

SOME DIFFERENTIAL COUNTS OF THE CELLS IN
THE LYMPH OF THE DOG: THEIR BEARING
ON PROBLEMS IN HÆMATOLOGY.¹

By F. PEYTON ROUS, M.D.

*Instructor in Pathology, the University of Michigan.
(From the Pathological Laboratory of the University of Michigan.)*

Our knowledge of the cell-formula of the lymph is nearly comprised in the statement that small mononuclear elements make it up almost entirely. No systematic differential counts of the lymph-cells in man or in other animals are on record. According to the most recent work, that of Weidenreich (1), whose paper is as yet only reported in brief, one finds in the thoracic duct of the rabbit, the dog, the cat, the guinea-pig and the monkey, non-granular cells in large number, "especially little lymphocytes, but next to these large leucocytes with round nuclei, in which last all stages of mitosis are met. . . . Finely granular leucocytes (neutrophile or amphophile) are few, as are eosinophile leucocytes."

As an aside to another investigation I have had opportunity to observe cells from the lymph of a number of healthy dogs. Differential counts of these have disclosed facts which seem worthy of report.

There exists a good general description of the elements found in the lymph of the dog—that of Biedl and v. Decastello (2). They noted in the fluid all the cells of the animal's blood except the mast-cell. Polymorphonuclear neutrophiles were present only as evidence of blood-contamination. Mitoses in the mononuclear leucocytes were rare. Once they found a large number of eosinophile cells independent of blood-admixture. These authors give no differential counts of the leucocytes. Delamere (3) mentions that out of 133 successive cells he noted 128 lymphocytes, three polymorphonuclear neutrophiles, and one eosinophile.

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Lymph for the counts here recorded was obtained by operation on animals anæsthetized with morphine and chloroform. A fine, glass cannula was inserted in the thoracic duct near its mouth, and cover-glass preparations immediately taken, to be colored later with Wright's stain. The counts, which for the most part total five hundred cells—never less than three hundred—were in each case derived from observations on several slips, made from different drops of lymph collected at short intervals. In this way an attempt was made to obtain the average cell-formula, but the individual spreads from the same animal proved to differ little in this regard.

The results are best taken up in the consideration separately of Tallqvist and Willebrand (4), Dawson (5), Busch and v. Bergen (6), render a description of most of these unnecessary. Appended (6) render a description of most of these unnecessary. Appended are complete counts from the lymph of twenty-three dogs.

1. *Polymorphonuclear Neutrophiles*.—These were observed only in the presence of many red corpuscles, and then, with one exception, in such small quantity as the presence of blood in the lymph would quite account for. A little blood in the dog's lymph is practically the rule, as many previous observers have noted. Often its source is discovered in an anastomosis of the thoracic duct with small veins in the superior mediastinum, but occasionally it gains entrance further back in the lymph-system. The white cells introduced into the lymph in this way are few. That the polymorphonuclear neutrophiles in the normal lymph are due wholly to contamination was several times shown with some exactness by quantitative counts of the red and white cells of the lymph and of the blood, conducted with another end in view (Rous (7)). In these cases the ratio between polymorphonuclear neutrophiles and red cells in the lymph coincided with that found in the blood.

In one instance, as noted above (Dog H1), polymorphonuclear neutrophiles were found in an abundance that admixture of blood could not account for—11.5 per cent. of the 4,500 white cells per cubic millimeter of lymph. At autopsy of this animal its organs seemed normal macroscopically. The instance remains unexplained.

2. *Eosinophiles*.—These striking cells were almost always found

in the lymph, and often in considerable numbers,—12 per cent. of the total white cells in one instance. They average 2.6 per cent. Their independence of blood-admixture is shown by their relative abundance when compared with the polymorphonuclear neutrophils. The finding has a special interest because of the well-known general relationship between the eosinophile and conditions of nutrition, and between the eosinophile and animal parasites. Accordingly steps were taken to find whether an eosinophilia of the lymph depends on these factors.

Heidenhain (8) first pointed out that the intestinal mucosa of the well-fed dog is thronged with eosinophiles. They are most abundant when the animal is on a mixed diet, and become fewer if meat is withdrawn from the food, or if there is overfeeding with it. Starvation causes a profound diminution in the cells. These observations have been confirmed on other animals. Opie (9) has recently shown that when food is withheld from the guinea-pig the number of eosinophiles in its blood, after a transient increase, falls off rapidly.

