The Role of the Lungs in the Removal of Transfused Lymphocytes*

By AUSTIN S. WEISBERGER, M.D., RICHARD A. GUYTON, M.D., ROBERT W. HEINLE, M.D. and JOHN P. STORAASLI, M.D.

MANY CONDITIONS are characterized by severe leukopenia or granulocytopenia in which an increase in the number of circulating leukocytes would be desirable. No effective means of achieving this desired effect has been found, however. The failure of whole blood transfusions to raise the leukocyte count in a manner analogous to raising the erythrocyte cell count in patients with anemia is a common clinical observation. It has been suggested that this is the result of either the short life span of leukocytes, their rapid disappearance from stored blood, or to possible incompatibilities in blood groups. Much time and effort is being expended in searching for better methods to collect and preserve leukocytes in the hope of obtaining sufficient numbers of viable cells for successful transfusion.

Previous studies in this laboratory showed that transfusion of concentrated suspensions of granulocytes did not increase the leukocyte count of recipient animals but was followed, within a matter of minutes, by leukopenia. This was shown to be due to a selective action of the lungs in removing transfused cells following intravenous or intra-arterial administration. The granulocytes used in these experiments were obtained from the peritoneal cavity of rabbits by distending it with normal saline. Inasmuch as granulocytes obtained from the peritoneal cavity may not be identical with cells present in the circulating blood, it was desirable to repeat these experiments with blood cells obtained in a more physiologic manner, as well as with cells of a different type. There has also been considerable speculation concerning the ultimate fate of lymphocytes normally present in the circulation, as well as those introduced by transfusion. The following experiments were undertaken, therefore, to study the fate of transfused lymphocytes obtained from the lymphatic system.

METHODS

Lymphocytes were obtained by cannulation of the intestinal lymphatics of rats by a modification of the method of Bollman et al. Rats weighing between 200 and 300 Gm. were anesthetized with ether, then were given 50 mg. of sodium pentobarbital per kg. hypodermically. A transverse upper abdominal incision extending from the right upper quadrant to the left flank beneath the costal margin was made, and the viscera reflected to the right. A relatively large lymphatic duct could then be exposed beneath the abdominal aorta just below the diaphragm. Visualization of the duct was facilitated by administration of a fat...
meal of linseed oil prior to the anesthesia. The aorta was retracted with a ligature and the lymphatic duct isolated by careful blunt dissection.

A small opening was made in the duct with a 27 gauge needle and a specially made glass cannula of appropriate bore inserted. The use of dissecting glasses proved to be helpful in this part of the procedure. The glass cannula was filled with heparin before insertion and care was exercised in tying and fixing it in place because any slight angulation interfered with the flow of lymph. The intestines were then distended with 5 to 10 ml. of normal saline to facilitate lymph flow, the viscera replaced and covered with moist cotton. During the period of collection, supplementary ether anesthesia was given as necessary.

Lymph was collected from 5 or 6 rats simultaneously over a period of approximately 4 hours, which provided the total of 12 to 15 ml. necessary for a single satisfactory transfusion experiment. The lymphocytes were separated by centrifugation at 1,000 r.p.m. for 5 minutes and washed twice with 10.0 ml of 0.85 per cent saline, then resuspended in 1.0 ml of 0.85 per cent saline. The final cell count of this suspension usually ranged from 30,000 to 65,000 cells per cu. mm. This cell suspension was then used for transfusion into either the tail vein, inferior vena cava, or carotid artery of another rat. Recipient animals weighed 150 to 200 Gm. To test the stability of the cell suspensions, several samples were stored at 4C. for 24 hours without any appreciable decrease in count. The cells retained normal staining characteristics.

Radioactive lymphocytes for tracer experiments were prepared by injecting the donor rats with 0.2 mc. of P32 intravenously or intraperitoneally 24 hours prior to cannulation. These cells were washed 3 times with saline before injection into a recipient rat, to remove any freely dialyzable P32. The effect of repeated washing on the cell count and radioactivity of the cell suspension in one experiment is shown in table 1. Negligible amounts of radioactivity were found in the wash water after the first wash.

