Role of the Lungs in Regulation of the White Blood Cell Level

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It is a common clinical observation that in agranulocytosis and related conditions the white blood cell level cannot be increased—or only slightly and fleetingly—by transfusion of large volumes of normal blood (1-3). Disappearance of infused white blood cells in the recipient was observed during exsanguination-transfusions in humans (4), in leukopenic cats (5) and eviscerated rabbits (6). Leucemic white blood cells rapidly disappeared after being infused into non-leukemic individuals or patients with a different type of leukemia (7-9). In cardiac catheterization studies in humans Bierman and associates (9, 10) found that most infused leucocytes disappeared in the pulmonary circulation. These same authors (10, 11) reported that the white cell filtering mechanism may be impaired in leukemic patients. Transfused stained (12-14) or P32-labeled (15-18) white blood cells were found to be rapidly deposited (in order of decreasing quantities) in the lungs, liver and spleen. In contrast to these findings Farr (19) reported that the bone marrow and lymphoid tissue were the main sites of removal of intravenously injected lymphocytes labeled with a fluorescent stain. Weidman and Bucher (20) and Braunusteiner, Giebisch, Kolder and Werner (21) perfused the lungs of rabbits and guinea pigs through the pulmonary artery with homologous and heterologous blood after washing out the residual blood with Ringer's solution. They found a decreased white cell count after a single passage through the lungs. Weisberger, Guyton, Heinle and Storaasli (16) discussed the possibility that the mechanism which removes white cells from concentrated cell suspensions may not be identical with that operating in more dilute suspensions. Moreover, data obtained with altered white blood cells or lungs may not be directly applicable to the problem of physiological white cell removal.

The experiments to be reported here have been undertaken to study the white blood cell filtering mechanism of the lungs and to investigate the possible physiological role of this mechanism in maintaining normal white blood cell level in the blood. These studies were divided into the following categories: a) Starling heart-lung preparations b) cross-circulation experiments c) long range and d) short range cardiac catheterization experiments e) single blood sample studies.

MATERIALS AND METHODS

Mongrel dogs of either sex weighing between 10 and 40 kg were used. The animals were observed for 2-8 days and in most cases routine hematological examination was performed before being used. A total of 130 dogs have been used in the experiments reported here. Intravenous pentobarbital anesthesia was applied; an initial dose of 25 mg/kg was supplemented with further doses if necessary.

Pulmonary and cardiac catheters were introduced according to the method of Stroud, Stetson and Rahn (22) under fluoroscopic guidance. Polyethylene catheters with mercury tips, prepared as described by Stroud et al. (22) were used in the right heart and pulmonary artery; Cournand cardiac catheters No. 7F to 10F were used in the left heart and pulmonary vein. Before use, the catheters were calibrated for internal volume and filled with physiological saline containing 0.1 mg/ml heparin. In all experiments heparin sodium was used containing 100 IU/mg. Before the blood samples were obtained a volume of blood and heparin solution equal to three times the quantity corresponding to the internal volume of the catheter was removed and discarded. One to five milliliters blood was
Polyethylene tubings (Gr and GJ to a Y-shaped poly-
bottom of the reservoirs were connected by means of
conventional type stirrers or placing the entire apparatus
parts were silicone coated. The cannula leading from
water circulated from a 38°C constant temperature
bath. Magnetic stirrers gently moved the blood in the
during the time of flow several inspirations and expirations occurred. Bierman, Kelley, Cordes, Petrakis, Kass and Shpil (23) described that human white blood cell counts may be different in expiration and inspiration. In short term catheterization studies where frequent sampling was required, the catheters were not filled between sampling with heparin, but were allowed to drip slowly all the time.