In the present instance the time that elapsed between the animal's last meal and the operation at which lymph-smears were obtained was purposely varied much. From the diet of half of the

TABLE I.
Series A.—Much Meat in Diet.

Dog.	Hours Since Last Meal.	Condition of Lymph.	Per Cent. of Eosinophiles in Lymph.	Parasites in Intestine.
Cl	5	Chylous.	11.6	No autopsy.
Hl	27	Thinly chylous.	2.4	One specimen of <i>Toxacara canis</i> .
Il	27	Slightly chylous.	7.8	<i>Dipylidium caninum</i> 0.5 gram.
Bl	27	Opalescent.	2.3	No autopsy.
Gl	27	Opalescent.	2.5	One specimen of <i>Toxacara canis</i> . <i>Dipylidium caninum</i> , 0.5 gram.
Ml	27	Opalescent.	6.9	<i>Tenia pisiformis</i> 1.7 grams.
Dl	30	Opalescent.	1.0	No autopsy.
Ll	52 ²	Quite chylous.	2.9	<i>Dipylidium caninum</i> 0.5 gram.
Nl	52	Opalescent.	0.4	<i>Dipylidium caninum</i> 0.8 gram.
Kl	52	Nearly clear.	1.0	No parasites.
Jl	52	Nearly clear.	1.1	<i>Toxacara canis</i> 1.8 grams.

² Autopsy shows recent feeding. Stomach and small intestine contain much food.

Series B.—Meat Almost Wholly Excluded from Diet.

Dog.	Hours Since Last Meal.	Condition of Lymph.	Per Cent. of Eosinophiles in Lymph.	Parasites in Intestine.
Bt	2	Thinly chylous.	0.0	Two specimens of <i>Toxacara canis</i> .
C ₂ l	3	Thinly chylous.	2.8	<i>Dipylidium caninum</i> 0.2 gram.
Ft	3½	Chylous.	2.3	<i>Tenia pisiformis</i> 17 grams.
Rl	4	Chylous.	3.3	No parasites.
Ql	20	Clear.	1.3	<i>Dipylidium caninum</i> 1.0 gram.
Sl	27	Opalescent.	0.0	<i>Toxacara canis</i> 3.0 grams.
Yl	27	Opalescent.	0.0	<i>Tenia pisiformis</i> 1.9 grams.
Tl	27	Slightly opalescent.	1.2	<i>Tenia pisiformis</i> 16.0 grams.
Zl	27	Clear.	0.6	<i>Tenia pisiformis</i> 9.4 grams.
Xl	52	Slightly opalescent.	1.1	<i>Tenia pisiformis</i> 11.5 grams.
Pl	52	Clear.	1.6	No parasites.

dogs meat was almost wholly excluded, carbohydrates being substituted. The results are made plain by the following table.

These figures show that a mixed diet rich in meat considerably favors the occurrence of eosinophiles in the lymph, as compared with one of which meat forms a slight part. Furthermore, when all food is withheld the number of eosinophiles in the lymph becomes much fewer. Series A. demonstrates this especially well.

To determine the influence of animal-parasites a record was kept of those that autopsy of the dogs disclosed. Only such as were prominent macroscopically were noted. These were washed, fixed in formalin, and then weighed. The table gives the data resulting. No clear relationship between the presence of parasites and the number of eosinophiles of the lymph is to be seen.

One may well ask, whence come these eosinophiles? Are they the result of a local formation in lymph-glands and intestinal mucosa, or do they represent a migration, more or less indirect, from the blood-stream? Biedl and v. Decastello concluded that the eosinophiles formed in the lymph-glands of the dog must enter the blood directly, since only once did they come upon these cells in the lymph. But the present findings destroy the ground for that conclusion. Simon (10) has noted a few eosinophile myelocytes in the intestinal mucosa of the dog, though not sufficient to account for its content in polymorphonuclear eosinophiles. It is conceded that these, in the main, gather there from the blood. The fluctuation in the lymph's content of eosinophiles agrees remarkably with

that observed by others in the intestinal mucosa, and the fact suggests that this mucosa may be the proximal source of the eosinophiles of the lymph.

