LYMPHOCYTES IN THORACIC DUCT, INTESTINAL AND HEPATIC LYMPH

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With the technical assistance of EMERY VAN HOOK

THE USE of polyvinyl or polyethylene plastic tubes as cannulae has made it possible to collect lymph for periods up to ten days from the thoracic duct or the lacteals, and for periods of one to four days from the hepatic lymphatics. After the initial operation, anesthesia was no longer required so that in a suitable cage, to prevent dislodgment of the cannulae, animals were maintained in apparently good health during the experimental period. Later, if desired, the cannula may in certain instances be removed without ill effects.

Three sites were used where these cannulations may most advantageously be made to collect the lymph draining from the various organs. The first of these sites is the thoracic duct just below the cisterna chyli. A lymph fistula established here will drain the intestinal lymph, the hepatic lymph and the lymph derived from all the muscles below the diaphragm, because at this point there are few lymphatic collateral channels. The second favorable site for cannulation is the intestinal lymphatic vessel which lies adjacent to the superior mesenteric artery. Although numerous anastomotic channels by-pass this lacteal, most of them are occluded by the cannulation procedure. Those that are not occluded do not develop into effective collateral channels because the pressure in a freely flowing lymph fistula is so much less than the pressure in the intact lymphatics that fluid flows preferentially into the cannula. We believe that such an intestinal lymph fistula drains all the lymph from the small intestine because all of the fat fed to an animal can be directly recovered from the fistula, and furthermore an acute deficiency of at least one fat-soluble vitamin promptly develops in these animals. Thirdly, the hepatic lymphatic vessel which lies close to the hepatoduodenal artery may be cannulated advantageously to collect hepatic lymph. Such lymph may be readily differentiated from intestinal lymph by its high protein content and by its clarity even after a fatty meal.

In all of these preparations, therefore, a reasonably constant region of tissue was drained completely of its lymph for a considerable period in a relatively intact animal. It seemed possible that a study of the cellular constituents of such lymph might yield new information which could not be discovered in short term observations on anesthetized animals previously employed.

This report describes attempts to collect such information. First, the numbers and the types of cells emerging from each of the foregoing lymph fistulas were studied over a period of several days. Second, the effect of the loss of these cells
on the animals' blood count was observed. Next, the effects of such physiologic factors as food intake and water balance were studied by simply fasting the animals and by greatly increasing their fluid intake. Lastly, efforts were made to alter the observed output of cells in the lymph, largely by injecting the collected lymph intravenously.

**Review of the Literature**

Many studies have been reported on the thoracic duct lymph in several species of anesthetized animals. Observers agree that the lymphocyte is by far the most common cell and that many more cells pass through the thoracic duct daily than are in the blood stream at any one time. Since, moreover, obstruction or external drainage of the thoracic duct produces lymphopenia, the lymphatics are generally considered to be the most important, although perhaps not the only, route by which lymphocytes enter the blood.

An important tissue which contributes lymphocytes to the thoracic duct is the intestine. While it is by no means concluded either that the intestine is the chief lymphopoietic organ or that it is the chief site of lymphocyte excretion, it certainly contains many lymphocytes, whatever be their direction of motion. Ehrich has shown, moreover, that gastro-enterectomy in rabbits produced lymphopenia which was not augmented by further extirpation of lymphatic tissue. This lymphopenia was at least as profound as that produced by more radical extirpation of lymphatic tissue in rats with relatively intact intestines. This seems to confirm Drinker's view that most of the cells in the thoracic duct lymph come from the intestine.

But if most of the lymphocytes enter the blood stream from the lymph and enter the lymph stream from the intestine, what are the factors which govern this motion? The immediate, mechanical factors which can temporarily augment this transfer were described long ago. While these, however, could perhaps temporarily induce lymphocytosis such as that described after administration of pilocarpine, they could hardly be the basic mechanism which regulates lymphopoiesis.

The older hypothesis of the function of the lymphocytes, that they carry fat, inactivate toxins or supply enzymes, similarly do not account for any such basic regulatory mechanism. If, as is widely recognized, the lymphocytes under certain circumstances may transform into macrophages, or, as has been suggested, differentiate into other blood and tissue cells, how many lymphocytes do so normally and how do such transforming cells fit in with the regulation of lymphopoiesis?