Heart lung preparations were made according to the classical method of Starling (24). The perfusion apparatus—diagrammatically shown in figure 1—was designed to offer the least possible opportunity for ad-

of white blood cells. Cannulas were prepared from polyethylene tubing. Polyethylene tubing was used throughout the perfusion apparatus, except for the joints, where silicone-coated rubber pressure tubing was used. In the joints, the polyethylene or glass parts
were touching each other, except for the sections ap-
approximately 1 cm long immediately preceding the cannulas A1, A2; these served for obtaining blood samples by puncturing the rubber tubing. All glass
parts were silicone coated. The cannula leading from the right upper part of the apparatus-diagrammatically shown in figure 1—was inserted over the constant temperature bath in order to avoid excessive evaporation from the lungs. The preparation was kept warm with an infra-red lamp. The opened chest cavity was covered with a transparent plastic sheet to avoid drying.

One milligram of heparin/10 ml blood was used where heparinized blood was desired. Horse and cattle blood was obtained by puncturing the jugular vein of unanesthetized animals or by cutting the vein immediately after killing the animal. Dog blood was obtained from the femoral vein or artery of anesthetized dogs. Leucocyte-poor blood was prepared by removing the buffy coat after centrifugation, then separately centri-
being mixed with the infused blood. Through the
horizontal branch of the Y tube the blood flowed di-
rectly into one of the reservoir bottles (01 or 02) which
were about 40 cm above the level of the animal's heart. No special resistance was necessary in this system. In experiments where no anticoagulants were used, these
tubes contained a few grains of heparin powder. Samples
were then taken up with N.B.S. certified Sahli, white
tubes were used as described above. No anticoagulants were
administered.

Blood samples were obtained by simultaneously
counting the connecting rubber pieces above the
arterial and venous cannulas using 26-gauge needles
and 5-ml silicoled syringes. The blood was immediately
discharged into silicone-coated serological tubes. In experiments where no anticoagulants were used, these
tubes contained a few grains of heparin powder. Samples
were then taken up with N.B.S. certified Sahli, white
tubes were used as described above. No anticoagulants were
administered.

In the preliminary experiments, several types of resistances
were found to trap white cells. The reservoir bottles
were surrounded with jackets (E1 and E2) in which
water circulated from a 38°C constant temperature
bath. Magnetic stirrers gently moved the blood in the
reservoirs in order to prevent settling. This proved to be
superior and less injurious to white cells than conven-
tional type stirrers or placing the entire apparatus
on a vibrator platform. Glass tubes extending from the
bottom of the reservoirs were connected by means of
polyethylene tubings (G1 and G2) to a Y-shaped poly-
ethylene tube (H) which led into a venous cannula.
Two reservoirs were used side by side in order to be
able to switch the infused blood samples rapidly. The reservoir not in use could be excluded by a pinchcock placed on the rubber tubing of the corresponding branch of the Y tube. Circulating heparinized dog or horse blood through this apparatus over a period of two hours resulted in neither loss of white blood cells nor morphologically detectable damage.

Artificial respiration was maintained by means of
Harvard respiratory pumps. The air intake tube was
inserted over the constant temperature bath in order to
avoid excessive evaporation from the lungs. The prepara-
tion was kept warm with an infra-red lamp. The
opened chest cavity was covered with a transparent plastic sheet to avoid drying.

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bottom of the reservoirs were connected by means of
polyethylene tubings (G1 and G2) to a Y-shaped poly-
For leucocyte counts, one or two pipettes were filled from each blood sample. Each pipette was counted in each of eight squares of single or duplicate chambers. Depending on the number of pipettes to be counted and the type of diluent used, the shorter or longer of the above methods was employed. Conventional clinical methods were used to take red blood cell counts, hemoglobin (Sahli) and hematocrit (Van Allen) values.