3. *Mast-cells*.—These were not seen in the lymph.

4. *Lymphocytes*.—In the study of the dog's blood several classes of these may be separated with reference to staining properties of nucleus and cytoplasm, relative proportion of the two, and size of the cell as a whole (Dawson). But in dealing with the lymph it was soon found a sufficient task to make one group of these cells as distinct from the large mononuclears. Botkin long ago called attention to the effect of pressure as determining the appearance of lymphocytes in spreads, and he held this responsible for much of the individual variation seen. The factor comes into special play in preparations made from the lymph, because this fluid lacks the "body" necessary to the production of uniform films. Frequently cells at the periphery of a lymph spread average twice the size of those at the center.

As a standard to separate large mononuclears from small the size of the polymorphonuclear neutrophile in the same specimen of lymph, or that failing, in the animal's blood, was adopted. A mononuclear element of which the nucleus was smaller than an entire polymorphonuclear neutrophile was classed as a lymphocyte, no matter what its amount of cytoplasm. Such elements constituted from 69.8 to 96.8 per cent. of the total white cells, with an average of 87.6 per cent.

5. *Large Mononuclear Leucocytes*.—Mononuclear elements with a round or oval nucleus larger than that just defined for the lymphocyte were classed as "large mononuclears." Occasional mitoses were observed in them. From 1.8 to 18 per cent. of such cells were found in the lymph, with an average of 5.1 per cent. The relation of these to the like elements in the blood is discussed further on.

6. *Transitional Leucocytes*.—The characteristic transitional cell of the dog's blood is slightly larger than the polymorphonuclear neutrophile. The nucleus is indented, often bilobed or deeply cleft into two or three irregular, blunt branches. With Wright's stain the cytoplasm is a distinct blue, usually of light tint, as compared

with the pink, more or less granular, cytoplasm of the polymorphonuclear neutrophile; and the nucleus is a medium dark blue in contrast with the dense, purplish blue of that of the latter cell. The most complex nucleus of a transitional is thick and clumsy as compared with that of a neutrophile, and the chromatin is much less compact. The cytoplasm occasionally shows a few neutrophile granules. All gradations between this cell and the typical large mononuclear exist, but, as has been brought out, it can always be sharply distinguished from the polymorphonuclear neutrophile.

In my preliminary observations on the lymph transitional leucocytes were but seldom noted, indeed much less frequently than polymorphonuclear neutrophiles. To test this finding, in the later counts all mononuclear elements with indented nucleus (even cells in evident process of division, and those with reniform nucleus) were put under the head of "transitionals." Yet despite this latitude the group remained a small one. In one case (Dog P1) 1.6 per cent. of these cells were present; but altogether, out of 10,511 leucocytes counted, only forty-one were "transitionals." At least half of these forty-one were mononuclear elements in process of division. In the lymph of six of the twenty-three animals "transitionals" were not met with during an average count of five hundred cells.

This is in marked contrast to the frequency of transitional leucocytes in the dog's blood. For the purposes of such contrast special differential counts were made of smears taken at time of operation from the animals yielding the lymph. The counts of the authors already quoted could not be utilized, since in these the transitional and large mononuclear leucocytes are lumped under one head. In the special counts (which are appended in full) cells with a merely reniform nucleus are termed "large mononuclears," and only those with a nucleus more deeply indented are accepted as transitional leucocytes. Thus the class of transitional forms is made radically narrower for the blood than for the lymph. Yet in the blood these cells occur in the proportion of 1 to every 3.1 lymphocytes, whereas in the lymph there is only 1 to every 224 lymphocytes. The table shows this fact.

TABLE II.
Transitional Leucocytes and Lymphocytes.