Many observations of the morphology of lymphatic tissue have been made and its degree of activity has been inferred usually from its weight and appearance, sometimes from the blood lymphocyte count, and in a few instances from the lymph lymphocyte count. This type of investigation, plus certain immunologic and microchemical determinations, led to the current hypothesis stated by Dougherty and White of a hypophysis-adrenal hormonal mechanism which induces cellular dissolution of the lymphocytes resulting in the formation of antibodies. The supply of cells to the lymph and to the blood would thus be under hormonal control. This hypothesis has recently been reviewed completely by Valentine, Craddock and Lawrence. They pointed out that, although there is no doubting the effect of the adrenal cortical hormone on the lymphatic tissues, and although a transient lymphopenia has been reported after its administration, the available studies of the effect of the hormone on the level of lymphocytes in the lymph stream are flatly contradictory.

This whole problem of the regulation of lymphocytes entering the lymph is further complicated by the possibility that some of the cells may have actually filtered into the lymph from the blood stream instead of coming directly from the lymphatic tissues; in other words, a circulation of lymphocytes per se may occur. Yoffey and Drinker, after comparing the cell concentrations in the peripheral and central lymph of dogs, concluded that "such a circulation does occur but on a small scale," so that about one cell passes from the blood to the lymph for every thirty cells passing from the lymph channels to the blood. From the morphologic appearance of lymph nodes, Heiberg has suggested that after one or more circulations, lymphocytes may return to the nodes and be destroyed in the secondary nodules; new ones perhaps are formed from their remnants.

These ideas have recently been reviewed by Ehrich.
METHODS

Male albino rats of the Sprague-Dawley strain, weighing from 180 to 240 Gm., were used. All had been fed a standard commercial ration prior to operation except those which were specifically fasted. After a preoperative blood count, cannulations of the lymph vessels were done by the technics previously described. In some animals, polyethylene tubes were also inserted into the femoral vein to facilitate subsequent intravenous injections.

After operation, the animals were placed in special cages, where they had constant access to either water, 0.2 per cent saline solution or 0.8 per cent saline solution, depending on whether a moderate, large or very large lymph flow was desired. A standard, balanced, mixed diet was provided them by placing it periodically either in the cages or in special feeding boxes.

Clotting of lymph in the cannula was a troublesome problem, especially in thoracic duct and hepatic lymph fistulas. About one-half of these fistulas clotted during the first twenty-four hours of flow or produced less than 10 cc. of lymph and were discarded from the series. In rats with intestinal or thoracic duct fistulas from which the lymph flowed longer than eighteen hours, hypoprothrombinemia developed so that thereafter the lymph rarely clotted. The lymph from hepatic lymph fistulas invariably clotted and rarely flowed longer than twenty-four hours. Heparin and dicumarol were used in isolated instances but most of the animals did not receive an anticoagulant, because doses adequate to prevent coagulation of lymph were also prone to cause postoperative bleeding.

Lymph collections were made over twenty-four hour periods. To prevent the coagulation of the lymph and the deterioration of the lymphocytes during such a long time, a solution of 1.0 per cent sodium citrate and 1.0 per cent formaldehyde was used as an anticoagulant and fixative. From 1.0 to 6.0 cc. of this solution was pipetted into an Erlenmeyer flask of from 25 to 115 cc. capacity, the size of the flask and the volume of the solution depending on the anticipated lymph flow. Repeated cell counts on the same lymph specimen showed that a dilution of 1 cc. of this fixative to 20 cc. of lymph was adequate to prevent deterioration of the cells for more than four days. Without such a fixative repeated counts of the same lymph specimen gave inconsistent results after only a few hours.

After the volume of each day’s collected lymph had been measured, the flasks were thoroughly shaken and the concentration of white blood cells in the collected specimens was determined by standard hemato-logic methods. Determinations were done in duplicate and, if they varied by more than 10 per cent, the lymph was resuspended and the cells were recounted. From the average observed white blood cell count, the measured lymph volume and the volume of fixative used, one could, of course, easily calculate the actual lymph volume, the actual concentration of cells and the total number of white cells present in the entire twenty-four hour lymph specimen. In a number of animals, smears of lymph were made daily.

