THE EFFECT OF PILOCARPINE ON THE NUMBER OF SMALL LYMPHOCYTES IN THE CIRCULATING BLOOD FOLLOWING LIGATION OF THE THORACIC DUCT.

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(Received for publication, October 13, 1923.)

INTRODUCTION.

In a previous communication the writer has shown that following the intrathoracic ligation of the thoracic duct in cats, the number of small lymphocytes in the circulating blood is temporarily decreased (Lee (1922, *a*)). In view of this observation experiments were undertaken to determine what effects an artificially produced lymphocytosis would have upon the number of small lymphocytes in the blood after the thoracic duct had been tied.

Materials and Method.

In this series of experiments eight adult male cats were used, of which number two served as controls. The absolute number of small lymphocytes in the blood was calculated from the total white blood cell determination and from a differential count of 300 or more cells from a smear stained with Wright's blood stain. Specimens of blood were examined before the ligation of the thoracic duct, and at definite intervals following the intraperitoneal administration of pilocarpine nitrate as shown in Table I. The thoracic duct was ligated in the chest under sterile precautions according to the method previously described (Lee (1922, *b*)).[1] About 24 hours later pilocarpine nitrate, sterilized by boiling gently for 5 minutes in a freshly prepared aqueous solution, was injected into the peritoneal cavity to the extent of 3.3 mg. per kilo of body weight. Food was withheld for a period extending from 24 hours before the ligation of the thoracic duct until 24 hours after the administration of the pilocarpine; water, however, was always provided. From 2 to 3 days after the injection of pilocarpine the animals were placed under ether anesthesia, and the mesenteric lymph vessels were injected in a centripetal

[1] All operations were performed under ether anesthesia.

direction in order to determine whether the thoracic duct had been completely ligated. Tissue from the animals was placed in 10 per cent commercial acid formalin and subsequently sectioned in paraffin. The postoperative course of all the animals showed that they were free of infection.

OBSERVATIONS.

Aside from minor variations the results were comparatively uniform. The following protocol and Table I, together with Text-fig. 1, represent an average case.

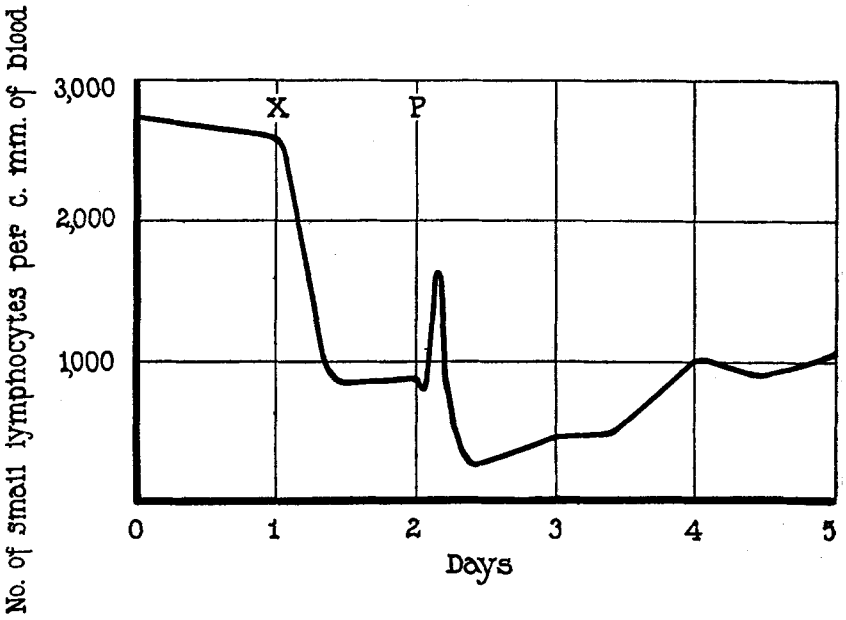
TABLE I.

