B.C. remains 32,650/cu. mm. with 86 per cent eosinophils.

Marrow from each patient was normal except for increased eosinophils. Blood eosinophils from both patients were morphologically mature and normal both by light and electron microscopy.

**Blood Cell Counting**

Blood cells diluted in Isoton were counted in duplicate with a Model B Coulter Electronic Cell Counter. The Zaponin used to prepare samples for W.B.C. counting did not selectively destroy eosinophils. Differential counts were performed on Wright's stained cover slip smears.

**Isolation of W.B.C.**

Leukocytes were isolated from 20 ml. of blood by dextran sedimentation and hypotonic lysis of residual red cells.14 Lymphocytes, free from granulocytes and monocytes, were eluted with saline from blood incubated for 30 minutes at 37°C on a sterile column packed with cotton.15

**DNA Labeling and Counting**

Orthoradiophosphorus (³²P) was injected intravenously (2.5 μCi/Kg.) to effect DNA labeling. At appropriate intervals afterward, heparinized blood samples were drawn from the opposite arm.

Blood leukocyte DNA was extracted as previously described16 and hydrolyzed in 0.5 NHClO₄ in a boiling water bath for at least three hours or until the volume was reduced to 0.5 ml. The volume was adjusted to 1.2 ml. with phosphate-free water and duplicate 0.1 ml. samples were taken to measure phosphorus content.16 Then 0.5 ml. portions were added to 10 ml. of scintillation mixture (83 per cent toluene and 17 per cent Triton-X100 containing 0.5 per cent 2,5-diphenyloxazole and 0.01 per cent 1,4-bis-2-(4-methyl-5-phenyloxazoly) benzene dimethyl) per liter. Additional Triton-X100 was sometimes required to produce a clear emulsion. The specific activity was expressed as counts per minute per μgm. of DNA-phosphorus (DNA-P).

**⁵¹Cr-labeling Studies**

Blood leukocytes were labeled in vitro with sodium radiochromate (⁵¹Cr) by methods previously described.17-19 A portion of the labeled cells was removed to measure total and differential cell counts and leukocyte specific activity and the remainder infused intravenously within 1-2 minutes. At intervals afterward, venous blood samples were ob-
Table 1.—The Specific Activity of Blood Leukocytes Labeled In Vitro with Na$_{31}$CrO$_4$

<table>
<thead>
<tr>
<th>Sample</th>
<th>Milliliters</th>
<th>Eosinophils</th>
<th>Polymorphonuclear Leukocytes</th>
<th>Lymphocytes</th>
<th>Monoocytes</th>
<th>$\mu$Ci Added $\mu$Ci/ml.</th>
<th>cpm/10$^8$ Cells</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>30</td>
<td>81</td>
<td>5.5</td>
<td>11.5</td>
<td>2</td>
<td>1.26</td>
<td>6116 7477 0.82</td>
</tr>
<tr>
<td>B</td>
<td>75</td>
<td>20</td>
<td>4.5</td>
<td>75.5</td>
<td>0</td>
<td>1.70</td>
<td>6815 9453 0.72</td>
</tr>
</tbody>
</table>

W.B.C. sedimented in dextran, collected and resuspended in homologous plasma before adding label.

Whole blood (400 ml) centrifuged at 50 $\times$ g. for 30 minutes; upper layer of plasma, rich in lymphocytes, collected and label added.

*Lymphocytes were isolated by incubating a portion of each sample on cotton for 30 minutes at 37$^\circ$ C. and eluting with saline.*

Results

Recovery of Eosinophils During Isolation of Blood Leukocytes

The differential leukocyte count on each of 10 venous blood samples was compared with the differential count of the leukocytes isolated from it. Eosinophils constituted 83.8 per cent (SD ± 1.8% of the W.B.C. in R. H.'s blood and 85.4 per cent (SD ± 1.5%) of the W.B.C. isolated from it. For E. W., the respective percentages were 81.1 ± 2.5 and 89 ± 4.2. Thus eosinophils were not selectively lost during the isolation procedure.