The 95% confidence limits of the method used for counting white blood cells has been estimated on the basis of the formula of Berkson, Magath and Hurn (28). According to this:

\[ V_L = \sqrt{\frac{(100)^2}{n_b} + \frac{(4.6)^2}{n_c} + \frac{(4.7)^2}{n_p}} \]

Where: \( V_L \) = coefficient of variation; \( n_b \) = total number of cells counted; \( n_c \) = number of chambers and \( n_p \) = number of pipettes

The percentage error (\( V \)) obtained from the above may be converted to 95% confidence limits by multiplying \( V \) by student’s \( t \) value for \( P = 0.05 \), i.e. 1.96. The 95% confidence limits are shown in table I for leucocyte counts under several conditions. The total number of cells counted was never less than 400 cells, in most cases 1000 cells. Thus the 95% confidence limits varied between 11 and 16.3%.

**RESULTS**

**Fate of White Blood Cells in Starling Heart-Lung Preparations.** White blood cells from heparinized dog blood rapidly disappeared in the heart-lung preparation until a certain level was reached. This level was then maintained (in one experiment for 5 hours); introduction of new blood resulted in filtration to the same level. The level appears to be independent of the original white cell count of the blood; it was the same for the same heart-lung preparation whether the blood originated from a donor dog or from the dog from which the preparation was made (fig. 2). Similar results were obtained with defibrinated dog blood and with either heparinized or defibrinated horse blood (fig. 3). The white blood cells disappeared so rapidly from the perfusion fluid that it is difficult to judge whether heterologous leucocytes are removed faster than homologous ones. Certainly the level finally attained appears not to be different in either case. During the phase of rapid filtration a large venous-arterial difference was evidenced. This rapidly decreased until the level was reached. In some experiments, after obtaining the level, the preparation was perfused with fresh blood from another reservoir and then with the previously used sample of blood. As may be seen in figure 4, when blood was passed a second time through the preparation no appreciable filtration occurred. This suggests that it is not the introduction of a new blood sample, but the leucocyte count of the blood which is responsible for the filtration; if the count corresponds to the level maintained by the preparation, no further changes occur. Within the useful life of a heart-lung preparation it was impossible to exhaust the filtering ability of the lungs. Introduction of fresh blood every 10–30 minutes over a period of 3 hours resulted in unaltered filtration each time fresh blood was introduced. Figure 5 demonstrates that the fall of the total leucocyte count in the heart-lung preparation is mainly due to a fall of granulocytes, excepting eosinophiles. None of the experiments demonstrated a significant change in erythrocyte counts, hemoglobin concentration or hematocrit values. Blood flow (measured periodically by allowing the blood to flow into
a graduated cylinder instead of the reservoir) remained reasonably constant.

It appeared to be of interest to study whether or not this extensive filtration of leukocytes is an artifact brought about by injuring the white blood cells, thus rendering them more susceptible to removal. Weisberger, Heinle and Hannah (29) described an extract that may be obtained from disintegrated leukocytes which causes leukopenia in rabbits. Samples of pooled dog blood were divided into three fractions. No. 1 was kept in siliconed bottle at 37°C before being introduced into the preparation. No. 2 was vibrated on a vibrator platform for 5 hours at room temperature. No. 3 was centrifuged at 1900 rpm for 30 minutes, resuspended and kept at room temperature for 5½ hours before being introduced into the preparation. No essential difference was evidenced in the rate of filtration or the level obtained (fig. 6).

To investigate whether the filtration of leukocytes is connected with phagocytic activity of reticulo-endothelial elements, Thorotrast, a powerful inhibitor of the latter function (30, 31) was injected intravenously in a dose of 6 ml/kg to six dogs. The heart-lung preparation was made on two occasions 24 hours after the injection, on two occasions 6 hours, and on two occasions 12 hours afterwards. No evident differences were observed between the filtering ability of these and nontreated lungs.

