

CHANGES IN THE NUMBER OF SMALL LYMPHOCYTES
OF THE BLOOD FOLLOWING LIGATION OF THE
THORACIC DUCT.

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INTRODUCTION.

The important work of Murphy and his collaborators has added great interest to the study of the physiology of the lymphocyte. These investigators have shown particularly well the rôle that the lymphocyte plays in the resistance and susceptibility of mice to cancer grafts. Previously the lymphocyte was studied considerably in regard to the extent of its appearance in the thoracic duct under ordinary conditions or its appearance in the blood stream following splenectomy (Biedl and von Decastello), after extirpation of groups of important glands (Ehrlich and Reinbach), after the administration of pilocarpine (Rous), and following ligation of the thoracic duct (Davis and Carlson; Bunting and Huston).

During the progress of work on the ligation of the thoracic duct recently reported (Lee), the opportunity offered itself for studying the effect of the ligation of that vessel on the number of small lymphocytes in the blood.¹

Materials and Methods.

Young adult male cats were used for all of the experimental work. From blood which was procured from the ear of the animal, a white blood count and a smear were obtained. Wright's stain was applied to the smear and a differential count of 300 cells or more was made. Two control counts were made; one was obtained on the day preced-

¹ All operations were performed under ether anesthesia.

ing operation, and the other was taken 24 hours later, on the morning of the day of operation. The care of the animal before, during, and after the operation was the same as reported previously; likewise, the operation, which was essentially an intrathoracic ligation of the thoracic duct and the periaortic lymphatic plexus, was similar. 5 hours after operation the blood was examined again. On the following day counts were made in the morning and afternoon, while from then on the examinations were made in the morning of the next 7 successive days, and after that on alternate days. At the end of about 3 weeks the lymphocytes were again up to the preoperative level, and the animal was then sacrificed after the mesenteric lymphatics had been injected with Berlin blue. Blocks of tissue from the various organs were fixed in formaldehyde for further study, and a dissection of the collateral circulation of the thoracic duct was made.

Since the data from the entire series of five animals are about the same, it is only necessary to report the findings in two animals in order to illustrate the changes in the number of small lymphocytes in the circulating blood.

In the differential counts here reported the white blood cells have been classified under the following headings: polymorphonuclear neutrophils, eosinophils, basophils, small lymphocytes, large lymphocytes, large mononuclears, and transitionals. The polymorphonuclear neutrophils need no discussion. The eosinophils were not constant with respect to their granulation; the size of the granules was constant for any particular cell, though it varied for the different cells. Busch and Van Bergen have classified eosinophils into three groups according as the granulation was fine, medium, or coarse; yet it is obvious that such a classification is difficult. In all the smears that were examined a typical basophil was not seen. Very infrequently a cell was seen which had an irregularly shaped nucleus with a slightly granular purplish red cytoplasm; but the granulation was not typical of a basophil.

The small lymphocyte was taken to be a cell smaller than the polymorphonuclear neutrophil, round, with a round nucleus which was sometimes slightly indented. The nucleus was clearly outlined, and occupied almost the entire cell; its chromatin was dense, often aggregated, and deeply purple-stained. The cytoplasm was scant,

slightly blue-stained as a rule, with a small colorless rim around the nucleus, and it frequently contained a few fine azurophil granules.

The large lymphocyte differed from the small lymphocyte mainly in size, since the staining was essentially the same; there is a possibility, then, of considering these two cells as belonging to the same class. The nucleus of the large lymphocyte was larger than that of the small lymphocyte, the cytoplasm surrounded the nucleus definitely, and the azurophilic granules were larger and more numerous.

The large mononuclear was the largest blood cell observed in the cat; it was often several times as large as the polymorphonuclear neutrophil. The nucleus was large, often oblong, sometimes slightly indented; the chromatin was loose and skein-like and did not stain heavily. The cytoplasm was abundant, usually slightly granular or mottled, and contained, as a rule, numerous fine azurophil granules. It was often difficult to distinguish between this cell and the large lymphocytes, and undoubtedly the tables herewith submitted suffer because of this difficulty.

The transitional cell was considered to be similar to the large mononuclear and different mainly in that it had a markedly indented nucleus.