Dog.	Lymph.			Blood.		
	Transitionals.	Lymphocytes.	Total Count.	Transitionals.	Lymphocytes.	Total Count.
Bl	4	452	526			
Cl	3	477	684	11	78	512
Dl	0	445	502			
Fl	1	409	500	10	47	315
Gl	0	504	550	25	16	311
Hl	0	405	545	21	45	317
Il	0	431	510	15	72	344
Jl	5	332	363	27	51	320
Kl	1	284	303	14	41	306
Ll	1	478	519	21	106	305
Ml	2	457	536	14	69	310
Nl	0	467	492	12	64	400
Pl	5	257	307	9	80	400
Ql	1	261	300			
Rl	1	442	509			
Sl	2	484	500	41	36	500
Tl	1	370	402	19	36	400
Xl	4	358	402	15	60	320
Yl	2	371	400	13	32	306
Zl	3	461	500			
Bt	3	314	335			
C ₂ l	0	461	526			
Ft	2	226	300			
	41	9,206	10,511	267	833	5,366

Plainly the transitional leucocytes of the blood cannot be furnished to it as such through the thoracic duct. True, it is possible to suppose that the production of transitional forms ceases immediately that the animal is operated upon; hence the scarcity of these elements in the lymph. But the other cells are not so affected, and we have no reason to believe that this occurs. The conclusion seems justified that the transitional leucocytes of the blood do not come to it as such in the lymph. They either develop in the blood from other lymph elements or are derived from another source.

Furthermore, if the cell-character of the lymph represent the general type of contribution made by all lymphoid tissue to the blood, then one is forced to conclude that lymphoid tissue either furnishes transitional leucocytes to the blood under the guise of some other element, or else has no hand in their production.

Are similar conclusions for the large mononuclear leucocyte justified? The counts show the existence of from 1.8 to 18 per

cent. of these cells, with an average of 5.2 per cent. in the lymph of the thoracic duct; and these are, of course, added as such to the blood. Nevertheless, a comparison of the ratios between the large mononuclear leucocytes and lymphocytes in the blood and in the lymph, respectively, gives results similar to, though not so striking as, those for the transitional leucocyte. For this comparison it is necessary to add to the number of "large mononuclears" of the lymph that of the few transitional leucocytes found, because cells with reniform nucleus found in this fluid were classed as "transitionals," whereas in the differential counts of the blood they were called "large mononuclears." Yet, despite this inflation of the number of "large mononuclears" of the lymph through the real transitional leucocytes introduced under this head, the ratio of large mononuclear leucocytes to lymphocytes in the lymph is 1 to 17, as compared with a 1 to 4.4 ratio in the blood.

TABLE III.
Large Mononuclear Leucocytes and Lymphocytes.

Dog.	Lymph			Blood.		
	Large Mononuclears.	Lymphocytes.	Total Count.	Large Mononuclears.	Lymphocytes.	Total Count.
Cl	38	452	526			
Bl	85	477	684	19	78	512
Dl	45	445	502			
El	17	469	500	9	47	315
Gl	20	504	550	3	16	311
Hl	10	405	545	7	45	317
Il	16	431	510	6	72	344
Jl	18	332	363	16	51	320
Kl	0	284	303	15	41	306
Ll	14	478	519	15	106	305
Ml	20	457	536	9	69	310
Nl	19	467	492	20	64	400
Pl	27	257	307	18	80	400
Ql	13	261	300			
Rl	11	442	509			
Sl	14	484	500	13	36	500
Tl	12	370	402	7	36	400
Xl	37	358	402	20	60	320
Yl	19	371	400	13	32	306
Zl	24	461	500			
Bt	8	314	335			
C ₁	17	461	526			
Ft	54	226	300			
	544	9,206	10,511	190	833	5,366

Despite these figures it is not necessary to suppose, as for the transitional leucocytes, that large mononuclear forms develop in the blood from other lymph elements, or else have a source outside the lymph. For, in the first place, the lymph certainly does add to the blood a considerable quantity of large mononuclear cells as such; and, second, a relative accumulation of large mononuclear cells might occur through their persistence in the blood for a longer period than the lymphocytes. Third, in the counts on which this discussion is based, the separation of the large from the small mononuclear elements was accomplished through their difference in size alone—a criterion which permits of large error from the subjective side. The separation of the transitional leucocytes rested on peculiarities of morphology and of tint which could not be overlooked.

7. *Unclassified.*—A considerable number of elements were put under this head. These were for the most part cells distorted

LYMPH COUNTS.