All blood counts were made in duplicate on blood secured by puncture of the heart. Determinations of the lymphocytic distribution in the blood were made preoperatively and then on the second, fourth and sixth days after operation. Some animals were bled four times, others only twice at varying intervals. Differential counts were made and the absolute concentrations per millimeter of blood of neutrophilic leukocytes and of lymphocytes were calculated. All smears of blood and lymph were stained by the May-Grunwald-Giemsa method.

In the control series a sham operation was performed on 6 rats and polyethylene tubes were inserted into their peritoneal cavities, but their lymphatic vessels were not disturbed. They were then treated exactly like the test animals. After three days, operation was again performed on these controls; on 3 of them a sham operation was performed a second time, while in the other 3, an intestinal lymph fistula was established. A blood count was taken prior to each operation and three days after the second operation.

Various procedures were employed in attempts to alter in some way the number of cells collected from the lymph fistulas. In one group, lymph was intravenously reinjected in an attempt to replace that which was drained away. Sometimes the animal’s own lymph and sometimes the lymph from 1 to 5 donor animals was injected. There were minor variations in technic from experiment to experiment, but basically two methods were used. Either refrigerated, heparinized lymph was intermittently injected with a syringe, or else a direct infusion method was employed. In the latter technic, 1 or 2 donor rats were elevated about 1½ feet (about 46 cm.) above a recipient rat and the donor’s lymph was made to flow through polyethylene tubes directly into the lower rat’s veins. A small dropper made of polyvinyl or
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silicon-treated glass permitted the observer to be sure that transfer of lymph was actually taking place although, of course, the actual volume of the lymph being infused could not be measured. Technically, this method seemed to work surprisingly well if the donor rat was heparinized or made hypoprothrombineemic. Because of the well-known effects of malnutrition on lymphatic tissue, 4 animals were fasted, 1 for one day and 1 for seven days. Since hypoprothrombinemia and hypoproteinemia develop in lymph fistula rats, 1 animal was given vitamin K and another was given large quantities of rat plasma in order to prevent these particular changes.

### Table 1. Average Blood Counts and Range of Blood Counts Encountered in 46 of the Normal Rats Used in These Experiments

<table>
<thead>
<tr>
<th></th>
<th>Average count</th>
<th>Range of counts</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(X 10^9/mm^3)</td>
<td>(X 10^9/mm^3)</td>
</tr>
<tr>
<td>Total white blood cells</td>
<td>18.9</td>
<td>9.5-32.5</td>
</tr>
<tr>
<td>Lymphocytes</td>
<td>15.6</td>
<td>7.6-18.4</td>
</tr>
<tr>
<td>Neutrophils</td>
<td>2.9</td>
<td>1.9-7.5</td>
</tr>
</tbody>
</table>