The effect of altering the composition of the leukocyte sample on labeling with sodium chromate was studied in vitro using R. H.'s blood. These results are shown in Table 1. Lymphocytes labeled slightly less than the other cells, irrespective of their concentration in the labeling medium.

Figure 1 depicts the blood survival in vivo of the $^{51}$Cr of eosinophils labeled in vitro. These studies in the two subjects show obvious qualitative differences. Quantitative differences between R. H. and E. W. may, in part, reflect differences in degree of cell labeling and number of labeled cells infused. The disappearance of label during the first eight hours is quite different in the two subjects (Fig. 1A). In R. H., this curve is exponential with a T/2 of about 4.5-5.0 hours; in E. W., it is flat. The shape of the two curves otherwise, is somewhat similar (Fig. 1B). Both show a net loss of labeled eosinophils during the first 24 hours and both also show a definite rise in leukocyte radioactivity during the second 24 hours. The validity of the observed increases in W.B.C. radioactivity at 24 hours depends upon the reliability of the technique used to determine white cell radioactivity. To insure accuracy, all samples were counted on two different scalers and the radioactivity related both to...
Fig. 1.—In vivo survival of W.B.C. labeled in vitro with $^{51}$Cr. Blood W.B.C. specific activity, determined at times indicated following infusion of labeled cells into R. H. and E. W. (A) Log plot. (B) Arithmetic plot.

Table 2.—Specific Activity of Blood Lymphocytes vs. Mixed Blood Leukocytes Following Transfusion of $^{51}$Cr Labeled Cells

<table>
<thead>
<tr>
<th>Time After Transfusing $^{51}$Cr-Leukocytes</th>
<th>Eluate Cells from Cotton (100% Lymphocytes)</th>
<th>Mixed Leukocytes (82–87% Eosinophils)</th>
</tr>
</thead>
<tbody>
<tr>
<td>15 min</td>
<td>116</td>
<td>119</td>
</tr>
<tr>
<td>2 hours</td>
<td>36</td>
<td>111</td>
</tr>
<tr>
<td>1 day</td>
<td>61</td>
<td>103</td>
</tr>
<tr>
<td>2 days</td>
<td>37</td>
<td>76</td>
</tr>
<tr>
<td>3 &quot;</td>
<td>21</td>
<td>45</td>
</tr>
</tbody>
</table>

* Labeled cells infused comprised 89 per cent eosinophils, 9.5 per cent polymorphonuclear leukocytes, and 1.5 per cent lymphocytes.

cell number and to cell nitrogen in the samples. Also note that the eight-hour point in R. H. falls precisely on the exponential regression line and that the 24-hour point is greater by a factor of two, a difference far greater than the error inherent in this method. In addition, this rise in radioactivity is not ascribable to label in lymphocytes. Each of the blood samples drawn from R. H. at 15 minutes, two hours, and at one, two, and three days was divided; pure lymphocytes were obtained from one portion, mixed leukocytes from the other. The lymphocytes in all samples had considerably less radioactivity than the corresponding mixed leukocytes (Table 2) and in each sample they constituted less than 10 per cent of the total leukocytes. Therefore, they contributed little to the total leukocyte radioactivity in R. H. Lympho-
cytes isolated from E. W.'s blood two hours and 24 hours after the infusion of labeled cells contained no detectable radioactivity. Such an increase in blood leukocyte radioactivity during the second day has not been observed following infusion of $^{51}$Cr-labeled neutrophils into normal subjects.$^{18,19}$

$^{32}$P-labeling Study

Plasma $^{32}$P-clearance in these subjects was similar to that observed in normal individuals and in patients with various hematological disorders.$^{14}$ In both subjects inorganic $^{32}$P disappeared rapidly from plasma during the first two hours and gradually achieved a slowly changing low concentration after four days as it equilibrated with the body's pool of inorganic phosphate.$^{15}$