The recipient animals were sacrificed 10 to 15 minutes following the injection of suspensions of radioactive lymphocytes. The lungs, liver, spleen and kidneys were removed and the radioactivity in these tissues determined by methods described previously.2 The distribution of radioactivity following intravenous and intra-arterial injection of radioactive lymphocytes was compared with the distribution of radioactivity in control animals which had received comparable amounts of intravenously administered radioactivity in the form of inorganic phosphate ion (table 2).

**RESULTS**

1. The Effect of Transfused Lymphocytes on the Leukocyte Count

The injection of 1.0 ml of a concentrated suspension of lymphocytes containing from 30,000 to 65,000 cells per cu. mm. into the tail vein of a rat resulted in a drop in the total leukocyte count in 4 of 5 rats within 5 minutes. The average control leukocyte count was 19,700 per cu. mm. (fig. 1). In spite of the injection of large numbers of intact, evenly dispersed lymphocytes, the total leukocyte count averaged 10,900 cells per cu. mm. 5 minutes after injection. There was no
increase in the number of circulating lymphocytes and the fall in the total leukocyte count was paralleled by a proportionate drop in the absolute numbers of both lymphocytes and neutrophils. There was no subsequent rise in the number of lymphocytes, but the neutrophils returned to, or slightly above, pretransfusion

Table 2.—Distribution of Intravenously Injected $^{32}$P in the Form of Inorganic Phosphate Ion

<table>
<thead>
<tr>
<th>Rat</th>
<th>Lung</th>
<th>Liver</th>
<th>Spleen</th>
<th>Kidney</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>% per Gm.</td>
<td>Total %</td>
<td>% per Gm.</td>
<td>Total %</td>
</tr>
<tr>
<td>C 1</td>
<td>1.14</td>
<td>1.12</td>
<td>1.89</td>
<td>15.7</td>
</tr>
<tr>
<td>C 2</td>
<td>1.26</td>
<td>1.68</td>
<td>1.82</td>
<td>11.9</td>
</tr>
<tr>
<td>C 3</td>
<td>1.80</td>
<td>1.91</td>
<td>5.03</td>
<td>27.8</td>
</tr>
<tr>
<td>C 4</td>
<td>2.74</td>
<td>3.20</td>
<td>3.49</td>
<td>23.0</td>
</tr>
<tr>
<td>C 5</td>
<td>1.72</td>
<td>1.81</td>
<td>2.95</td>
<td>22.9</td>
</tr>
<tr>
<td>Average</td>
<td>1.73</td>
<td>1.94</td>
<td>3.04</td>
<td>20.2</td>
</tr>
</tbody>
</table>

Animals received 20,000 counts per minute of inorganic $^{32}$P and were sacrificed after 10 minutes by injecting air.

Fig. 1.—Leukocyte counts after administering a concentrated suspension of lymphocytes.

values at the end of 2 hours and remained at this level for the remaining 3 hours of observation. There was a further decrease in the total number of circulating lymphocytes after the first hour which was inversely proportional to the increase in neutrophils.
2. Distribution of Radioactivity Following Intravenous Injection of Lymphocytes Labeled with P\textsuperscript{32}

Rats, sacrificed 10 to 15 minutes after the injection of lymphocytes labeled with radioactive phosphorus, had the greatest percentage of radioactivity in the lungs. In 12 rats, an average of 19.6 per cent of the total amount of radioactivity injected (as radioactive lymphocytes) was recovered per Gm. of lung tissue. In comparison, the lungs of control animals, receiving comparable amounts of radioactivity as inorganic P\textsuperscript{32}, contained only 1.73 per cent per Gm. (tables 2 and 3, fig. 2). The spleen contained an average of 0.36 per cent of the radioactivity per Gm., the liver 2.65 per cent per Gm. and the kidneys 2.39 per cent per Gm. In control animals, given inorganic P\textsuperscript{32}, the spleen contained an average of 2.01 per cent of the radioactivity per Gm., the liver 3.04 per cent per Gm. and the kidneys 2.80 per cent per Gm.