### Table 2. Thoracic Duct Lymph*

<table>
<thead>
<tr>
<th>Rat no.</th>
<th>Given to drink ad libitum</th>
<th>Volume of lymph (cc)</th>
<th>WBCs/mm^3 in lymph (X 10^9)</th>
<th>Total no. of WBCs in 24-hour specimen (X 10^9)</th>
</tr>
</thead>
<tbody>
<tr>
<td>T11</td>
<td>Water</td>
<td>13.0</td>
<td>54.1</td>
<td>703</td>
</tr>
<tr>
<td>T14</td>
<td>Water</td>
<td>18.8</td>
<td>45.5</td>
<td>780</td>
</tr>
<tr>
<td>T17</td>
<td>Water</td>
<td>25.5</td>
<td>38</td>
<td>905</td>
</tr>
<tr>
<td>T19</td>
<td>Water</td>
<td>19.4</td>
<td>16.3</td>
<td>510</td>
</tr>
<tr>
<td>T21</td>
<td>Water</td>
<td>2.5</td>
<td>53.8</td>
<td>673</td>
</tr>
<tr>
<td>T22</td>
<td>Water</td>
<td>11.5</td>
<td>38.7</td>
<td>445</td>
</tr>
<tr>
<td>T25</td>
<td>Water</td>
<td>18.5</td>
<td>57.4</td>
<td>1,060</td>
</tr>
<tr>
<td>T31</td>
<td>Water</td>
<td>15.8</td>
<td>46.2</td>
<td>730</td>
</tr>
<tr>
<td>T32</td>
<td>Water</td>
<td>15.3</td>
<td>75.8</td>
<td>1,110</td>
</tr>
<tr>
<td>T33</td>
<td>Water</td>
<td>14.8</td>
<td>55.1</td>
<td>815</td>
</tr>
<tr>
<td>T36</td>
<td>Water</td>
<td>17.3</td>
<td>57.5</td>
<td>995</td>
</tr>
<tr>
<td>T38</td>
<td>0.8% saline</td>
<td>95.0</td>
<td>10.9</td>
<td>1,040</td>
</tr>
<tr>
<td>T39</td>
<td>0.8% saline</td>
<td>48.0</td>
<td>16.3</td>
<td>782</td>
</tr>
<tr>
<td>T41</td>
<td>0.8% saline</td>
<td>38.5</td>
<td>17.6</td>
<td>678</td>
</tr>
<tr>
<td>T42</td>
<td>0.8% saline</td>
<td>12.2</td>
<td>40.5</td>
<td>697</td>
</tr>
<tr>
<td>T45</td>
<td>See below*</td>
<td>75.0</td>
<td>19.5</td>
<td>878</td>
</tr>
<tr>
<td>T77</td>
<td>0.2% saline</td>
<td>40.5</td>
<td>15.7</td>
<td>717</td>
</tr>
<tr>
<td>T78</td>
<td>0.2% saline</td>
<td>17.9</td>
<td>56.1</td>
<td>1,000</td>
</tr>
<tr>
<td>T79</td>
<td>Water</td>
<td>20.0</td>
<td>30.2</td>
<td>604</td>
</tr>
<tr>
<td>T80</td>
<td>Water</td>
<td>33.5</td>
<td>28.3</td>
<td>948</td>
</tr>
<tr>
<td>T81</td>
<td>Water</td>
<td>18.5</td>
<td>88.1</td>
<td>1,630</td>
</tr>
<tr>
<td>T83</td>
<td>Water</td>
<td>25.0</td>
<td>43.2</td>
<td>1,080</td>
</tr>
</tbody>
</table>

* The lymph volume, white blood cell concentration, and the total number of white blood cells (volume x concentration) in the thoracic duct lymph of 42 rats, collected over the first twenty-four hours of lymph flow. All rats were given a standard mixed diet and the fluid indicated above, except for rat T50. This animal received 55 cc. of 0.8 per cent saline solution intravenously over the twenty-four hour period.
Results

Because of the marked variations which have been reported in the blood counts of normal rats from different colonies and because of the important differences which the various staining technics seem to make, data were assembled from normal rats of this colony (table 1). These data showed that the normal lymphocyte count was rather high and that the range of normal values was very wide.

The quantitative data recorded during the first day's flow from the thoracic duct fistulas of 22 rats (table 2) show the general range and the wide variations observed in the volume of lymph, in the cell concentration and in the total number of cells present. Under the conditions of these experiments, the most important factors governing the volume of lymph flow were the amount of food and the volume of fluid each animal took. Most rats drank far more if salt was added to their drinking water. This effect is clearly indicated (table 2) in the four instances in which rats were given 0.8 per cent saline solution ad libitum. This increase in lymph flow, however, did not produce an increase in the total number of cells emerging from the fistula, for the concentration of cells was proportionately diminished as the volume of lymph increased so that the total cellular output was unchanged. These data are portrayed graphically (fig. 1).
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A similar set of values obtained during the first day's flow from intestinal lymph fistulas of 8 rats is shown in table 3. The striking point here is that the figures are virtually identical with those obtained from the thoracic duct lymph fistula (table 2). The data on the hepatic lymph collected from fistulas of 4 animals (table 4) over a period of twenty-four hours were, on the contrary, very different from those recorded for intestinal and thoracic duct fistulas. The liver seems to contribute relatively few cells to the thoracic duct lymph.

