The Intravascular Life Span of Transfused Leukocytes Tagged with Atabrine

By Laurens P. White, M.D.

The life span of the leukocyte has been repeatedly studied, and the range of reported values is wide. Dissimilar technics and the different species used have probably accounted for part of these discrepancies. The shortest life span, 23 minutes for neutrophils and 170 minutes for lymphocytes, was reported by Van Dyke and Huff using a technic of irradiating parabiotic rats. Values of approximately 10 to 12 hours were reported by several groups using methods of thoracic duct lymph collection in cats. Lawrence et al. reported a life span of 16 hours for leukocytes in cats rendered agranulocytic by infection with the virus of feline agranulocytosis and subjected to cross transfusion with normal cats. Recently, studies in man using P uptake in DNA have reported leukocyte survival values of 13 days in seven normal patients and 30 days in patients with chronic lymphocytic leukemia.

The intravascular phase of the life span of leukocytes has lent itself to scrutiny, if not elucidation, through studies on the survival in the circulating blood of transfused leukocytes. Minot and Isaacs, in 1925, studied a patient with lymphosarcoma, who received 450 cc. of blood from a patient who had chronic lymphocytic leukemia with a count of 80,000/cu. mm. In the recipient the lymphocytes trebled in absolute number at the completion of the transfusion, and returned to pretransfusion levels in thirty minutes.

Twenty-five years later, Lanman, Bierman, and Byron reported data on seven transfusions of blood containing large numbers of leukocytes. They observed only small increase in arterial leukocyte number during the transfusion with return to control levels thereafter and concluded that the pulmonary circulation was the major site of disappearance of the transfused cells. In 1951, Bierman, Byron, and Kelly, extending their previous investigations, found that prolonged transfusions of blood from leukemic donors into patients with various types of leukemia resulted in a significant elevation in the recipients' leukocyte count, demonstrating a difference in response between nonleukemic patients and patients with leukemia.

The limitations of this method were immediately apparent in that the only method of identifying transfused cells was by their morphology. It was not possible to differentiate donor cells from recipient cells of the same type. Thus, tracing of individual cells was impossible. Moreover, studies with blood containing low numbers of leukocytes could not be carried out.

To circumvent this difficulty, Weisberger et al. labelled rabbit leukocytes with P and transfused these tagged cells into rabbits. They found most of the
radioactivity in the lungs thirty minutes after the transfusion; this would lend support to Bierman's conclusion that the lung is involved initially as the site of disappearance of transfused leukocytes. The inability to distinguish between individual transfused and recipient cells using isotopic labelling methods limited the interpretation of this valuable data.

Seyderhelm and Oesterreich,11 in 1927, conducted a series of experiments in which they stained leukocytes with a vital dye, Congo red. They then injected these leukocytes into rabbits and dogs. In animals killed immediately, the stained (transfused) cells were found in the lungs, suggesting the role of the lungs in the disappearance of transfused leukocytes. Further, in animals killed three to four hours after transfusion, the stained cells were found in the liver, where many were disintegrating. Both of these findings were remarkably similar to those of Weisberger et al. However, Congo red was found to be markedly toxic to leukocytes in the amounts used, and the authors concluded that the transfused cells had not been viable, which vitiated some of the validity of their data.

The in vivo staining of leukocytes with fluorescent compounds provided a technic that could be useful in the solution of this problem.

In 1929 Ellinger and Hirt12 described the staining of nuclei of various cells with acriflavine. Twenty-one years later DeBruyn and Farr13,14 extended this work, using a wide variety of compounds related to acridine, specifically certain diaminoacridines. Farr,14 utilizing this method of intravital staining, followed the course of tagged lymphocytes after injection into rabbits.