The recent work of Sabin has shown how vitally stainable granules may serve as a specific criterion for the differentiation of the three strains of the white blood cells of the chick; and there is reason to believe that this method may be applied generally to all blood examinations and can thus supplement the routine differential blood counts. Simpson has described how the vital dyes may serve to differentiate large mononuclear cells from transitional cells. Thorne and Evans also have employed vital dyes in showing the very low monocyte count of lymph in rabbits.

EXPERIMENTAL DATA.

With these standards for the identification of the cells in the differential counts made with Wright's stain, the experimental observations were carried out as described. The general technical procedures are indicated in the protocol of Cat 1, which is given below in condensed form.

Cat 1.—May 9, 1921. Young adult male cat, black; in stock since May 6, 1921. 10 a.m. Count made.

May 10. Food withheld for the last 24 hours. Weight 3,135 gm. 10 a.m. Count made. 11 a.m. Ligation of thoracic duct according to method already described. 5 p.m. Count made.

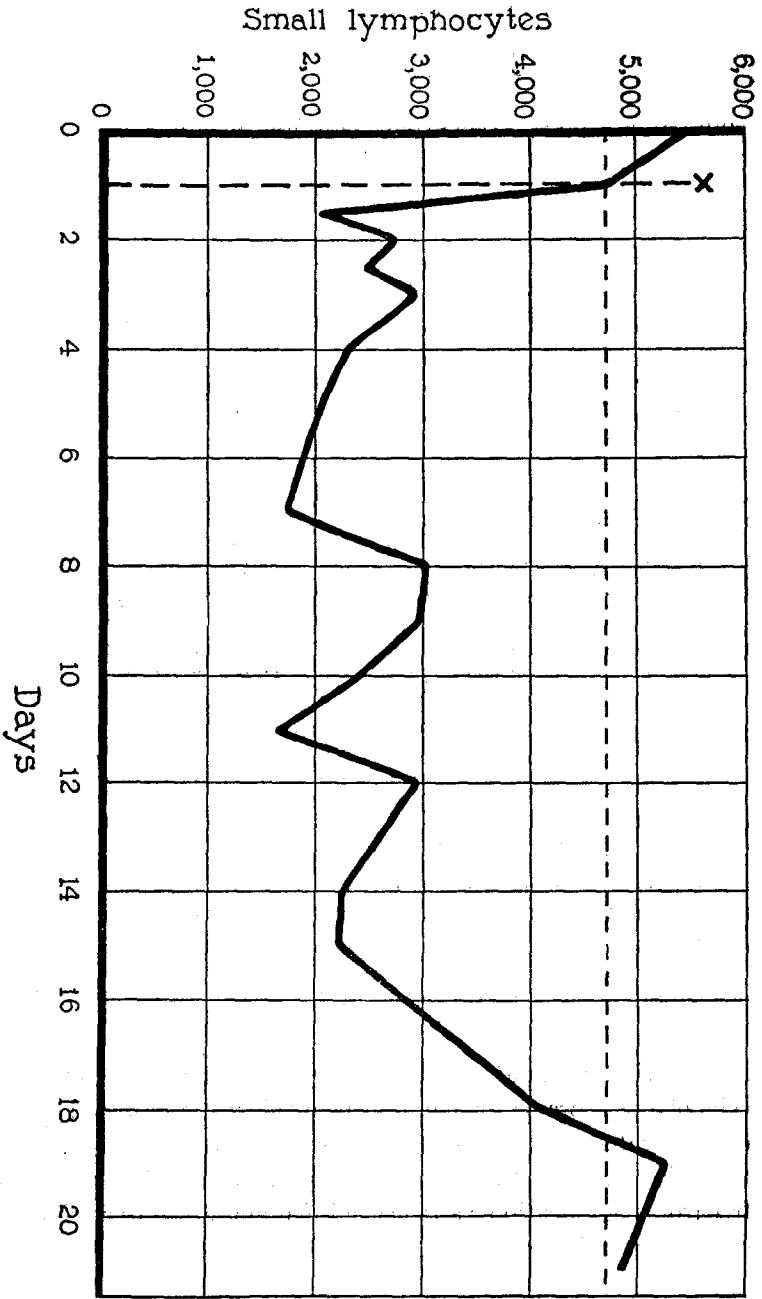
TABLE I.

Cat 1.