### Table 3. Intestinal Lymph*

<table>
<thead>
<tr>
<th>Rat no.</th>
<th>Volume of lymph (cc.)</th>
<th>WBC's/mm.³ in lymph (x 10⁶)</th>
<th>Total no. of WBC's in 24-hour specimen (x 10⁶)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>23.2</td>
<td>34.8</td>
<td>807</td>
</tr>
<tr>
<td>15</td>
<td>37.0</td>
<td>18.3</td>
<td>677</td>
</tr>
<tr>
<td>19</td>
<td>33.0</td>
<td>26.3</td>
<td>868</td>
</tr>
<tr>
<td>110</td>
<td>25.5</td>
<td>26.8</td>
<td>683</td>
</tr>
<tr>
<td>111</td>
<td>15.3</td>
<td>37.3</td>
<td>571</td>
</tr>
<tr>
<td>112</td>
<td>16.7</td>
<td>60.5</td>
<td>1,010</td>
</tr>
<tr>
<td>123</td>
<td>24.6</td>
<td>46.7</td>
<td>1,150</td>
</tr>
<tr>
<td>130</td>
<td>25.2</td>
<td>16.0</td>
<td>655</td>
</tr>
</tbody>
</table>

* The lymph volume, white blood cell concentration, and the total number of white blood cells (volume x concentration) in the intestinal lymph of eight rats, collected over the first twenty-four hours of lymph flow. All animals were given a standard mixed diet and 0.1 per cent saline solution to drink ad libitum.

### Table 4. Hepatic Lymph*

<table>
<thead>
<tr>
<th>Rat no.</th>
<th>Volume of lymph (cc.)</th>
<th>WBC's/mm.³ in lymph (x 10⁶)</th>
<th>Total no. of WBC's in 24-hour specimen (x 10⁶)</th>
</tr>
</thead>
<tbody>
<tr>
<td>H1</td>
<td>2.1</td>
<td>19.9</td>
<td>41.8</td>
</tr>
<tr>
<td>H2</td>
<td>0.9</td>
<td>16.7</td>
<td>15.0</td>
</tr>
<tr>
<td>H6</td>
<td>3.9</td>
<td>6.5</td>
<td>25.4</td>
</tr>
<tr>
<td>H7</td>
<td>1.1</td>
<td>6.2</td>
<td>6.8</td>
</tr>
</tbody>
</table>

* The lymph volume, white blood cell concentration, and the total number of white blood cells (volume x concentration) in the hepatic lymph of four rats, collected over the first twenty-four hours of lymph flow. All animals were given a standard mixed diet and 0.2 per cent saline solution to drink ad libitum.

When lymph was collected for longer periods than twenty-four hours an interesting change in cellular output was observed (figs. 2 and 3). Whatever may have been the initial twenty-four hour total output of white blood cells, either from a thoracic duct fistula or from an intestinal lymph fistula, that output became progressively less with each successive day of lymph flow. This effect was quite independent of either lymph flow or lymph cell concentration (fig. 1). Sometimes (figs. 2 and 3) an individual rat's total cellular output would remain about the same or even increase slightly on two succeeding days, but by the third day, it would invariably fall.

A decrease in the number of circulating lymphocytes was shown to occur in
Fig. 1.—The total white cell output with each consecutive day's lymph flow from the thoracic duct lymph fistulas of 5 representative animals (lymph volume × lymph cell concentration × 10⁶).

Fig. 3.—The total white cell output with each consecutive day's lymph flow from the intestinal lymph fistulas of 5 representative animals (lymph volume × lymph cell concentration × 10⁶).
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the blood of these fistulous animals which showed such marked reductions in the number of lymphocytes of the lymph. The total white count was altered only slightly during the six-day period of lymph fistula drainage, depending on whether or not leukocytosis had developed, but the absolute concentration of circulating blood lymphocytes consistently dropped and attained levels usually far below those ever observed preoperatively (fig. 4).

