FATE OF THE LYMPHOCYTE.

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The normal function of the lymphocyte, as a cell, and the fate of the lymphocytes that enter the blood circulation are questions which apparently have not been solved. It seems to be generally accepted that lymphocytes are produced in the lymphoid tissue of the body and only to a minor extent, if at all, in the bone marrow. From the lymphoid tissue they gain entrance to the efferent lymphatics, and are, in the words of Davis and Carlson,[1] "as much a part of the lymph as the erythrocytes and leucocytes are of the blood." Eventually the lymphocytes, which have entered the lymph stream, in part at least, reach the blood circulation through the major lymph trunks, and chiefly through the thoracic duct. The content of the thoracic duct lymph is high in lymphocytes. Thus, in eleven dogs studied by Rous[2] the leucocytes per c. mm. varied from 2,400 to 13,120. Of these cells, the majority was lymphocytes. Davis and Carlson[1] found the number in dogs to vary from 1,000 to 30,000 leucocytes per c. mm., of which from 95 to 100 per cent were lymphocytes.

While it is not certain that the rate of flow of lymph from the thoracic duct through a cannula represents the normal flow into the jugular vein, especially since the animal is anesthetized,[3] yet, when such flow remains fairly constant over a period of hours, one must assume that it approximates the normal flow. If, with this point in mind, we calculate, from fourteen dogs in Rous'[2] series on a basis of the lymphocyte content of the thoracic duct per cubic millimeter and the rate of flow during fixed intervals of his experiments, we find a surprisingly large number, 3,300,000,000 cells, as the total number that

[1] Davis, B. F., and Carlson, A. J., *Am. J. Physiol.*, 1909–10, xxv, 173.

[2] Rous, P., *J. Exp. Med.*, 1908, x, 238, 329, 537.

[3] All operations were performed under ether anesthesia.

would enter the blood stream during 24 hours. This is a larger number than that of lymphocytes present in the blood stream at any one time. Davis and Carlson agree with this estimate in their statement that "many more lymphocytes enter the blood with the lymph in the course of 24 hours than can be found in the blood at any given time."

Given these findings, then, of the entrance of an enormous number of lymphocytes into the blood each day, and the further findings of a relatively constant count of such cells in the circulating blood, a problem of great interest is presented as to the fate of these cells. As many cells must disappear from the circulation each day as are introduced into it, or we would find not a constant count but an ever increasing accumulation of these cells in the blood stream. It is with this problem that the present paper is concerned. There would seem at the outset only three possibilities as to the fate of the lymphocyte: (1) It might be changed in development into some other type of leucocyte. (2) It might disintegrate in the blood stream. (3) It might leave the blood stream. Since the first possibility seems remote, as a result of modern work on hematology, we think that it may be dismissed from consideration and have therefore confined our work to the two other possibilities.

EXPERIMENTAL.

Our work has been done on rabbits, and although chiefly the fate of the lymphocyte has been studied, a few observations have been made on these cells in the thoracic duct and on the rate of flow. Direct counts, obtained not on freely flowing fluid, but from fluid from the duct immediately after the death of the animal, showed from 20,000 to 50,000 cells per c. mm. in four animals, with a proportion of 80 per cent of small lymphocytes to 20 per cent of larger lymphoid cells in counts of 1,000 cells Granular polymorphonuclear cells and red cells do not appear as normal constituents of the thoracic duct lymph of the rabbit. Agglutinated masses of poorly staining lymphocytes, apparently disintegrating, are quite numerous in stained films of duct lymph. The possible significance of these will be referred to later. We have, in one of two attempts, succeeded in catheterizing the thoracic duct in the rabbit and we obtained lymph flow at the rate of 1 cc. in 10 minutes, in an etherized animal. This does not

necessarily represent the normal rate of flow. The animal is in an abnormal position; the rate and depth of its respiration are abnormal and seem to determine the progress of the column of fluid through the cannula. As the cannula must be of almost capillary size, capillary attraction must be a factor in rate of delivery; on the other hand, the viscosity of the lymph prevents free dropping from the end of the cannula. Withal, it may be assumed that a very considerable number (a billion or more) of lymphocytes enter the blood from the thoracic duct in the course of a day.