Quinacline (atabrine) is such a fluorescent acridine compound. After accurate methods for determining the level of atabrine in biologic fluids were developed in 1943,15,16 a high percentage of the whole blood atabrine was found to be in the leukocyte component.17,18 It was shown that atabrine reacts strongly to form a complex with deoxyribose nucleic acid. At high concentration of atabrine the complex precipitated, while at lower concentrations there was apparently no change in the physical state of the complex.19,20 DeBruyn reported that acriflavine and several other acridine derivatives had no effect on the motility or macrophage formation of leukocytes in tissue culture and pointed out that at therapeutic levels there could be no significant tissue toxicity of these materials. Beck21 and Haas22 have noted inhibition of purified flavoprotein enzymes with small amounts of atabrine. Wright and Sabine23 demonstrated some inhibition of respiration of liver slices in the presence of atabrine. Although the question has not been settled, one could assume from the evidence that, at lower concentrations, atabrine had no effect on the viability of leukocytes.

METHODS

A Spencer phase microscope with a turret condenser was used for the determination of the presence of fluorescence in leukocytes. The light source was a GE AH 4 mercury vapor lamp. A front surfaced mirror reflected the light beam through an Abbe condenser.* A drop of mineral oil was placed between the condenser and slide.24

A Corning 5840 UV transmitting filter, with maximum (55 per cent) transmission at 365 m, was placed in the incident beam, which was also passed through a chamber containing 7.5 per cent Cu SO₄, to remove the small quantity of infra red light passed by the 5840 filter.

* Although atabrine has a maximum E of 2700A,14 fluorescence is readily excited by the longer wave length UV light, and therefore quartz optics are not necessary.
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A disc of Eastman A2 gelatin was fixed into the eye piece to suppress any UV light which passed to that level.

Preliminary investigations were performed to determine the amount of atabrine needed to produce visible fluorescence of leukocytes. It was found in four patients that more than 200 mg. given intravenously was the minimum dose required to produce such tagging of leukocytes. Patients given 100 and 200 mg. showed no visible fluorescence of leukocytes under the microscope. With doses of 300 mg. of atabrine, fluorescence of all the leukocytes was observed in smears of arterial blood. This fluorescence persisted for twenty-four to forty-eight hours after the administration of atabrine.

In vitro, 40 to 60 mg. of atabrine, added to 450 to 600 cc. of blood, produced bright fluorescence in all the leukocytes. This fluorescence persisted for four days when blood was incubated at 37 C. The addition of this amount of atabrine to the blood caused no alteration in the microscopic appearance of leukocytes, and no change in their number.

Since bright fluorescence of leukocytes could be obtained in vitro with amounts of atabrine insufficient to produce tagging in vivo, it was concluded that the technique could be used to differentiate between donor and recipient cells. All tagged cells could be assumed to be from the donor since the possibility of transfer of visible amounts of atabrine had been excluded. Further, the disappearance of tagged cells from the blood could not represent simple loss of tag, since the tag had been shown to persist for twenty-four to forty-eight hours.

On the day of the transfusion, blood from the two patients involved was carefully cross matched. Atabrine dihydrochloride* (10 mg./cc. in sterile distilled water) was introduced into a Baxter vacuum bottle containing 125 cc. of ACD solution. Amounts of atabrine used ranged from 90 mg. in the initial study to 50 mg. after the latter was found to be the optimum quantity; 500 to 650 cc. of blood was then withdrawn from the donor and at the same time a control leukocyte count taken. The blood was immediately transfused into the recipient using large-bore needles so that the duration of the infusion approximated ten minutes. The procedure in the auto transfusions was identical, except that the patient's own blood was removed, tagged in the bottle, and then reintroduced.

All blood specimens from the recipients were drawn through a Cournand needle placed in a femoral artery. Control specimens were taken in each case. Specimens thereafter were taken at timed intervals from the start of the transfusion, every five minutes for the first half hour and every ten minutes for the next half hour. In several studies, counts were continued for periods up to seven and one-half hours.