When the total amount of radioactivity recovered per organ is calculated, (table 3, fig. 3), the greatest percentage of radioactivity was again in the lungs. The lungs contained slightly more radioactivity than the liver, even though the liver weighs more than 5 times as much as the lungs.

In 12 rats, the average amount of radioactivity recovered in the lungs was 28.3 per cent of the amount injected compared to 1.94 per cent in controls. An average of 6.36 per cent was found in the spleen compared to 1.16 per cent in the controls. The liver contained an average of 23.0 per cent compared to 20.2 per cent in the controls, and the kidneys contained 5.23 per cent compared to 5.52 per cent in the controls.

Because of the reported lymphopenia produced by the administration of ACTH and cortisone, the effect of these substances on transfused lymphocytes was
studied. Four rats, weighing approximately 200 Gm., were injected intramuscularly with 10 mg. of cortisone (Cortone acetate, Merck) 16 hours preceding transfusion and 20 mg. 4 hours preceding transfusion. Two rats were given 10 mg. each of adrenocorticotrophic hormone (Wilson) 4 hours before transfusion. The amounts of radioactivity recovered in the lungs, liver, spleen and kidneys of these animals did not differ significantly from that recovered in rats not prepared with these hormones (table 3).

Comparison of the distribution of radioactivity in rats transfused with intact radioactive lymphocytes with that in 4 rats who were given comparable injections of radioactive lymphocytes disintegrated by supersonic vibration, revealed marked differences (table 5, figs. 2 and 3). In the latter instance no selective uptake of radioactivity occurred in the lungs and spleen, and the distribution of radioactivity was comparable to that which occurred after administration of inorganic phosphate ion.

3. Distribution of Radioactivity Following Intra-Arterial Injection of Radioactive Lymphocytes

To determine whether the concentration of radioactivity in the lungs following intravenous injection of radioactive lymphocytes represented capillary sequestration by the first large capillary bed encountered, the effect of intra-arterial injec-
tion was investigated. Suspensions of radioactive lymphocytes were injected into carotid arteries of 5 recipient rats and flushed into the arterial system with 0.2 to 0.3 ml. of saline. The greatest part of the radioactivity was again recovered in the lungs on a per cent per Gm. basis. The spleen likewise contained increased

The affinity of the lungs for the radioactive lymphocytes following either intravenous or intra-arterial administration is again strikingly demonstrated when compared to the distribution of radioactivity in the controls.

Table 4.—Distribution of Radioactivity Following the Injection of Radioactive Lymphocytes into the Carotid Artery

<table>
<thead>
<tr>
<th>Rat</th>
<th>Lung</th>
<th>Liver</th>
<th>Spleen</th>
<th>Kidney</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>% per Gm.</td>
<td>Total %</td>
<td>% per Gm.</td>
<td>Total %</td>
</tr>
<tr>
<td>1</td>
<td>25.2</td>
<td>29.8</td>
<td>3.72</td>
<td>40.5</td>
</tr>
<tr>
<td>2</td>
<td>7.4</td>
<td>12.7</td>
<td>2.20</td>
<td>23.3</td>
</tr>
<tr>
<td>3</td>
<td>13.2</td>
<td>22.4</td>
<td>1.85</td>
<td>15.1</td>
</tr>
<tr>
<td>4</td>
<td>11.3</td>
<td>19.25</td>
<td>2.87</td>
<td>29.4</td>
</tr>
<tr>
<td>5</td>
<td>15.2</td>
<td>25.9</td>
<td>5.85</td>
<td>24.8</td>
</tr>
<tr>
<td>Average</td>
<td>14.4</td>
<td>22.01</td>
<td>3.30</td>
<td>26.6</td>
</tr>
</tbody>
</table>

The amount of radioactivity as compared with controls which had received inorganic P³². The amount of radioactivity recovered from the liver and kidneys did not differ significantly from the controls (tables 4 and 5, figs. 2 and 3). The lungs contained an average of 14.4 per cent per Gm. of the total amount of radio-
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activity injected, the spleen 7.90 per cent per Gm., the liver 3.30 per cent per Gm. and the kidneys 1.6 per cent per Gm.