Determination of the Rapidity of the Disappearance of Lymphocytes from the Blood.

In a series of experiments on the rabbit, we attempted to cut off the inflow of lymphocytes into the circulation and thus to determine the rapidity of their disappearance from the blood. As a primary procedure, animals were splenectomized under ether anesthesia to exclude the direct entrance into the blood of lymphocytes from the Malpighian corpuscles. Then, after a sufficient interval for recovery and for return of the leucocyte formula to approximately normal, the thoracic duct was occluded by a ligature which included both the thoracic duct and the jugular vein just below the entrance of the former into the jugular. In later experiments the neck lymphatic trunks on the right and left sides were also tied. Since the results of these experiments were uniform, as far as the trend of the change in the lymphocyte count is concerned, although there was a variation in degree, it does not seem necessary to report the series in detail, especially as the procedure is not new and the results do not differ from those of others. The two protocols below illustrate the results.

Rabbit 1.—Male; weight 1,500 gm. December 1, 1920. Spleen removed under ether and with aseptic precautions. Recovery, apparently without complications. December 9. With careful surgical technique one ligature was placed around thoracic duct and left jugular, and a second about the main right cervical lymph trunk. Blood counts, made from the right ear with free flow of blood, are given in Table I.

In this animal there is a drop in lymphocytes of 1,826 per c. mm. in 5 hours and 1,955 in 10 hours after partially cutting off the supply,

with a gradual return toward the normal number. On the basis of 75 cc. (5 per cent of body weight) of blood this primary drop represents the disappearance from the blood stream of 136,950,000 lymphocytes in 5 hours. The simultaneous drop in polymorphonuclears is definitely due to migration into the operative field.

TABLE I.

Time.	Total No. of leucocytes.	Lymphocytes.		Neutrophils.		Basophils.		Large mononuclears.	
		Per cent.	No.	Per cent.	No.	Per cent.	No.	Per cent.	No.
Before splenectomy.....	8,000	28	2,240	61	4,880	6	480	5	400
" tying thoracic duct.	8,500	34	2,890	60.5	5,142	2.5	212	3	255
5 hrs. later.....	5,600	19	1,064	70	3,920	3	168	8	448
10 " "	5,500	17	935	66	3,630	4	220	13	715
24 " "	8,000	24	1,920	58	4,640	5	400	13	1,140
2 days "	7,600	23	1,748	59	4,484	4	304	14	1,064
3 " "	6,900	22	1,516	61	4,209	4	276	13	897
4 " "	8,300	22	1,826	60	4,980	5	415	13	1,079
6 " "	8,100	25	2,116	58	4,698	4	324	12	972

TABLE II.

Time.	Total No. of leucocytes.	Lymphocytes.		Neutrophils.		Basophils.		Large mononuclears.	
		Per cent.	No.	Per cent.	No.	Per cent.	No.	Per cent.	No.
Before splenectomy.....	4,100	51	2,091	41	1,678	3	123	5	205
" tying thoracic duct.	9,000	53	4,770	36	3,240	5	450	6	540
6 hrs. later.....	4,500	8	340	85	3,825	4	180	3	135
12 " "	6,200	5	310	86	5,332	5	310	4	248
24 " "	6,700	15	1,005	73	4,891	5	335	7	469
48 " "	12,000	11	1,320	78	9,160	2	240	9	1,080
4 days "	9,700	13	1,251	72	6,984	3	291	12	1,164
6 " "	7,600	25	1,900	66	5,016	3	228	6	456
10 " "	7,700	40.5	3,118	50.5	3,888	3.5	277	5.5	423

Rabbit 2.—Male; 2,000 gm. December 7, 1920. Splenectomy. Recovery took place without apparent complications and none was found at subsequent autopsy. January 5, 1921. The thoracic duct and jugular were included in a ligature; a second was placed above to include the left cervical trunk; the right cervical main trunk was tied, and an attempt was made to dissect free the lymphatic trunks in the neighborhood of the right jugular and subclavian junction. The tissues were closed in layers by interrupted sutures. The blood counts in this animal are given in Table II.