The blood was collected in clean glass tubes with heparin added as the anticoagulant. Heparin as supplied commercially is slightly fluorescent but does not act as a fluorochrome, i.e. does not induce fluorescence in otherwise untreated leukocytes. Smears were made as soon as possible after the specimen was drawn, and a leukocyte count performed using NBS certified Trerser pipets and hemocytometers.

The cover slip smears were then mounted on glass slides in mineral oil, and examined microscopically.

With the use of phase optics, the leukocytes in each field were located and counted. The phase diaphragm was then spun out, the filter inserted, and the fluorescent cells in the same field counted. Atabrine tagged cells show a characteristic bright yellow-green fluorescence against the dim blue black background of plasma and blackness of erythrocytes (figs. 1 and 2). Untagged leukocytes are invisible in UV light. Using this method of “differential” counting, a minimum of one hundred leukocytes were examined. In patients with elevated counts (above 10,000) two hundred cells were inspected.

* The atabrine used in these studies was generously supplied by Winthrop-Stearns through the kind offices of the late Mr. Robert Haight.
Fig. 1.—Low power photos showing the same field under UV illumination (left) and phase illumination (right). Details of the cells are not clear in either.

Fig. 2.—High power magnification of the same cells, again showing the fluorescence of tagged leukocytes under UV light on the left, and the same cells in phase lighting on the right.

When no fluorescent cells were found in the first one hundred examined, the entire slide was scanned under UV light for fluorescent cells. Counts reported as less than 1 per cent represent cells found in scanning, though not in the first one hundred to two hundred cells encountered.

Wright's stain inhibited the fluorescence of atabrine and could not be used.
Because of this difficulty, neutrophils and mononuclear cells were not separated, and the data throughout refer only to the total leukocyte numbers.

Subjects

Ten patients, all with far-advanced neoplastic diseases, received transfusions of atabrine tagged blood. Auto transfusions were performed in five of these cases (two with carcinomatosis, three with leukemia) and simple heterotransfusions in the other five. Of this latter group, two patients with nonleukemic malignancies received leukemic blood, two others with carcinomatosis received blood from other patients with carcinomatosis, and one patient with leukemia received blood from a patient with carcinoma, who had a granulocytic leukocytosis secondary to infection.

Results

Tagged cells were found in arterial blood of all patients during and immediately after the intravenous transfusion of blood to which atabrine had been added. In no case was there a significant rise in leukocyte number following the transfusion (table 1).

In other respects the results fell into two classes. The first, consisting of the nonleukemic recipients, showed a relatively low number of fluorescent (tagged) cells even at the peak of their appearance, and these cells disappeared rapidly from the circulating blood, often within 30 minutes (table 1, fig. 3). Case 5, in whom a slightly higher peak of tagged cells was found, received more leukocytes by transfusion than the other patients of the nonleukemic group; in this patient the tagged cells disappeared very rapidly (fig. 3).

The leukemic recipients, on the other hand, all demonstrated a rise in tagged cells to relatively high levels, and the decrease in these cells occurred slowly, so that, with the exception of case 9, 2 to 16 per cent tagged cells persisted at 60 minutes (table 1 and fig. 4). Even more striking is the comparison of the persistence of tagged cells at 30 minutes between leukemic and nonleukemic patients (table 1). This difference in peak number of tagged cells and rate of their disappearance was a true difference and not dependent on the number of cells given, since this factor was similar in the two groups.

The exception to these findings was case 6, a patient with multiple myeloma, who showed a response similar to that of leukemic patients (fig. 5). This woman had been studied at the time of cardiac catheterization, with samples presumably from the pulmonary artery. However, blood oxygen determinations revealed oxygen saturation in specimens from the venous catheter equal to or greater than that of femoral artery blood. More critical study of spot films of the heart, taken during catheterization, led to a strong suspicion that the catheter tip had been in a pulmonary vein, presupposing the presence of an interauricular septal defect. Such a defect was found three months later at autopsy.