Figure 2 shows the changes in specific activity of blood leukocyte DNA-P following injection of $^{32}$P. In both subjects little activity appeared for three days. DNA-$^{32}$P specific activity began to rise between days 3 and 4 and reached a maximum at about day 9; thereafter, it declined gradually. In subject E. W. on day 29, the specific activity of the leukocyte DNA-P was equivalent to that of the plasma inorganic phosphate. The curves representing blood leukocyte DNA-P specific activity are not smooth. Between days 5 and 10 they show peaks and troughs that are defined only by the frequent samples; this would not have been evident with only single daily determinations. During this study, until hydrocortisone was given, the concentration of circulating leukocytes and eosinophils was remarkably constant.
Fig. 3.—Changes in concentration of blood eosinophils/ cu. mm. during eight hours following administration of hydrocortisone. (A) Log plot. (B) Arithmetic plot.

On day 11 for R. H. and day 10 for E. W., after obtaining the blood samples, 200 mg. of hydrocortisone was injected intravenously. In both subjects, this caused a marked fall in the concentration of circulating leukocytes as a consequence of a decrease in the eosinophils; the proportion of blood neutrophils increased. But despite this alteration in the composition of blood leukocytes in the posthydrocortisone samples, there was no significant change in the leukocyte DNA-32P specific activity in either subject. Since lymphocyte DNA labeling contributes little to the shape of the blood leukocyte DNA labeling curves, these results suggest that both neutrophils and eosinophils were labeled to about the same degree.

Hydrocortisone Infusion Study

Figure 3 depicts the effects of infusing hydrocortisone on the level of circulating eosinophils. In R. H., regardless of the rate of hydrocortisone infusion there is a similar decrease in the concentration of circulating eosinophils for seven–eight hours. Following rapid injection, the concentration of
eosinophils reached a nadir at about seven hours and increased to preinfusion levels within two–three days. But on another occasion, when the same dose of the drug was infused slowly over a period of three hours, the concentration of eosinophils remained low for a longer time; return to preinfusion levels was not complete for several weeks. The significance of this observation is not clear. The curves describing the fall in concentration of circulating eosinophils after hydrocortisone injection appear to be different in the two subjects. In R. H. it best describes an arithmetic fall (Fig. 3B), but in E. W. it appears to be exponential (Fig. 3A). In both subjects, however, the nadir occurs at about the same time.

In vitro studies suggest that hydrocortisone alters the concentration of blood eosinophils other than by direct lysis. In heparinized blood obtained from both subjects just after hydrocortisone injection and kept at 37°C, the concentration of eosinophils did not fall during the subsequent eight hours as it did in vivo. In addition, hydrocortisone added to heparinized blood in vitro (both 5 μg./ml. and 50 μg./ml.) and kept at 37°C for 21 hours, failed to alter the concentration of eosinophils from that of control blood without hydrocortisone.

**DISCUSSION**

Information about eosinophils has been derived mostly from studies in either rodents or horses. Since there are so few eosinophils in normal blood, the kinetics of the production and movement of these cells in humans has not been studied extensively. Thus, additional information about human eosinophil kinetics is not apt to accumulate rapidly unless it is obtained from patients with disorders involving eosinophilia. Neither of our two subjects with eosinophilia has developed the clinical picture of granulocytic leukemia. One (R. H.) remains well and now has a normal leukocyte count with only 15 per cent eosinophils. Transient hilar adenopathy in the absence of other demonstrable causes, suggests sarcoidosis as the cause of his eosinophilia; a similar case has been described by Miale. The other (E. W.) continues to manifest Raynaud’s phenomenon and perhaps has vasculitis. Similar cases, primarily with cardiac manifestations and massive eosinophilia, have recently been reported by Roberts et al.