Considering these striking quantitative changes in the cellular distribution within the blood and lymph, there were surprisingly few morphologic alterations in the individual cells. In the lymph of these fistulous animals, as was to be expected, the predominant cell was the small lymphocyte; only a few medium sized lymphocytes were usually present. Polymorphonuclear cells and eosinophilic leukocytes were very rarely seen in lymph smears and then only in those made of unusually bloody lymph. Five days after lymphatic cannulation, as far as could be detected the morphology of the individual cell types remained unchanged. Platelets were not seen in any of the slide preparations.

The small lymphocytes, characteristic of lymph, are also the most common white cells in the blood. After lymphatic cannulation, however, these cells practically disappear from the blood stream. The normal differential count of the rat (about 80 per cent lymphocytes, 20 per cent neutrophils) was often very nearly reversed as a varying number of mature, normal appearing neutrophils entered the blood. Those lymphocytes that did remain, moreover, were predominantly of the medium sized type rather than the small lymphocyte and appeared to have the normal morphology of the medium sized lymphocyte. There were no

FIG. 4.—The total white blood cell counts, blood lymphocyte counts and blood neutrophil counts of animals with thoracic duct lymph fistulas (left) and intestinal lymph fistulas (right) (×10⁶ per cubic millimeter).
changes observed in the blood eosinophilic leukocytes after lymphatic cannulation. There were considerable increases in the percentage of mature myeloid elements in the bone marrow, but more work must be done before sound conclusions can be drawn concerning the factors incident to this myeloid stimulation.

Adequate controls were necessary to make certain that these changes in the blood and lymph of animals with lymph fistulas were due actually to the loss of lymph and not to some other factor—the operation, the cage, and so forth. The results of a control experiment are shown graphically (fig. 5). The blood lympho-

![Control series](image)

**Fig. 5.—**Control series; the effects of sham operation on the blood counts and total white cell output in the lymph from intestinal lymph fistulas. A sham operation was performed on 6 rats; three days later, a sham operation was again performed on 3 of them and in the other 3 an intestinal lymph fistula was actually established. The blood counts for the animals on which sham operations were performed are indicated in outline, while the counts for the animals with lymph fistulas are indicated in black (×10⁸ per cubic millimeter).

cyte level was considerably lower in the 3 rats which had actually lost lymph through their fistulas than it was in the 3 which underwent the sham operation. Furthermore, when the lymphatic cannulations were made on the third day after a sham operation, the same high initial lymphocyte output occurred and the same progressive daily decrease was observed as though the sham operations had not been performed. It seemed, therefore, that the lymphopenia and the decrease in lymphocyte output were induced by the loss of lymph.

Logically, then, it would seem, one could prevent these changes by reinjecting lymph. The results obtained from such reinjection experiments were either equivocal or else clearly failures (table 5). None of the animals into which lymph injections were made, moreover, ate as well or appeared to be in as good condition generally as the usual lymph fistula animal does. The daily intravenous in-
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TABLE 5.—Results of Reinjection of Lymph and Other Procedures