There was no apparent difference in the results between heterotransfusion and autotransfusion. The number of cases was too small to detect any difference in response of leukemic patients with various types of leukemia. There did not seem to be any difference in results between high and low count leukemias, although again, the number of cases was too small for any conclusions.

It can be seen from table 1 that half of the patients showed a peak of tagged cells greater than that which could be accounted for by dilution of the transfused
TABLE 1.—Summary of Data on Ten Transfusions of Tagged Leukocytes

<table>
<thead>
<tr>
<th>CASE NO., SEX, AGE, DIAGNOSIS</th>
<th>DONOR DIAGNOSIS</th>
<th>BLOOD GIVEN</th>
<th>LEUKOCYTES GIVEN</th>
<th>ATABRINE MG</th>
<th>CONTROL</th>
<th>MAXIMUM % TAGGED CELLS</th>
<th>MAXIMUM NO. TAGGED CELLS</th>
<th>% TAGGED AT 30 MIN</th>
<th>% TAGGED AT 1 HR</th>
<th>MAXIMUM NO. TAGGED CELLS</th>
<th>THEORETICAL MAXIMUM NO. TAGGED</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 Male, 63, Ca. stomach</td>
<td>Pt.</td>
<td>555 cc.</td>
<td>7.7 x 10^9</td>
<td>75</td>
<td>6,300</td>
<td>6,800</td>
<td>7</td>
<td>0</td>
<td>0</td>
<td>450</td>
<td>0</td>
</tr>
<tr>
<td>2 Male, 51, Ca. nasopharynx</td>
<td>Pt.</td>
<td>500 cc.</td>
<td>7.2 x 10^9</td>
<td>50</td>
<td>7,900</td>
<td>2,300</td>
<td>11</td>
<td>3</td>
<td>1</td>
<td>800</td>
<td>50</td>
</tr>
<tr>
<td>3 Male, 54, Ca. prostate</td>
<td>Ca. colon</td>
<td>650 cc.</td>
<td>9.2 x 10^9</td>
<td>40</td>
<td>8,000</td>
<td>4,000</td>
<td>11</td>
<td>3</td>
<td>1</td>
<td>800</td>
<td>50</td>
</tr>
<tr>
<td>4 Female, 42, Ca. rectum</td>
<td>Fibrosarcoma</td>
<td>487 cc.</td>
<td>1.3 x 10^9</td>
<td>50</td>
<td>3,500</td>
<td>3,600</td>
<td>18</td>
<td>3</td>
<td>1</td>
<td>500</td>
<td>40</td>
</tr>
<tr>
<td>5 Male, 30, Hepatoma</td>
<td>Myelogenous leukemia</td>
<td>523 cc.</td>
<td>1.2 x 10^9</td>
<td>50</td>
<td>4,100</td>
<td>4,100</td>
<td>28</td>
<td>3</td>
<td>1</td>
<td>1,000</td>
<td>0</td>
</tr>
<tr>
<td>6 Female, 48, Multiple myeloma</td>
<td>Myelogenous leukemia</td>
<td>475 cc.</td>
<td>1.8 x 10^9</td>
<td>150</td>
<td>6,600</td>
<td>6,600</td>
<td>12</td>
<td>3</td>
<td>1</td>
<td>2,000</td>
<td>0</td>
</tr>
<tr>
<td>7 Female, 51, Stem cell leukemia</td>
<td>Ca. maxillary sinus</td>
<td>505 cc.</td>
<td>7.3 x 10^9</td>
<td>60</td>
<td>1,000</td>
<td>3,600</td>
<td>63</td>
<td>23</td>
<td>16</td>
<td>1,920</td>
<td>430</td>
</tr>
<tr>
<td>8 Female, 66, Lymphoecytic leukemia</td>
<td>Pt.</td>
<td>800 cc.</td>
<td>8.3 x 10^9</td>
<td>60</td>
<td>2,100</td>
<td>1,500</td>
<td>53</td>
<td>13</td>
<td>2</td>
<td>3600</td>
<td>300</td>
</tr>
<tr>
<td>9 Male, 44, Myelogenous leukemia</td>
<td>Pt.</td>
<td>600 cc.</td>
<td>1.2 x 10^9</td>
<td>60</td>
<td>2,100</td>
<td>2,000</td>
<td>32</td>
<td>7</td>
<td>0</td>
<td>650</td>
<td>0</td>
</tr>
<tr>
<td>10 Male, 69, Lymphoecytic leukemia</td>
<td>Pt.</td>
<td>620 cc.</td>
<td>7.5 x 10^9</td>
<td>60</td>
<td>11,500</td>
<td>13,500</td>
<td>10</td>
<td>5</td>
<td>5</td>
<td>4500</td>
<td>670</td>
</tr>
</tbody>
</table>