Date.	Total white blood count.	Poly-morpho-nuclear neutrophils.	Eosino-phils.	Small lympho-cytes.	Large lympho-cytes.	Large mono-nu-clears.	Transi-tionals.	Absolute No. of small lympho-cytes.
		<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	
1921 May 9	15,720	51.3	11.0	35.0	1.0	1.3	0.3	5,502
" 10, a.m.	13,880	50.3	11.7	34.0	1.3	1.7	1.0	4,689
Operation.								
May 10, p.m.	30,600	88.3	0.7	6.7	2.7	1.0	0.7	2,050
" 11, a.m.	23,640	83.3	0.7	11.7	2.0	1.7	0.7	2,765
" 11, p.m.	22,800	84.0	1.0	10.7	2.0	1.3	1.0	2,439
" 12	19,900	75.7	4.3	14.7	2.7	1.7	1.0	2,925
" 13	14,280	70.7	10.7	16.3	1.3	0.7	0.3	2,327
" 14	13,880	71.3	11.7	15.0	0.7	1.0	0.3	2,080
" 16	13,760	73.7	9.0	12.3	4.0	0.7	0.3	1,692
" 17	17,400	70.7	4.3	17.3	3.7	3.3	0.7	3,010
" 18	13,800	66.7	6.0	21.3	3.3	1.7	1.0	2,939
" 19	13,600	68.7	6.3	17.7	3.0	3.3	1.0	2,407
" 20	10,560	71.7	7.3	15.7	1.0	3.3	1.0	1,657
" 21	12,000	65.3	7.0	24.7	1.0	1.3	0.7	2,964
" 23	12,520	70.7	8.7	18.3	0.7	1.0	0.7	2,291
" 24	10,800	71.3	6.3	20.3	1.0	0.7	0.3	2,192
" 27	16,640	60.7	10.0	24.7	3.3	1.0	0.3	4,109
" 28	18,000	56.3	8.7	29.0	2.7	2.7	0.7	5,220
" 30	17,440	60.7	9.7	27.3	1.3	0.7	0.3	4,861

Cat made uneventful recovery; the incision healed perfectly. Counts made are listed in Table I, and shown in Text-fig. 1.

May 30. Weight 3,420 gm. Since counts showed that the number of small lymphocytes in the circulating blood had reached the same level as before operation, the animal was sacrificed; the mesenteric lymphatic vessels were injected as described, and it was found that a lymphatovenous communication had established itself between the thoracic duct and the ninth left intercostal vein. The specimen was fixed in formaldehyde.



In all the figures the horizontal broken line indicates the level at the time of operation.
TEXT-Fig. 1. Curve to show the absolute number of small lymphocytes per cubic millimeter of blood in Cat 1 in which the thoracic duct was ligated at X. On the 19th day the number of small lymphocytes had again reached its preligation level.

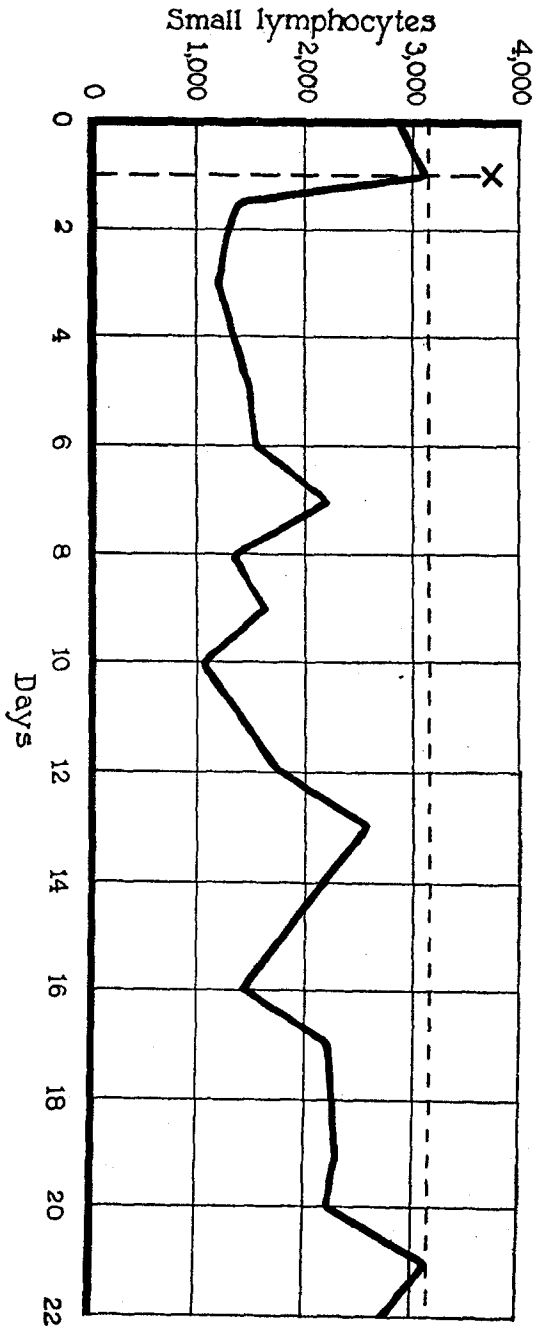
In this animal (No. 1) the number of small lymphocytes decreased 56 per cent immediately following the ligation of the thoracic duct. This reduction in the number of lymphocytes in the circulating blood endured until the preligation level was attained on the 21st day.