By introducing leucocyte-poor blood into the preparation, a gradual release of white blood cells was evidenced (fig. 7). During the release, arterial counts were higher than the venous counts until the original level was

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4 We are greatly indebted to the Heyden Co., N. Y., for generously supplying Thorotrast.
reached. In contrast to the leucocyte filtering mechanism, the releasing activity could easily be exhausted by introducing several samples of new white cell-poor blood. Each time this was done a lower level was obtained (fig. 1D). In some release experiments, a few milliliters of heparinized chicken blood were introduced into the reservoir. The nucleated erythrocytes could be easily detected during qualitative counting. While leucocyte release was apparent, nucleated erythrocytes could also be seen despite several switches to new reservoir bloods. In two experiments P32-labeled red blood cells were introduced into the heart-lung preparation. Horse red cell suspensions were incubated with P32 at 37°C for 2 hours; the cells were subsequently washed three times with plasma and resuspended in the original plasma. By placing directional shields over the polyethylene tubing, radioactivity was recorded on an Esterline recorder connected to a rate meter. New leucocyte-poor horse blood was poured constantly into the reservoir, the arterial blood being discarded rather than recirculated. Twelve minutes elapsed before the initial radioactivity was removed. Release of white blood cells was evidenced for 10 minutes. Thus it appears
that the leucocyte releasing activity of the lungs as observed in heart-lung preparations is at least partly based on the mixing of the introduced white cell free blood with residual blood in the preparation.

Cross Circulation Experiments. Heart-lung preparations were supplied with blood originating from both femoral arteries or veins of donor dogs. The arterial blood from the preparations was reintroduced into the carotid artery of the donor dog. If the donor blood had a sufficiently high leucocyte count, filtration occurred as in the previous experiments (fig. 8). If the donor blood was close in leucocyte count to the level of the preparation, no filtration occurred (fig. 9). Since no anticoagulant was used in these experiments, it seems to be unlikely that filtration of leucocytes was caused by the presence of heparin in the preparations of the previous series. Moreover this type of experiment excluded possible injury to leucocytes due to handling, storing, etc.

Long range cardiac catheterization studies were performed on anesthetized dogs in an attempt to approach the question of the physiological significance of leucocyte removal by the lungs in animals with intact hematopoietic system. Unfortunately, the presence of catheters in the heart or pulmonary vessels for a longer time caused greater fluctuations in the white cell counts than usually encountered in dogs. For the most part, a decrease of leucocyte level occurred, this being due mainly to a decrease in granulocytes (fig. 2D). Often leucocytosis followed the leucopenic phase (figs. 10, 11). Initially, venous counts were somewhat higher than arterial ones, probably indicating physiological filtration by the lungs. During the development of leucopenia venous counts were mostly higher than arterial ones. During the development of leucocytosis this process was reversed. Nevertheless, in some instances it seemed to be clear that leucocytosis was mainly of extrapulmonary origin. No explanation can be offered at present for these phenomena. It made no difference whether leucopenia or leucocytosis was developing in vivo; heart-lung preparations from these dogs rapidly filtered leucocytes from their own or donor blood, until the level characteristic for the preparation was reached (fig. 11, fig. 3D).

Short Range Cardiac Catheterization Studies. Since the chronic presence of cardiac catheters in the cardio-pulmonary system appeared to induce changes in leucocyte levels, experiments were designed to obtain a larger number of counts within a maximum of 25 minutes. In order to avoid irritation to cardio-pulmonary structures as far as possible and to reduce the time required to complete the operation, a polyethylene catheter was placed into the right ventricle, while blood which passed through the lungs was obtained through a cannula in the carotid artery. Out of 15
dogs in which this operation has been successfully completed, data of 13 will be reported here, since in two, conditions were not strictly identical. The detailed data are deposited with the American Documentation Institute,8 a summary as well as statistical analysis is shown in Table 2. It appears from these data that the total leucocyte count is higher in blood entering the lungs than in blood leaving the lungs.

In only six dogs were there taken five or more differential counts. These data are summarized in Table 3. The difference in total leucocyte count (T) between the right auricle and carotid artery in these dogs is significant, but to a lesser extent than in the data presented before. This is probably due to the smaller number of dogs used. The difference in polymorphonuclear neutrophiles (P) appears to be significant although the significance does not quite reach the 5% level. In none of the other cell types were there observed significant differences.