Dog.	Lymphocytes.	Large Mononuclears.	Transitionals.	P.4ymorphonuclear Neutrophiles.	Eosinophiles.	Unclassified.	Total Counted.	Remarks.
Bl	452	38	4	0	12	20	526	
Cl	477	85	3	4	79	34	684	
Dl	445	45	0	0	5	7	502	
El	469	17	1	1	0	12	500	
Gl	504	20	0	2	14	10	550	
Hl	405	10	0	63(!)	13	54	545	Only slight blood admixture.
Il	431	16	0	7	39	17	510	
Jl	332	18	5	3	4	3	363	
Kl	284	6	1	0	3	9	303	
Ll	478	14	1	1	15	10	519	
Ml	457	20	2	12	37	8	533	Considerable blood admixture.
Nl	467	19	0	4	2	0	492	
Pl	257	27	5	8	5	5	307	
Ql	261	13	1	4	4	17	300	
Rl	442	11	1	6	17	32	509	
Sl	484	14	2	0	0	0	500	
Tl	370	12	1	0	5	14	402	
Xl	358	37	4	0	3	0	402	
Yl	371	19	2	3	0	5	400	
Zl	461	24	3	3	0	9	500	
Bt	314	8	3	1	0	9	335	
C,l	461	17	0	1	14	33	526	
Ft	226	54	2	3	7	8	300	
	9,206	544	41	126	278	316	10,511	
	87.6%	5.2%	0.39%	1.2%	2.6%	3.0%		

while the smears were being made, owing to the lymph's lack of "body." A few typical "basket cells" were observed.

Résumé.—Eosinophile cells are a frequent constituent of the lymph of the normal dog. Sometimes they are quite abundant: in one case noted they formed 12 per cent. of the total white cells and they averaged 2.6 per cent. When food is withheld from the animal their number becomes much fewer. The kind of food on which the dog has subsisted also exerts an influence, a mixed diet largely of meat favoring eosinophiles, as compared with one mostly of carbohydrates. The finding parallels so nearly that of Heidenhain in his study of the eosinophiles of the intestinal mucosa as to suggest this mucosa as the proximal source of the lymph's content of these cells. A systematic record of the animal parasites in the

BLOOD COUNTS.

Dog.	Polymorpho-nuclear Neutrophiles.	Eosinophiles.	Lymphocytes.	Large Mono-nuclears.	Transitionals.	Unclassified.	Total.	Remarks.
CI ³	350	44	78	19	11	10	512	
EI ³	216	22	47	9	10	11	315	Two normoblasts seen during count.
GI ³	248	15	16	3	25	4	311	
HI ³	205	32	45	7	21	7	317	
II ³	207	39	72	6	15	5	344	Two mast cells and normoblasts seen during count.
JI ³	203	19	51	16	27	4	320	Three normoblasts seen during count.
KI ⁴	216	16	41	15	14	4	306	
LI ⁴	141	15	106	15	21	7	305	Two mast cells and nine normoblasts seen during count.
MI ⁴	195	19	69	9	14	4	310	One normoblast seen during count.
NI ⁴	271	21	64	20	12	12	400	
PI ⁴	274	10	80	18	9	9	400	
SI ⁴	360	38	36	13	41	12	500	
TI ⁴	289	48	36	7	19	1	400	
XI ⁴	202	14	60	20	15	9	320	
YI ⁴	233	11	32	13	13	4	306	

³ Smears made just after incision.

⁴ Smears made just before operation.

dogs used did not bring out any definite relation between these and the eosinophiles of the lymph.

Mast-cells are not a constituent of the lymph of the normal dog. Polymorphonuclear neutrophiles are only present as a result of blood admixture. Lymphocytes, by which are meant non-granular cells with a round or oval nucleus smaller than an entire polymorphonuclear neutrophile from the same animal, form an average of 87.6 per cent. of the cells.

Typical transitional cells are rare in the lymph. Evidently those of the blood must either develop in it from other lymph-elements or must come from another source than the lymph, and thus from another source than adenoid tissue in general.

Large mononuclear cells—non-granular mononuclear elements larger than the lymphocyte as above defined—average 5.2 per cent. of the lymph's leucocytes.

Owing to the lymph's lack of "body" smears made from it show many distorted cells. Thus the class of "unclassified" elements is rendered comparatively large.

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