* This represents the per cent of one hundred to two hundred leukocytes which showed fluorescence, and usually occurred just at the end of the transfusion.

Fig. 3.—See legend, opposite page.

Fig. 4.—See legend, opposite page.
cells in the total (estimated) blood volume. This occurred only during the transfusion; following its completion the number of tagged cells found was, in every case, less than the theoretic maximum number of these cells.

It is known from dye dilution studies of blood volume that a period of 15 to 20 minutes is required for thorough mixing of injected particulate matter, and specimens taken before this time result in falsely low estimates of blood volume. Since these high peaks of fluorescent cells appeared only during the first 5 to 10 minutes of the study, it seemed justified to assume that the transfused leukocytes were being diluted in less than the total blood volume at that time, full mixing occurring thereafter. This would be a reasonable explanation for the transient rise of tagged cells to numbers apparently greater than the total given.

**DISCUSSION**

The data indicate that transfused atabrine tagged leukocytes do not remain in the peripheral blood for more than one to two hours and do not produce a change in the leukocyte count of the recipient. These findings are in accord with those of Minot and Isaacs and of Lanman et al.

It is also apparent that there is a difference in the intravascular persistence of tagged cells between leukemic and nonleukemic recipients. In the latter the tagged cells appear in relatively low numbers and quickly disappear. On the other hand, in leukemic recipients, tagged cells rise to a considerably greater percentage and disappear more slowly. These findings are independent of the original count in the recipient or the number of cells transfused.

**FIG. 5.**—Case 6, patient with myeloma and interauricular septal defect, who showed a high peak of tagged cells and slow disappearance thereof.

**FIG. 3.**—Case 5, nonleukemic, showing sharp peak of tagged cells at end of transfusion (10 min.) and rapid decrease to none at 40 minutes.

**FIG. 4.**—Case 7, leukemic, showing peaks of 63 per cent tagged cells at 10 min., slow decrease to 16 per cent at 60 minutes. This patient received approximately three-fifths as many leukocytes as case 5 (fig. 3) but these cells appeared in greater numbers for a longer time than did such tagged cells in case 5.
The single exception to these differences was a patient with multiple myeloma and interauricular septal defect. It is generally held that the direction of flow of blood through an unguarded septal defect is from left to right. This results in right heart hypertrophy and a great increase in the amount of blood traversing the pulmonary circulation. Late in the disease, occasional right to left flow of blood may occur, as may paradoxical embolization. If there was right to left shunt of blood in this patient, and oxygen determinations suggest this possibility, a considerable amount of blood may have by-passed what Lamman et al. consider to be the "normal" pulmonary leukocyte barrier. Alternatively, the marked alteration of normal pulmonary blood flow might alter the normal mechanism of leukocyte retention in the lung. It is of primary interest that the single patient who demonstrated a leukemic response, also had a marked abnormality in circulation.