Table 4 shows the analysis of covariance for the differences in (T) and (P). The significance of the reduction is measured as the F ratio of the mean square for reduction to the mean square for residuals. This ratio is highly significant, \( P < 0.001 \) indicating a strong correlation or dependence of (P) upon (T). Thus it appears that the magnitude of the difference in polymorphonuclear neutrophile count is directly proportional to the magnitude of the difference in total white blood cell count, when measured in the same dog.

### Single Simultaneous Blood Sample Studies

In order to further decrease the effect of the presence of cardiac catheters upon white blood cell levels, the following experiments were designed. In any experiment the maximal time from anesthetizing the animal to obtaining the last samples was not more than 20 minutes. The maximal time from the first incision or other manipulation to obtaining the sample was 4 minutes. In most dogs only one pair of data were obtained, in some a few pairs from different locations. In some instances samples

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8 See footnote 5.
were obtained by direct puncture rather than by catheterization. No anticoagulants were used. All samples where minute clots were found in the hemocytometer have been eliminated. Some of the dogs in this series were subsequently used in isolated organ studies.

The detailed data are deposited with the American Documentation Institute; a summary and statistical analysis is given in table 6. Values from the first pair of data obtained in the 13 dogs of the previous series are included in the last row (right auricle-carotid artery). When all samples obtained from these 13 dogs were taken in consideration, the differences were found to be significant, as discussed above. Yet, regarding the first samples only, the differences were not significant. Analysis of variance considering the magnitude of differences revealed that only the differences between the vena cava superior and aorta and between the right auricle and left auricle were significant. On the other hand according to the 'sign' test, which only considers the direction of the difference, significant differences were found between the right auricle and the aorta, no significant differences were found between the right auricle and carotid artery, while all other differences were inconclusive. If the values of all the samples obtained from locations leading blood into the lungs are pooled and compared with the pooled values of samples from locations receiving blood from the lungs, the differences are significant. This is true whether the first samples of the 13 dogs mentioned above (which are in themselves insignificant) are included or not. Thus it appears that if sufficient number of paired data are considered a significant difference is present in the leucocyte count of the blood entering the lungs and leaving the lungs.

**DISCUSSION**

Heart-lung preparations of dogs removed leucocytes from the blood circulating through them. This removal does not appear to depend primarily on the degree of injury to the white cells during preparation. Removal cannot be inhibited by Thorotrast, an agent which inhibits phagocytic activity of cells of the reticulo-endothelial system.

Anoxia is known to increase viscosity of blood and thus may promote the margination of leucocytes. All experiments in which partial anoxia could be suspected were eliminated.
TABLE 2. MEAN DIFF. IN TOTAL LEUCOCYTE COUNTS

Right Auricle Minus Carotid Artery

<table>
<thead>
<tr>
<th>Dog No.</th>
<th>No. of Diff.</th>
<th>Diff.*</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>10</td>
<td>+471</td>
</tr>
<tr>
<td>2</td>
<td>10</td>
<td>-460</td>
</tr>
<tr>
<td>3</td>
<td>10</td>
<td>+446</td>
</tr>
<tr>
<td>4</td>
<td>10</td>
<td>+1387</td>
</tr>
<tr>
<td>5</td>
<td>9</td>
<td>+1000</td>
</tr>
<tr>
<td>6</td>
<td>10</td>
<td>+1365</td>
</tr>
<tr>
<td>7</td>
<td>9</td>
<td>-217</td>
</tr>
<tr>
<td>8</td>
<td>10</td>
<td>+335</td>
</tr>
<tr>
<td>9</td>
<td>10</td>
<td>+538</td>
</tr>
<tr>
<td>10</td>
<td>10</td>
<td>+1390</td>
</tr>
<tr>
<td>11</td>
<td>9</td>
<td>+2497</td>
</tr>
<tr>
<td>12</td>
<td>10</td>
<td>+585</td>
</tr>
<tr>
<td>13</td>
<td>10</td>
<td>+730</td>
</tr>
</tbody>
</table>

Weighted mean diff. \( N = 127 \)

95% spread \(-2000\) to \(+3000\)

Actual spread \(-2300\) to \(+4400\)

95% confidence limits of mean diff. \(+334\) to \(+1280\)

Significance of mean diff. \( P < .01 \)

* + difference means: right auricular count higher than count in carotid artery; - difference means: right auricular count lower than count in carotid artery.