<table>
<thead>
<tr>
<th>Rats</th>
<th>Type of lymph fistula</th>
<th>Procedures attempted</th>
<th>Quantity given</th>
<th>Duration of experiment (days)</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Thoracic duct</td>
<td>Intravenous injection every four hours of refrigerated, heparinized autogenous lymph</td>
<td>Total lymph output restored daily (40-60 cc.)</td>
<td>3</td>
<td>Daily decrease in white blood cells in lymph slower than usual (815 x 10^6 on 3rd day) (cf. table 2). Rat in poor condition and died on third day.</td>
</tr>
<tr>
<td>2</td>
<td>Intestinal</td>
<td>Intravenous injection every two to four hours of refrigerated heparinized, autogenous lymph</td>
<td>Total lymph output restored daily (6-35 cc.)</td>
<td>5</td>
<td>Contradictory. One animal had more white blood cells in lymph than usual; the other, less. Both rats in poor condition but survived.</td>
</tr>
<tr>
<td>3</td>
<td>Intestinal</td>
<td>Intravenous injection every two hours of sterile, heparinized homogenous lymph from 3 to 5 dicumarolized donor rats</td>
<td>15-28 cc. of lymph per day containing 780-1,400 x 10^6 cells</td>
<td>3</td>
<td>Usual white blood cell output observed in lymph (cf. fig. 3). Animal in very poor condition and died of infected retroperitoneal hematoma.</td>
</tr>
<tr>
<td>4</td>
<td>Intestinal</td>
<td>Continuous direct intravenous lymph infusion by gravity drip from 1 or 2 donor rats</td>
<td>Could not be determined</td>
<td>2-5</td>
<td>Usual white blood cell output observed in lymph (cf. fig. 3). Animals' condition generally poor.</td>
</tr>
<tr>
<td>5</td>
<td>Intestinal</td>
<td>Intermittent intravenous injection of fresh rat plasma</td>
<td>15 cc. of plasma daily</td>
<td>3</td>
<td>Usual white blood cell output observed in lymph (cf. fig. 3). Animal in excellent condition.</td>
</tr>
<tr>
<td>6</td>
<td>Thoracic duct</td>
<td>Animals fasted after lymph fistula had been established</td>
<td>—</td>
<td>1</td>
<td>Usual white blood cell output observed in lymph (736 &amp; 1,240 x 10^6 cells on 1st day) (cf. table 2).</td>
</tr>
<tr>
<td>7</td>
<td>Intestinal</td>
<td>Animals fasted six days before and one day after lymph cannulation</td>
<td>—</td>
<td>1</td>
<td>Usual white blood cell output observed in lymph (610 &amp; 970 x 10^6) (cf. table 3). Animals' condition satisfactory.</td>
</tr>
<tr>
<td>8</td>
<td>Intestinal</td>
<td>Vitamin K subcutaneously daily</td>
<td>1 mg. per day</td>
<td>5</td>
<td>Usual output of white blood cells in lymph (cf. fig. 3). Animal's condition excellent.</td>
</tr>
</tbody>
</table>
jection of very large quantities of fresh rat plasma, although apparently well tolerated, likewise failed to prevent the progressive daily decrease in the lymphocyte output (table 5). Vitamin K similarly had no effect.

Surprisingly too, fasting the animal during the period of lymph drainage, or withholding food for periods as long as six days before lymph drainage was instituted, did not reduce the number of lymphocytes collected from the cannula during the first twenty-four hours of lymph flow (table 5).

**Comment**

The total daily output of lymphocytes from the thoracic duct is higher than would be expected from previous short term observations by a different technic.11, 12 The normal white blood cell count of the rats of this colony is somewhat higher than average values in the literature, although the animals were maintained in excellent condition. The complete independence of total lymphocyte output and total volume of lymph seems a reasonable finding provided that the volume of lymph was sufficient to maintain free flow through the lymphatic channels. Decrease in lymphocyte output with decrease in lymph flow, as has been observed in short term experiments,40, 41 may well be due to complete cessation of flow from considerable portions of the region drained as the volume of lymph falls. In all the foregoing experiments care was taken to maintain free flow of lymph; animals from which free continuous flow was not obtained were not used.

An analogy may be drawn between these studies and studies on urine in which the total output of urinary constituents is independent of total volume. It would appear equally important in studies on lymph as on urine to obtain reliable twenty-four hour outputs since concentrations observed at any time are likely to reflect principally changes in fluid balance rather than changes in the constituents being studied.

The importance of the intestine in contributing cells to the thoracic duct lymph of the animal under anesthesia has been previously emphasized in the literature.47 Under the conditions of these experiments with the animal unanesthetized and freely feeding but limited in its activity, the intestine still appears to be practically the exclusive source of the lymphocytes in the lymph.

The foregoing finding is not surprising in view of the large mass of lymphoid tissue in the intestine, but the spontaneous daily decline in lymphocyte output from the lymph fistulas draining this tissue is the most striking and at the same time most puzzling observation in this research.