The arithmetic fall in the concentration of blood eosinophils in patients given hydrocortisone is thought to result from a shut down of eosinophil input from marrow, coupled with age dependent exit from blood. Thymidine labeling studies support the thesis that cortisone produces a reversible sequestration of eosinophils in some unknown compartment and may delay their entry from marrow into blood. Results of our studies, however, do not confirm that intravenous cortisone consistently produces an arithmetic decrease in the concentration of circulating eosinophils. The one study in E. W. shows clearly an exponential decrease, while two studies in R. H. show what probably are arithmetic decreases. The explanation for these differences is not readily apparent. Neither do these data indicate whether eosinophils that leave the blood in response to cortisone return or are destroyed. If cortisone merely interrupts the flow of eosinophils from marrow
to blood, the curves would depict their manner of departure. The in vitro studies, confirming results of others\textsuperscript{22,26}, clearly indicate that hydrocortisone in the doses used has no direct lytic effect on eosinophils. A possible explanation for the exponential decrease seen in E. W. is the presence of active disease that might affect cell utilization.

The DNA labeling curves from both subjects are thought to reflect the characteristics of eosinophil labeling and the movement of these cells into and out of blood. In support of this are the following observations: (1) Lymphocytes label poorly with DNA labels and lymphocyte labeling contributes little to the general shape of the total blood leukocyte DNA labeling curves.\textsuperscript{21} (2) The level of DNA labeling achieved in these studies is consonant with that obtained in other studies in which the predominant blood leukocyte was the neutrophil and, in view of the very high eosinophil concentration (85–90\%), it is not likely that the few neutrophils in the samples labeled high enough to produce this much radioactivity. (3) Altering the percentages of eosinophils and neutrophils in the samples by giving the patients hydrocortisone did not alter the DNA specific activity, suggesting that the DNA of eosinophils and neutrophils was about equally labeled.

These curves closely resemble those reflecting neutrophil kinetics in normal individuals and suggest that the eosinophils in these two subjects, like neutrophils in normal man, undergo mitosis and maturation in the marrow and enter the blood mostly in an orderly manner. The initial low plateau of blood W.B.C. DNA\textsuperscript{32P} activity for three days, the transit time of cells through the marrow postmitotic pool, is a little shorter than that observed for neutrophils in normal man. A shorter eosinophil marrow transit was also reported in another human subject studied with tritiated thymidine.\textsuperscript{27} Thus the postmitotic pool of eosinophils in these patients was sufficient to supply mature cells to the blood for three or four days. The remainder of the curves is also similar to the curves of neutrophils labeling, suggesting that other parameters of eosinophil kinetics resemble the corresponding ones for the neutrophils. The apparent cyclic undulations of DNA labeling of these cells resembles those clearly shown for neutrophils\textsuperscript{58} but their precise meaning is not entirely clear. A possible explanation for them, along with mathematical analysis of similar data for neutrophils, is in press.\textsuperscript{29} The generation of cyclic fluctuations in DNA label content is determined by label availability and the essentially asynchronous mitotic process in marrow. In an asynchronously replicating population of cells, given a high concentration of label in the precursor pool for an interval short in relation to the cell’s generative cycle, a cyclic undulation of DNA label will occur. The period of the undulations is determined by the length of the generative cycle. The preservation of these undulations so that they are visible when the cells in the blood are examined depends on an orderly transit of these cells through the postmitotic storage pool in marrow. The amplitude is determined not only by the characteristics of cell labeling, but also by the cells’ intravascular sojourn time and the manner in which they depart from the blood.

The pattern of blood leukocyte DNA labeling in these patients clearly differs from that obtained in patients with chronic granulocytic leukemia.\textsuperscript{14}
Thus this kind of study may help differentiate "benign" eosinophilia from "eosinophilic" leukemia.

The second rise in $^{51}$Cr activity at about 24 hours after infusing eosinophils labeled in vitro suggests that these cells recirculate, since reutilization of label is unlikely. It is impossible to judge from these data whether these cells merely marginate along vessel walls or actually leave the vessels and reenter. No secondary rise in $^{32}$P radioactivity has been reported in studies of neutrophils, but computer analysis of $^{32}$P labeling studies indicate that, within the constraints of the experimental data, substantial neutrophil recirculation could occur.

REFERENCES


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J. C. HERION, R. M. GLASSER, R. I. WALKER and J. C. PALMER