TABLE II.

Cat 2.

Date.	Total white blood count.	Poly-morpho-nuclear neutrophils.	Eosino-phils.	Small lympho-cytes.	Large lympho-cytes.	Large mono-nu-clears.	Transi-tionals.	Absolute No. of small lympho-cytes.
		<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	
1921								
May 25	13,400	71.0	4.7	21.0	1.3	1.0	1.0	2,814
" 26, a.m.	13,080	69.3	4.7	24.0	1.0	0.7	0.3	3,139
Operation.								
May 26, p.m.	38,160	94.0	0.8	3.6	0.6	0.8	0.2	1,373
" 27, a.m.	19,240	90.7	0.3	6.7	1.3	0.7	0.3	1,288
" 28	20,760	92.0	1.0	5.7	2.0	1.0	0.3	1,183
" 30	26,360	90.0	0.7	5.7	1.3	1.0	1.3	1,482
" 31	22,320	86.3	3.0	7.0	1.3	2.0	0.3	1,562
June 1	20,600	79.7	5.0	11.0	2.7	1.3	0.3	2,266
" 2	16,800	84.3	4.0	7.7	2.7	1.0	0.3	1,293
" 3	18,000	86.3	2.3	9.0	2.0	0.3	0	1,620
" 4	21,280	90.0	2.0	5.0	1.3	1.3	0.3	1,064
" 6	20,840	82.7	5.3	8.3	1.7	1.0	1.0	1,729
" 7	19,400	77.0	6.0	13.3	2.3	1.0	0.3	2,580
" 8	19,120	80.7	4.7	11.3	1.3	1.0	1.0	2,160
" 10	17,000	83.7	5.0	8.3	1.0	1.3	0.7	1,411
" 11	16,800	77.0	5.7	13.3	1.3	1.3	1.3	2,234
" 12	11,480	72.3	3.3	20.0	1.7	1.7	1.0	2,296
" 14	12,960	72.7	6.3	17.3	2.0	1.3	0.3	2,242
" 15	14,640	69.0	3.7	21.3	4.3	1.3	0.3	3,118
" 16	18,400	80.3	2.0	14.7	1.3	1.3	0.3	2,704

At this time an anatomical pathway capable of affording ingress of cells of the thoracic duct lymph into the systemic blood stream was demonstrated as a lymphatovenous communication between the thoracic duct and the ninth intercostal vein. The experiment was free from complications; absence of infection served to render the blood counts dependable.



TEXT-FIG. 2. Curve to show the absolute number of small lymphocytes per cubic millimeter of blood in Cat 2 in which the thoracic duct was ligated at X. On the 21st day the number of small lymphocytes had reached its preligation level.

A somewhat similar condition was shown by Cat 2, which weighed 3,030 gm. before operation, and 3,000 gm. when sacrificed. In this case, which likewise was free from concurrent infection, the lymphato-venous connection was between the thoracic duct and the azygos

TABLE III.

Cat 3.

Date.	Total white blood count.	Poly-morpho-nuclear neutrophils.	Eosino-phils.	Small lympho-cytes.	Large lympho-cytes.	Large mono-nu-clears.	Transi-tionals.	Absolute No. of small lympho-cytes.
<i>1921</i>		<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	
Apr. 25	15,920	78.0	3.3	12.7	2.7	1.3	2.0	2,021
" 26, a.m.	14,840	71.0	6.0	18.3	3.0	1.0	0.7	2,715

Operation.