In this animal, with probably better exclusion of lymphocytes, there is a drop in the number of lymphocytes of 4,430 per c. mm. in 6 hours, or, on a basis of 100 cc. of blood, a total disappearance from the circulation of 443,000,000 lymphocytes, a fact well confirmed by the count after 12 hours. This is followed, as in the previous animal, by a gradual return toward normal.

In this last animal the most successful exclusion of lymphocytes was obtained. Success depends upon the character of the main lymph trunks. The rabbit is not satisfactory for such experiments since there is frequently a right thoracic duct as well as a left, as pointed out by Gage[4] and as our injections of experimental animals, post mortem, have indicated. There are also many anastomosing lymph vessels near the entrance of the duct into the jugular. Injection of the thoracic duct through the capsule of the mesenteric lymph glands in one of our animals showed anastomosing vessels leading through the thymus toward the right side. It is through the utilization of such channels that the lymphocyte count in the blood is gradually restored.

Fate of the Lymphocyte.

With these indications of a large supply of lymphocytes and, as is consistent with this, a rapid disappearance of these cells when the supply is cut off, the question as to their fate is still left unanswered. Do they disintegrate in the blood stream or do they leave it? Occasional disintegrating lymphocytes are found in stained blood films made from normal and from diseased individuals, and it seems unquestionable that some lymphocytes may go to pieces in the blood. In an attempt to answer the question as to whether this is the fate of the large majority of lymphocytes, an experiment was performed, which, though not leaving them in a strictly physiological state, simulated this condition as closely as possible. After the number of lymphocytes in the freely flowing blood from the marginal vein of the right ear had been established, a section of the right jugular vein full of blood was isolated between carefully applied double ligatures. Counts of the cells in this section were made 6 hours later in some

[4] Gage, S. H., in Buck, A. H., Reference hand-book of the medical sciences, New York, 2nd edition, 1902, v, 624.

animals and 24 hours later in others. These experiments are open to objection since the cells are probably exposed to a reduced oxygen tension in the stagnant blood and the varied ameboid motion of cells and the settling of corpuscles cause an uneven distribution at the end of 24 hours which makes the counts somewhat questionable. Yet, as they have a bearing on the length of life of the lymphocyte, three counts are given (Table III).

TABLE III.

Rabbit No.	Site of count.	Total count.	Lymphocytes.		Neutrophils.		Basophils.		Large mononuclears.	
			Per cent.	No.	Per cent.	No.	Per cent.	No.	Per cent.	No.
3	Count from right ear.	7,800	41	3,198	52	4,056	5	390	2	156
	" " jugular section 6 hrs. after ligation.	7,250	63	4,567	30	2,175	5.3	384	16	116
4	Count from right ear.	7,250	52	3,770	37	2,682	8	580	4	295
	" " jugular section 24 hrs. after ligation.	4,600	62	2,852	32	1,372	4	184	2	92
5	Count from right ear.	9,000	36	3,240	50	4,500	2	180	13	170
	" " jugular section 24 hrs. after ligation.	6,800	53	3,604	40	2,720	2	136	5	340

In spite of the inaccuracies of the method, there is apparently a greater disappearance of polymorphonuclear cells than of lymphocytes. This was explained by histological sections made through the vein wall, which showed not that these cells were destroyed, but that there was a marked migration of them toward the operative field. In the stained film from the jugular blood, the most striking feature is the fact that the lymphocytes show no tendency toward disintegration but are as sharply stained and clear-cut in the differentiation of nucleus and protoplasmic rim as in films from the freely circulating blood. We believe that the experiments, though faulty, indicate that there is not the rapid disintegration of the cells which would be necessary to explain the rate of their disappearance from the blood when the supply is cut off. They must leave the blood stream.