Copley (32) and Dameshek and Miller (33) demonstrated that heparin may cause platelet and leucocyte thrombi in the cheek pouch of hamsters. Essex and Grau (34) obtained similar results using the ear chamber technique. Fleck (35) observed leucocyte agglutination in vivo following heparin injections. Lutz, Fulton and Akers (36) found increased adherence of thrombocytes and leucocytes to the endothelium in hamsters after heparinization, \( S. aureus \) infection and in advanced neoplasia. Heparin was used in most of our experiments, but since similar results were obtained with defibrinated blood and in cross transfusion experiments where no anticoagulants were used, this appears not to be the major factor in the above phenomenon. There was no attempt to use sterile technique, however the relatively short periods of time involved suggests that infection did not play a major role.

Several authors demonstrated the presence of blood groups in dogs (37-40). Cross matching was done only in some of our experiments, always with negative results. Moreover since the isoagglutinin titers are usually insignificant, the agglutination of white blood cells is generally questionable (41) and since no exception has been seen to the filtering activity of the lungs, blood group differences do not seem likely to be responsible for the removal of white blood cells.

The maintenance of the leucocyte level of blood in heart-lung preparations goes about with small fluctuations of alternate release and filtration as evidenced by alternating arterio-venous and veno-arterial differences about the base line (fig. 4). This reminds one of homeostatic mechanisms of the body. Yet the role of experimental errors must also be considered. This alone does not seem to be responsible for the above findings because in serial observations in the phase of maintenance an increase of the venous count is usually accompanied by a decrease in arterial count and vice versa.

The release of white cells from the heart-lung preparation following the introduction of white-cell poor blood appears to be at least partly due to mixing with residual blood. It is possible that a balance exists between circulating and marginated leucocytes. If the former decreases, more cells are detached from the endothelium. The release phenomenon seems to be a small, easily exhaustable process, certainly not an important factor in the homeostatic mechanism of maintaining normal white blood cell levels. Filtration by the lungs, on the other hand, may play a role in the regulation of leucocyte levels. The fate of removed white blood cells is under investigation at the present time.

Cardiac and pulmonary catheterization experiments indicate that removal of leucocytes, particularly polymorphonuclear neutrophiles, may be a physiological function of the lungs. In long range catheterization experiments the data were somewhat obscured by changes in leucocytic level presumably due to the presence of the catheters. In short range catheterization experiments as well as single sampling studies these differences could be more clearly demonstrated. Halonen and Kyllön (43) reviewed the literature on white blood cell counts in blood samples obtained from various locations in rodents. According to most references quoted, higher leucocyte counts were found in samples obtained from the periphery (tail vein, marginal ear vein,
Table 3. Mean difference in white blood cell counts

Right Auricle Minus Carotid Artery

<table>
<thead>
<tr>
<th>Dog No.</th>
<th>No. of Diffs.</th>
<th>Type of Count and Diff.</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Total</td>
</tr>
<tr>
<td>1</td>
<td>10</td>
<td>$+471$</td>
</tr>
<tr>
<td>2</td>
<td>6</td>
<td>$-300$</td>
</tr>
<tr>
<td>3</td>
<td>5</td>
<td>$+1377$</td>
</tr>
<tr>
<td>4</td>
<td>10</td>
<td>$+1640$</td>
</tr>
<tr>
<td>5</td>
<td>5</td>
<td>$+594$</td>
</tr>
</tbody>
</table>

Weighted mean diff. $+769$ $+668$ $+29$ $+25$ $+45$

95% Spread†

$-1600$ to $+3200$ $-1800$ to $+3200$ $-870$ to $+930$ $-1000$ to $+1000$ $-300$ to $+400$

Actual spread

$-3100$ to $+3480$ $-2150$ to $+3480$ $-800$ to $+1200$ $-3000$ to $+1688$ $-848$ to $+439$

95% Confidence † limits of mean diff.