Apr. 26, p.m.	18,040	88.0	0.3	7.7	1.3	2.0	0.7	1,389
" 27, a.m.	15,680	71.3	3.7	20.7	2.3	1.3	0.7	3,245
" 28	14,120	70.0	2.0	20.0	5.3	1.7	1.0	2,824
" 29	15,920	73.7	2.3	21.0	1.3	0.7	1.0	3,343
" 30	12,320	59.3	3.3	34.0	1.7	0.7	1.0	4,188
May 2	12,960	63.3	1.7	32.7	1.3	0.7	0.3	4,237

Cat 4.

Apr. 25	8,480	54.7	3.0	38.3	2.0	1.3	0.7	3,247
" 26, a.m.	8,360	61.0	3.0	32.7	2.0	1.0	0.3	2,733

Operation.

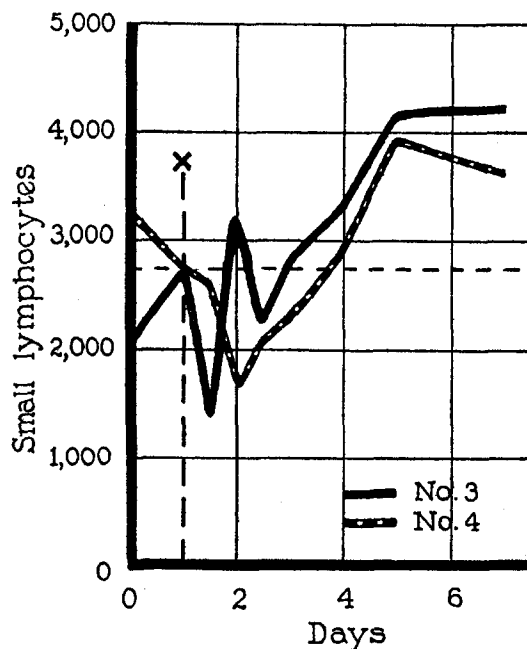
Apr. 26, p.m.	17,640	79.7	0.7	14.7	3.0	1.3	0.7	2,592
" 27, a.m.	9,680	77.3	1.7	16.7	1.7	2.0	0.7	1,616
" 27, p.m.	9,840	75.7	1.3	21.0	1.0	0.7	0.3	2,066
" 28	7,560	61.7	5.0	30.0	1.7	1.3	0.3	2,268
" 29	9,200	58.3	7.3	32.0	1.3	0.7	0.3	2,944
" 30	11,920	57.3	7.0	33.3	1.0	1.0	0.3	3,969
May 2	11,280	58.3	5.0	32.0	1.7	1.7	1.3	3,609

vein at the ninth interspace. The number of lymphocytes in the circulating blood also decreased by 56 per cent immediately following ligation, but gradually returned to the preligation level on the 19th day. Table II and Text-fig. 2 show the counts in this animal.

In order to check up the findings reported above, two control experiments were made. These animals were treated identically like

the ones above mentioned, with the exception that instead of performing the actual ligation in the chest, the operative procedure was limited to a slight dissection of the anterior periaortic tissue. The animals made a good recovery, and their blood counts are listed in Table III, and demonstrated in Text-fig. 3.

From the data herewith submitted it is seen that the intrathoracic ligation of the thoracic duct caused an immediate fall of about 56



TEXT-FIG. 3. Curve to show the absolute number of small lymphocytes per cubic millimeter of blood in Cats 3 and 4 which were used as controls. On the 4th day the number of small lymphocytes had already reached its preoperative level.

per cent in the number of small lymphocytes in the circulating blood, and that the number gradually returned to the preoperative level about 3 weeks after ligation (Text-figs. 1 and 2). In the one case the lymphatovenous connection was with the ninth left intercostal vein; and in the other the connection was with the azygos vein directly. It seems then that at the end of 21 days these lymphatovenous connec-

tions were of sufficient size to perform the functions previously exercised by the thoracic duct. Whether one can use the number of small lymphocytes in the blood as an absolute criterion for the degree of establishment of the collateral circulation is still questionable; nevertheless, the counting of the small lymphocytes is one of the more obvious and accessible methods for attacking this problem.