On a total organ basis, the lungs contained an average of 22.01 per cent of the total radioactivity injected, the spleen 4.14 per cent, the liver 26.6 per cent and the kidneys 4.4 per cent. These values are comparable to those obtained with intravenous administration with the exception that, following intra-arterial injection, the liver contained slightly more radioactivity than the lungs.

<table>
<thead>
<tr>
<th></th>
<th>Lung</th>
<th>Liver</th>
<th>Spleen</th>
<th>Kidney</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>%/Gm.</td>
<td>Gm.</td>
<td>%/Gm.</td>
<td>Gm.</td>
</tr>
<tr>
<td>Control (Inorganic P32)</td>
<td>1.73</td>
<td>1.94</td>
<td>3.04</td>
<td>20.2</td>
</tr>
<tr>
<td>I.V. Injection of Radioactive Lymphocytes</td>
<td>19.6</td>
<td>28.3</td>
<td>2.65</td>
<td>23.0</td>
</tr>
<tr>
<td>Carotid Injection of Radioactive Lymphocytes</td>
<td>14.4</td>
<td>22.01</td>
<td>3.30</td>
<td>26.6</td>
</tr>
<tr>
<td>I.V. Injection of Radioactive Lymphocytes Disintegrated by Supersonic Vibration</td>
<td>1.45</td>
<td>1.9</td>
<td>2.4</td>
<td>19.1</td>
</tr>
</tbody>
</table>

DISCUSSION

The injection of large numbers of lymphocytes obtained from the lymphatic system failed to increase the number of circulating lymphocytes even temporarily. Following the injection of radioactive lymphocytes either intravenously or intra-arterially in 17 animals, the majority of the radioactivity was recovered from the tissues of the lungs, liver, spleen and kidneys. Of these organs, the lungs and spleen contained amounts of radioactivity far in excess of the amounts found in animals which received comparable intravenous doses of inorganic P32.

It may be concluded, therefore, that the lungs and spleen were the organs most active in removing transfused lymphocytes from the peripheral circulation. The amount of radioactivity in the liver and kidneys was essentially the same whether the animals received intact radioactive lymphocytes, disintegrated radioactive lymphocytes or inorganic P32. This suggests that the radioactivity of these organs resulting after administration of intact radioactive cells may be due to the accumulation of P32 from cells destroyed in other parts of the body. The possibility has not been eliminated, however, that the liver and kidney, like the lung and spleen, also contribute to the sequestration of intact cells, although their activity in this process is much less than that of the lung and spleen when compared on a per Gm. basis.

Although considerable amounts of radioactivity are recovered from the spleen on a per cent per Gm. basis, it is probable that, because of its small size, the spleen does not contribute significantly in removing lymphocytes from the circulation under normal circumstances. The importance of the lungs assumes even greater significance when it is considered that they contain more radio-
activity than the liver even though their weight is less than one-fifth that of the liver. On a per cent per Gm. basis the activity of the lungs in removing radioactive lymphocytes far exceeds that of any other organ (fig. 2). The removal of transfused lymphocytes by these organs appears to be specific. Nonspecific capillary sequestration is a minimal factor since the distribution of radioactivity after intra-arterial injection was similar to that resulting from intravenous injection.

The difference in the distribution of radioactivity after the injection of disintegrated cells indicates that the majority of the injected cells were removed from the circulation in an intact state. This was corroborated by the fact that the alveolar septa, seen on histologic section, were engorged with lymphocytes.

These experiments confirm the importance of the lung barrier in the transfusion of leukocytes. Transfused lymphocytes, like granulocytes, fail to appear in the peripheral circulation. In both instances the lungs appear to be the organ chiefly involved in removing the transfused cells. This suggests the possibility that the lungs may act as a homeostatic organ in maintaining the leukocyte count at normal levels.

Little is known of the factors which control the number and types of circulating leukocytes. Bunting and Hustin 4 showed that the number of circulating lymphocytes remains constant even though more lymphocytes entered the circulation in 24 hours via the thoracic duct, than could be accounted for by those present in the blood at any one time. These authors suggested that the number of lymphocytes was kept constant by mucosal excretion into the lumen of the stomach and intestines.