$+50$ to $+1500$ $-100$ to $+1500$ $-320$ to $+380$ $-150$ to $+200$ $-70$ to $+160$

Significance of mean diff.

$P < 0.05$ $P < 0.10$ N.S. N.S. N.S.

† Rounded-off values. $P = \text{Polymorphonuclear neutrophiles}$, $L_y = \text{Lymphocytes}$, $E_o = \text{Eosinophiles}$, $M_o = \text{Monocytes}$.

Table 4. Analysis of covariance

<table>
<thead>
<tr>
<th>Sources of Variation</th>
<th>D.F.</th>
<th>Sums of Squares and Products</th>
<th>Residuals</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>$[P]$</td>
<td>$[TF]$</td>
</tr>
<tr>
<td>Between dogs</td>
<td>5</td>
<td>15,394,771</td>
<td>14,247,506</td>
</tr>
<tr>
<td>Within dogs</td>
<td>35</td>
<td>19,689,420</td>
<td>34,233,900</td>
</tr>
<tr>
<td>Total</td>
<td>40</td>
<td>55,072,191</td>
<td>48,481,466</td>
</tr>
</tbody>
</table>

* Significant ($P < 0.01$).

etc.) than in blood obtained by cardiac puncture. In their own experiments these authors found higher total white blood cell counts in the right ventricle than in the left ventricle of 10 rats after a blow on the head and 10 rats, 5 rabbits and 2 cats anesthetized with various drugs.

In heart lung preparations removal of white blood cells is evident from inspecting the data. Results obtained in in vivo catheterization experiments required statistical evaluation. In lungs in which only a small degree of filtration was evidenced during cardiac catheterization in vivo, dramatic removal of leucocytes occurred after making a heart-lung preparation from the same. It must be considered that in the heart-lung preparation the same blood was continuously recirculated and exposed to the white blood cell filtering activity of the lungs. In vivo, new leucocytes are constantly produced by the hemopoietic system. Nevertheless other factors must be involved also since in simultaneous arteriovenous samples mostly larger initial differences were found in the heart-lung preparation than in vivo. It is not likely that injury to the leucocytes in the heart-lung preparation would account for this phenomenon, since no difference could be observed between blood samples exposed to various degrees of injury to the leucocytes. Moreover similar filtration occurred in cross transfusion experiments, where a minimum handling of the donor blood was required.

In vivo as well as in heart-lung preparations the polymorphonuclear neutrophiles were
TABLE 5. ANALYSIS OF VARIANCE

<table>
<thead>
<tr>
<th>Source of Variation</th>
<th>D.F.</th>
<th>S.S.</th>
<th>M.S.</th>
<th>F</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reduction due to correlation</td>
<td>1</td>
<td>29,534,907</td>
<td>29,534,907</td>
<td>99.13*</td>
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<tr>
<td>Residual variation</td>
<td>34</td>
<td>10,129,947</td>
<td>297,939</td>
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<tr>
<td>Total</td>
<td>35</td>
<td>39,664,914</td>
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</table>

* Highly significant ($P < 0.001$).