It will be noted that the decrease in the number of lymphocytes in the blood as shown by Tables I and II was invariably around 56 per cent; in the entire series this decrease has been between 54 and 64 per cent. This figure is somewhat lower than that of the only experiment in the series in which the ligation of both thoracic ducts alone was performed (No. 10, Biedl and von Decastello) in which a decrease of 79 per cent was obtained. In this case the thoracic duct on the left side, as well as all visible lymphatic vessels in the right side of the neck, was ligated. It is obvious that such a procedure shut off more lymphatic vessels than a simple ligation of the thoracic duct in the chest, and consequently one would expect a greater decrease in the number of lymphocytes in the circulating blood. For if the thoracic duct is obstructed in the chest, then the lymph of the upper extremities, head, neck, and upper part of the thorax, may still enter the blood stream through normal channels and thus furnish a considerable number of lymphocytes to the blood stream.

The control experiments showed that although there was also a decrease in the number of small lymphocytes when the duct had not been tied, yet this decrease was only temporary, for the preoperative level was exceeded on the 2nd or 3rd day (Text-fig. 3). Other cats which simply received an ether anesthetic for a half hour showed a temporary fall in the number of small lymphocytes.

DISCUSSION.

Many investigators have studied the thoracic duct with respect to the number and kind of cells for which it forms the pathway to the blood stream. Winternitz in 1895 concluded that the interruption of the lymph flow into the blood *via* the thoracic duct does not prevent leucocytosis. In 1901, Biedl and von Decastello described changes in the blood picture of dogs in which either a fistula of the duct was made with or without splenectomy, or in which the duct was tied under aseptic technique. They found that the mononuclears, including the small

lymphocytes, decreased in the blood from 62 to 79 per cent in absolute numbers. They recorded only two experiments, Nos. 9 and 10, in which they ligated the duct and studied the blood. In Experiment 9 ligation of the duct produced practically no change in number of mononuclears in the blood; this condition was accounted for by the demonstration of a branch of the thoracic duct below the point of ligation which connected with veins on the right side of the neck. In the other experiment, No. 10, the thoracic duct as well as all lymphatics on both sides of the neck were ligated, and it was found that the number of mononuclears in the blood diminished 79 per cent on the day following ligation, but on the 4th day after ligation the decrease was only 29 per cent. Blood counts were made until 1 week after operation. No attempt was made to determine whether the mononuclear cells in the blood would come back to the preligation level. Furthermore, the animal in Experiment 10 had an infection of the operative wounds; though the infection cleared up, nevertheless the inflammatory process undoubtedly brought in an unwelcomed factor which must have influenced the blood examination. The authors concluded that ligation of the thoracic duct or production of a fistula of the same did not entirely prevent the entrance of mononuclears into the blood stream; nor did injections of a colored solution serve to demonstrate connections between the lymph and blood systems. However, more recent work has shown that collateral paths may become established after the thoracic duct has been ligated.

In 1904 Selinoff reported experiments on dogs in which he cannulated the thoracic duct under aseptic precautions and studied the resultant change in the number of cells in the blood stream. He divided the white cells into three groups, young, adult, and old. This division corresponds essentially to lymphocytes, monocytes, and polymorphonuclears, respectively. In one experiment, No. 18, the lymphocytes dropped from 1,600 before operation to 20 on the 14th day after operation. However, the remarkable decrease in lymphocytes was attended also by a decided increase in the number of polymorphonuclears; thus the animal in the experiment cited above had on the 14th day polymorphonuclears present in the blood to the extent of 99.7 per cent. Not only did this experiment show a high polymorphonuclear content, but other experiments which were extended only to the 5th day after operation, such as No. 12, showed polymorphonuclears in the blood to the extent of 98.0 per cent. Obviously infection was present in all of the operative wounds of these experimental animals because of the thoracic duct fistula. It would seem then that in these cases the infection was characterized not only by a great increase in the number of polymorphonuclear leucocytes but also by an astonishing decrease in the number of lymphocytes in the circulating blood.