It was first thought that some extraneous factor—the loss of weight which these animals sustain despite good appetites, a slight infection not grossly apparent, the effect of immobilization or dehydration, or the trauma from the operation—might produce this decrease. The results obtained in the control series, the fasted animals, and the markedly hydrated saline-drinking animals all seemed to show, however, that these factors were not important. The introduction of a plastic tube into the lymphatic channel may in itself have some effect, either by
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foreign body action or by lessening lymph pressure, but it is hard to believe that such factors could cause this large spontaneous decrease in lymphocyte output.

If one accepts the decrease, then, as a valid observation, it is doubly hard to understand why reinjecting fresh lymph does not prevent it. In the absence of criteria for lymphocyte viability, however, it is impossible to say that the reinjected cells were normal. The relatively poor condition of reinjected rats compared to nonreinjected rats might indicate the presence in the lymph of some toxic decomposition product or the products of incomplete, unrecognized coagulation.

It is interesting that Erf22 failed to maintain the blood lymphocyte level of gastro-enterectomized rabbits by the intravenous injection of fresh lymphocytes.

In the literature mentioned previously, there is no hypothesis which readily explains this progressive decrease in lymphocyte output with continued loss of lymph. The effect could perhaps be produced by some hormonal regulatory mechanism which was activated by loss of lymph but not by a sham procedure. One might hypothesize that some lymph-borne substance was necessary for continued production of lymphocytes but that it was so labile that it could not be readily reinjected. So little is known of the physicochemical laws governing lymphocyte motion that such a factor could be proposed as the cause of this effect without fear of contradiction.

None of the foregoing, however, are very satisfactory explanations. Somewhat more attractive to us is the idea that in cannulating the major lymphatic vessels, we are interrupting a perhaps numerically small but somehow physiologically important group of normally circulating lymphocytes. If they were the ones largely responsible for lymphocyte production, say the more rapidly dividing ones, then their loss would sharply decrease lymphopoiesis.

It is clear, however, that at present we do not have sufficient data to prove this or any other hypothesis.

The lymph, a major body fluid, probably has a multitude of specific functions. One, for instance, was discovered incidentally during the course of this study. As was mentioned before, and as is reported elsewhere,3 in the rat with a complete intestinal lymph fistula a hemorrhagic tendency develops. Investigation of this hemorrhagic tendency showed that it was due to hypoprothrombinemia and was corrected by parenteral administration of vitamin K. The absorption of vitamin K, therefore, appears to be exclusively through the lymph.

If, however, one had not known from other sources the importance of the fat-soluble (hence lymph-borne) vitamin K in maintaining normal blood coagulation, then the investigation of the hemorrhagic tendency induced by loss of lymph would have been a difficult instead of a simple problem. Reinjection of lymph into vitamin K deficient animals, experiments have shown, is not adequate to correct the hypoprothrombinemia; a concentrated solution of the vitamin is required. Had not such a solution been readily available, most probably an explanation for the hemorrhagic tendency would not have been found immediately. Our present knowledge of lymphocyte production, unlike our knowledge of blood coagulation, is not yet complete enough to provide any such explanation for the spontaneous decrease in lymphocyte output from lymph fistulas,
much less any such remedy for it. The explanation of this, too, may possibly depend on some specific, but as yet unknown, function of the lymph.

Until this spontaneous decrease is explained, however, one must be cautious in interpreting the results of studies on the lymphocytes in the lymph.

**Summary**

In rats whose intestinal or thoracic duct lymph was drained externally for several days, lymphopenia occurred. Large numbers of cells were collected in the lymph each day, as many, apparently, from the intestinal lymph alone as from the thoracic duct. Hepatic lymph contributed relatively few cells.

Augmentation of lymph flow decreased the concentration of cells in the lymph but did not affect the total number of cells collected each day. Fasting for several days likewise did not decrease the first day's output. With each day's lymph flow, however, the daily output of cells spontaneously decreased. The decrease was not prevented by the intravenous injection of fresh lymph or of fresh rat plasma in large amounts. In view of this unexplained effect, one must be cautious in interpreting the results of experiments on the lymph lymphocyte.

**References**


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LYMPHOCYTES IN THORACIC DUCT, INTESTINAL AND HEPATIC LYMPH

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