Erf, 5 however, showed that large numbers of viable lymphocytes administered intravenously rapidly disappeared from the circulation of rabbits in which the stomach and intestines had been removed. This occurred both after transfusion of the recipient's own cells as well as cells from other donors, and it was suggested that the mechanism of removal was lysis of the injected cells. The demonstration of a lung barrier mechanism now explains the phenomena observed by these investigators.

Minot and Isaacs 6 and more recently Lanman et al. 7 have shown also, that transfused leukemic cells rapidly disappear from the circulation. Again, it is probable that the lung barrier is responsible for the removal of these cells from the peripheral circulation. It is possible that specific infectious diseases as well as hematopoietic disorders may alter the lung-barrier mechanism with resultant leukopenia or leukocytosis. Cross transfusion experiments indicate that x-irradiation may also affect this mechanism. Thus, when animals made leukopenic by irradiation are cross transfused with normal animals, some rise in the leukocyte count occurs in the leukopenic animals. 8 However, this is accompanied by a marked leukopenia in the normal (donor) animals at the same time.

Inability to raise the white blood count by repeated transfusions, even in severely leukopenic conditions, is a common clinical observation. It appears unlikely that attempts to overcome this difficulty by better preservation of leukocytes or by the use of freshly drawn blood will be successful. Furthermore, experiments attempting to determine the life span of leukocytes based on survival time in transfused animals may give erroneous results. It is possible
that the disappearance of cells in survival experiments may be mediated by a
different mechanism than the rapid removal of concentrated cell suspensions
by the lung. It seems reasonable, however, that when less concentrated cell
suspensions are administered, as in cross transfusion experiments, less stimula-
tion of the lung barrier mechanism would result and the rate of removal of the
cells would be slower than when more concentrated cell suspensions are used,
as in the present experiment.

The failure of ACTH and cortisone to affect the distribution of radioactivity
when radioactive lymphocytes were transfused indicates that, under the con-
ditions of these experiments, there was no immediate lysis or increased seque-
stration of the injected cells. The production of lymphopenia reported with
ACTH and cortisone either requires that the lymphocytes be exposed for a
longer time interval than that employed in these experiments or is mediated
by a different mechanism than increased destruction of circulating cells.

It is recognized, as noted in previous work, that once a leukocyte has been
removed from its natural environment it can no longer be considered “normal,”
however careful the manipulation may be. The lymphocytes used in this ex-
periment retained normal staining characteristics and did not disappear from
suspensions after storage for several hours. Granulocytes obtained from the
peritoneal cavity of rabbits in previous experiments and manipulated in a
similar manner were actively phagocytic for bacteria and retained normal
characteristics when studied with a supra-vital (James green and neutral red)
stain. In view of these observations plus the fact that the cells were administered
within a short time after their removal from the donor animals, the authors
believe that the cells were at least as viable, or more viable, than cells adminis-
tered in the usual indirect blood transfusion, or after storage for several days
in a blood bank. It is probable that the removal of these cells from the circulation
is representative of the fate of leukocytes administered in the usual clinical
transfusion and it is suggested, in addition, that removal by the lungs may be a
normal physiologic mechanism for controlling the numbers of circulating leuko-
cytes.

**Summary and Conclusions**

In rats, the intravascular injection of large numbers of lymphocytes obtained
from the lymphatic system fails to increase the number of circulating lympho-
cytes. This is the result, chiefly, of a specific selective action of the lungs in
removing the cells, after either intravenous or intra-arterial injection. The
spleen, and perhaps the liver and kidneys also play a part in removing the
transfused lymphocytes, but are not as active as the lungs in this respect. These
results are similar to those obtained with the transfusion of granulocytes ob-
tained from the peritoneal cavity of rabbits and raise the possibility that the
lungs may act as a homeostatic organ in controlling the level of the white blood
count. Under the conditions of this experiment, relatively large doses of ACTH
and cortisone had no significant immediate effect on transfused lymphocytes.

These results indicate that inadequate preservation of leukocytes in stored
blood does not account for their failure to appear in the circulation after trans-
fusion, and that better preservation will not, in all probability, alter the situation.
REFERENCES

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