Also in 1904 Banti, in reporting the work done in his laboratory by Crescenzi, stated that after splenectomy and subsequent establishment of a thoracic duct fistula in dogs the lymphocytes of the blood decreased from one-half to ten-elevenths, but returned to normal or above normal at the end of 4 days, at which

time, it was claimed, there did not exist any accessory lymphatic channels between the thoracic duct and the subclavian vein. He was at a loss to account for the manner in which the lymphocytes reached the blood stream and considered as a probability the entrance of lymphocytes directly into the blood stream while still in lymphatic organs.

In 1908 Rous, using an improved technique, in the collection of samples from thoracic duct fistulæ showed that in dogs the thoracic duct furnished the circulating blood more lymphocytes than had been usually supposed. Also, he stated that muscular activity and certain lymphagogues increased the lymphocyte output.

Davis and Carlson in 1909, in studying the leucocytes in the neck lymph, thoracic lymph, and blood of normal dogs, also noticed a decrease in the number of the mononuclear elements of the blood following ligation of the thoracic duct and neck lymphatics. Unfortunately the observations were carried out for only 48 hours following operation and the subsequent course of the lymphocyte content of the blood was not followed; likewise it is uncertain to what extent the operation did prevent the entrance of lymph into the blood stream, since the lymphatics were not subsequently injected.

Bunting and Huston were also interested in the fate of the lymphocyte,— a question which Davis and Carlson had previously considered. Bunting and Huston splenectomized rabbits and found that the number of lymphocytes in the circulating blood increased. About a week following splenectomy the left thoracic duct as well as the neck lymphatics on both sides were ligated, and it was found that the number of lymphocytes in the circulating blood decreased. They also observed lymphatics going from the left thoracic duct through the thymus to the right thoracic duct, and concluded that the lymphocyte count was restored through such channels; however, they did not keep their animals long enough to note the return of the lymphocytes to their preoperative level. The authors advanced the hypothesis that the lymphocytes may migrate for instance into the mucous membrane of the intestine and from there into the lumen of the intestine, since more lymphocytes enter the blood stream *via* the thoracic duct in 24 hours than are present in the blood at any one time.

From the above discussion it will be seen that although all the investigators are impressed with the importance of the thoracic duct as an avenue for the supply of lymphocytes to the circulating blood, yet no one has made a consistent attempt to analyze the changes that take place in the animal economy following ligation of the thoracic duct in order that the normal number of lymphocytes be again restored to the blood stream; nor has any one determined the approximate time following ligation of the duct at which a collateral circulation of the thoracic duct has mediated in the establishment of the normal preligation supply of lymphocytes to the blood stream.

Whether lymphocytes may enter the blood stream directly at their site of formation, as in the lymph glands and spleen, is another factor that enters into the problem. According to von Schumacher, who has studied the lymph glands in this respect, the blood in the vein leaving a lymph gland has more leucocytes than that in a neighboring artery. Unfortunately he did not study the blood in the artery going to the lymph gland in question; also, no figures were given to indicate the extent of the difference in the number of leucocytes in the afferent and efferent blood vessels.

Besides the definite decrease in the number of lymphocytes following ligation there were striking changes in other cell groups. The polymorphonuclear neutrophils invariably increased greatly in number immediately after the operation, but then gradually returned to a normal level. Contrariwise, the eosinophils decreased in number following operation but soon returned to their preligation level. The number of mononuclears and transitionals did not seem to have been influenced much by the operation.

The hematology of the laboratory animals does not seem to have been studied very extensively. Burnett, Busch and Van Bergen, Goodall, and Klieneberger and Carl all give figures for the differential counts of cat blood. Oddly enough the normal differential counts from Cat 1 corresponded to those of Goodall, while the counts from Cat 2 resembled those of Klieneberger and Carl. The latter were unable to find any mast cells in the blood.

SUMMARY.

This report has attempted to analyze the changes in the absolute number of small lymphocytes in the blood stream of the cat following the intrathoracic ligation of the thoracic duct. Such a ligation produced an immediate decrease in the number of small lymphocytes to the extent of 56 per cent, but it was found that the preoperative level was again reached at about the end of 3 weeks. One is led to believe that the gradual return of the number of small lymphocytes to the preligation level took place *pari passu* with the establishment of the collateral circulation of the thoracic duct, although there is no absolute proof of this. Yet it is definite that the thoracic duct is an important avenue for the entrance of small lymphocytes into the blood

stream, and that it is the pathway through which at least half of the small lymphocytes reach the circulating blood in the cat.

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