Several explanations for a short intravascular existence of transfused leukocytes seem possible. It must be re-emphasized that only the intravascular phase of the white cell was under scrutiny in these studies, and no information or conclusions as to the total life span could be obtained.

An allergic reaction to foreign cells as an explanation can be excluded by the similarity in results between auto- and heterotransfusion.

Were these tagged cells nonviable, it would be expected that they would quickly disappear in a linear fashion, with no variation between leukemic and nonleukemic patients; this was not observed. All of the evidence that atabrine is an enzyme inhibitor has been obtained using highly purified systems. Although Wright and Sabine demonstrated some inhibition of respiration in tissue slices by atabrine, marked or complete inhibition was not shown. Coggeshall and Craigie reported that atabrine did not affect oxygen utilization in isolated brain or testis. This would indicate that in vivo the enzymatic inhibitory effects of atabrine are minimal or absent. In the present investigation no obvious change in the appearance or number of leukocytes was noted after the addition of atabrine. Although cases of agranulocytosis have been reported following long continued administration of the drug, these were considered due to idiosyncrasy rather than general toxicity. ACD solution, while ultimately toxic to leukocytes, has not been considered immediately destructive. Scudder et al. have demonstrated that leukocytes incubated in ACD maintain motility and apparent viability for eight to eighteen hours.

Despite the lack of definite proof of this point, one could assume that the leukocytes given were not damaged appreciably, and therefore nonviability of the cells was not responsible for their short survival.

It is apparent that during the period of transfusion and shortly thereafter there is a change in the circulating leukocyte group. To explain the appearance of tagged cells during the transfusion when the total leukocyte number remains constant, one must assume that an equal number of untagged cells has disappeared from the blood. This would suggest that, at least for the period of the transfusion, there is a rapid emigration of untagged (i.e. recipient) cells from the circulating blood, followed by reappearance of the same or other untagged cells as the transfused cells disappear.

One may then visualize a state of rapid change in the leukocytes of the peripheral blood. As new cells enter the blood, some of those present leave. The trans-
fused cells, entering the intravascular pool of leukocytes, would then be subject to the same migrations as other cells, and disappear rapidly from the circulating blood. The present data, subject to such an interpretation, would suggest some difference in capacity and/or function of this system of leukocyte migration between nonleukemic and leukemic patients.

It would seem quite likely that such an ebb and flow of leukocytes exists at all times, capable of greater adaptation to increased stimuli, but not resulting suddenly from the abnormal stimulus of transfusion. On this basis, one may further theorize that leukocytes may have many intravascular life-spans which may be only a fraction of the total life span of the cell.

Conclusions

1. A method of tagging leukocytes in vitro using a fluorescent compound, atabrine, has been expanded to studies in man, in which ten patients received transfusions of blood containing leukocytes tagged with atabrine.
2. Following such transfusions, tagged cells were found in the peripheral blood of the recipient for periods ranging from 30 to 90 minutes, but not thereafter.
3. Greater numbers of the tagged cells appeared and persisted for longer periods in recipients who had leukemia, than in those who were not leukemic.

Summario in Interlingua

1. Le technica de etiquettar leucocytas in vitro per medio del composito fluorescente atabrina ha esite applicate a un studio del duration de vita intravasaular de leucocytas transfundite in humanos. Dece patietstes recipeva transfusiones de sanguine que contineva leucocytas etiquettate con atabrina.
2. Post tal transfusiones, cellulas etiquettate esseva trovate in le sanguine peripheric del recipiente durante periodos variante inter 30 e 90 minutas. Nulle cellulas etiquettate esseva trovate plus tarde.
3. In recipientes qui habeva leucemia, plus grande numeros de cellulas etiquettate appareva e persisteva durante periodos plus longe que in recipientes qui non eseva leucemic.

References

LIFE SPAN OF TRANSFUSED LEUKOCYTES

21 Beck, W. S.: Personal communication.
The Intravascular Life Span of Transfused Leukocytes Tagged with Atabrine

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