TABLE 6. AVERAGE LEUCOCYTE COUNTS IN VARIOUS BLOOD VESSELS OBTAINED BY SINGLE SIMULTANEOUS SAMPLING

<table>
<thead>
<tr>
<th>Blood Entering</th>
<th>Blood Leaving</th>
<th>Significance of</th>
<th>Significance of</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lungs (V)</td>
<td>Lungs (A)</td>
<td>Diff. V-A</td>
<td>Diff. V-A</td>
</tr>
<tr>
<td>Vena cava superior</td>
<td>Aorta</td>
<td>$P &lt; 0.01$</td>
<td>0/6 N.C.</td>
</tr>
<tr>
<td>Right auricle</td>
<td>Aorta</td>
<td>N.S.</td>
<td>$*/ */ P &lt; 0.01$</td>
</tr>
<tr>
<td>Right auricle</td>
<td>Left auricle</td>
<td>$P &lt; 0.01$</td>
<td>$*/ */ N.C.$</td>
</tr>
<tr>
<td>Right ventricle</td>
<td>Left ventricle</td>
<td>N.S.</td>
<td>$*/ */ N.C.$</td>
</tr>
<tr>
<td>Right auricle</td>
<td>Carotid artery</td>
<td>N.S.</td>
<td>$*/ */ N.C.$</td>
</tr>
<tr>
<td>7002</td>
<td>7280</td>
<td>130%</td>
<td>1288</td>
</tr>
</tbody>
</table>

Significance of the composite of all data marked with *
Significance of the composite of all data marked with * and $\dagger$

N.S. = not significant; N.C. = not conclusive.

primarily affected by the filtering activity of the lungs. The mechanism of this is not clear. It should be mentioned, however, that leucopenia following injection of colloids (43–46) is also associated with a fall of granulocytes. It is remarkable that eosinophiles are filtered to a less extent than other granulocytes. Because of the smaller number of eosinophiles in the hemocytometer or smear, the larger source of error in counting them must also be considered.

SUMMARY

White blood cells are rapidly removed from heparinized dog blood circulating in heart-lung preparations of dogs until a certain level is reached. This level is then maintained. The level appears to be independent of the leucocyte count of the infused blood. It is not possible to exhaust the filtering ability of the lungs during the useful life of the preparation. Similar results are obtained with defibrinated dog blood and heparinized or defibrinated horse or cattle blood. Similar disappearance of leucocytes is observed in cross transfusion of intact donor dogs with heart-lung preparations, without the use of anticoagulants. The fall of white blood cell counts is mainly due to disappearance of granulocytes. After introducing white cell-poor blood, release of leucocytes occurs from the lungs. This was found to be at least partly due to mixing with residual blood. Thorotrust, a powerful inhibitor of the phagocytic activity of the reticuloendothelial system, does not impair filtering ability of the lungs. Differences are found in long range cardiac catheterization experiments in vivo between leucocyte levels of blood before and after passage through the lungs. The chronic presence of the catheters appears to induce fluctuations in the white blood cell level. In short range cardiac catheterization experiments significant differences are evidenced in the total leucocyte count as well as the polymorphonuclear neutrophile count between the blood entering and that leaving the lungs. Regarding other cell types no significant differences are observed. Correlation could be established between the decrease in total leucocyte count and polymorphonuclear neutrophile count. Single simultaneous blood samples rapidly obtained from various locations revealed a small difference in white cell counts of the blood before and after passage through the lungs. The possible role of the lungs in regulating normal white blood cell levels is discussed.

We are greatly indebted to Drs. A. Cantarow, J. M. Cown, C. M. Gruber, C. M. Gruber, Jr., C. P. Kraatz and E. J. Thomas for helpful advice in the course of this study and/or for critically reviewing the paper; to Dr. R. C. Stroud for teaching us his method of pulmonary catheterization and to Drs. J. L. Cimminera and W. Baker for help in statistical analysis. Our thanks are due to Drs. M. W. Allam, J. D. Beck, F. C. Fielder, F. Kraal and W. E. LaGrange of the University of Pennsylvania Veterinary Medical School for allowing us to use their X-ray facilities and for supplying blood from various species. Pride, Inc. kindly provided us with samples of horse blood.

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