Since the ameboid motion of lymphocytes is generally accepted, there is no objection *a priori* to the proposition that they leave the blood stream under normal conditions in the numbers demanded by the figures given. The question arises as to where they go. The small accumulations of lymphocytes found in the organs generally will not account for the vast number that leaves the blood vessels, unless the cells are destroyed in the tissues as rapidly as we postulated their destruction in the blood stream, and of this there is no evidence in normal organs. Without destruction, of necessity there would be an increasing accumulation in these organs such as may be seen in lymphocytic leucemias.

A search of the organs in histological section leads to the conclusion that the great majority of these lymphocytes enter the mucosa of the gastrointestinal tract and, in addition, pass through it into the intestinal lumen. In sections of rabbit intestine removed immediately after the death of the animal and fixed rapidly in formaldehyde without opening or disturbing the contents, the number of cells in the mucosa, between the cells of the intact epithelial lining, and in the intestinal content is surprising, not only in the parts of the tract where lymphocyte production takes place, as in the lower ileum and the appendix, but also high in the duodenum. If the 40 or 50 lymphocytes which may be counted between the epithelial cells of the tip of a duodenal villus in a 10 micron section are multiplied by its probable relation to the surface area of the intestine, there is no difficulty in accounting for all the lymphocytes that disappear from the circulation. Where there is local production, as in the lymphoid nodules of the appendix, several hundred lymphocytes and large mononuclears may be found in a section through the mucus of a single crypt. In a section of the intestine made without disturbing the contents of the lumen, collections of lymphocytes and larger mononuclear cells are numerous close to the mucosa, especially in the small intestine, even, as stated above, high in the duodenum. In the rabbit colon, which contains inspissated fecal masses, there is little or almost no migration of lymphocytes. The scarcity of polymorphonuclear leucocytes throughout the intestine is as striking as the presence of the mononuclear cells.

Several attempts were made to count the free lymphocytes in the intestinal content by taking up into a calibrated capillary tube a

known amount of fluid close to the mucosa, spreading it over a small part of a cover-glass, staining, and counting the total number of cells. Such a count gave 100 definite lymphocytes per c. mm. for the upper duodenum, 480 for the ileum, and 700 for the appendix. These figures are without significance when taken in relation to the total volume of the intestinal contents, as the lymphocytes are not evenly distributed throughout the contents, at least in recognizable form, but lie close to the mucosa. However, they indicate that large numbers of lymphocytes enter the lumen normally. In fact, one may speak of a normal excretion of lymphocytes from the intestinal mucosa. This takes place not only from the blood stream but from the lymphoid nodules and patches, which are shown by sections to be delivering lymphocytes to the intestine on one side, and to the efferent lymphatics on the other. The latter cells must take an indirect route through the thoracic duct and the blood stream to reach the lumen. To a slight degree the same process is visible in the bronchial mucosa of the rabbit.

It would seem then that under normal conditions the lymphocyte fulfills its function to a large degree upon the surface of the intestinal mucosa. To attempt to designate that function would, with our present knowledge, be a matter of almost pure speculation. In attacking the problem, it is difficult not to be influenced by the immunity of the intestinal mucosa to the countless bacteria within its lumen and to their toxins. The lymphocyte is not phagocytic, but there are many indications that it may affix toxins, and among these may be cited the agglutination of lymphocytes noted in the thoracic duct.

CONCLUSION.

Although the count of circulating lymphocytes in the blood stream remains constant, more lymphocytes enter the blood from the thoracic duct during 24 hours than are present in the blood at any one time. This excess of lymphocytes is not destroyed in the blood stream. The cells migrate from the blood vessels into the mucous membranes and through them to their surface. This occurs chiefly in the gastrointestinal tract, and it is apparently in the mucosa and especially within the intestinal lumen that the function of the lymphocyte is normally performed.