Date.	Time.	Total white blood count per c.mm.	Polymorphonuclear neutrophils.	Eosinophils.	Small lymphocytes.	Large lymphocytes.	Large mononuclears.	Transitionals.	Absolute No. of small lymphocytes per c.mm.
1923			*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	
Apr. 23	10.00 a.m.	13,920	68.7	7.7	19.7	1.7	1.0	1.3	2,742
" 24	10.00 "	12,800	61.7	11.7	20.7	3.0	0.7	2.3	2,649
	11.00 "	Ligation of thoracic duct.							
	5.00 p.m.	16,900	90.7	0.3	5.3	1.0	1.0	1.7	896
" 25	10.20 a.m.	16,000	88.0	0.3	6.0	1.7	1.7	2.3	960
	10.30 "	8.8 mg. of pilocarpine nitrate injected intraperitoneally.							
	11.30 "	8,940	85.7	0.3	9.0	1.3	1.7	2.0	803
	12.45 p.m.	23,500	90.0	0	7.0	0.3	1.0	1.7	1,645
	2.00 "	12,000	90.7	0.3	5.7	1.0	1.3	1.0	684
	3.15 "	10,200	91.7	0	4.0	0.3	1.7	2.3	408
	4.45 "	10,080	93.7	0.3	2.3	0.3	1.3	2.0	231
" 26	10.00 a.m.	8,800	86.7	1.0	5.7	1.7	1.0	4.0	501
	4.30 p.m.	6,020	77.7	9.3	8.7	2.0	1.0	1.3	513
" 27	9.30 a.m.	7,600	71.3	9.3	14.7	1.0	1.7	2.0	1,017
	5.00 p.m.	7,100	72.7	8.7	13.0	1.7	3.0	1.0	923
" 28	9.00 a.m.	6,200	67.3	9.0	19.0	1.3	1.0	2.3	1,178

Cat 1.—Adult, gray, male cat; seemingly in good health; weight 2,580 gm. Aseptic intrathoracic ligation of thoracic duct on April 24. Intraperitoneal injection of 8.8 mg. of pilocarpine nitrate on the following day. This injection was followed by vomiting, diarrhea, sweating, lacrimation, and pronounced salivation; respiratory distress was not observed. It was difficult to secure blood for the two counts immediately following the injection. On the 3 following days the animal was in good condition; it ate some meat on the 2nd day after the administration of pilocarpine. On April 28 the mesenteric lymph vessels were injected in a centripetal direction with Berlin blue while the cat was under the influence of ether; lymph vessels to the liver and kidneys were markedly en-

larged; some retrograde injection of the mesenteric lymph vessels occurred; a slight amount of free fluid was found in the peritoneal cavity; the thoracic duct was definitely ligated; pieces of the various organs were placed in formalin for histological examination.

In this cat the increase in the absolute number of small lymphocytes in the circulating blood after the administration of pilocarpine was 104 per cent; the average increase for the six animals was 98.7 per



TEXT-FIG. 1. Curve to show the changes in the absolute number of small lymphocytes in the circulating blood of the cat, following the intrathoracic ligation of the thoracic duct (at *X*), and the intraperitoneal administration of pilocarpine nitrate (at *P*).

cent, whereas the increase for the two control cats was 100.8 per cent. The peak of increase was found to come within 2 hours after the injection, and in two animals a secondary rise appeared after the initial drop. However, the low point of the decrease following the peak was, as a rule, lower than the point from which the initial rise began; and from this low point the curve invariably went upwards and simulated the rise seen after the ligation of the thoracic duct.

Frequently the mesenteric lymph vessels were collapsed and difficult to inject at first; but after the injection was once successfully begun the other mesenteric lymph vessels became dilated because the injection mass displaced the lymph in the lymph glands and larger channels and thus forced it in a peripheral direction. In this dilated condition the lymph vessels were frequently injected in a retrograde manner.

The abdominal lymph glands, particularly the mesenteric glands, were always large and edematous. The lymph trunks leading to the liver and iliac glands were also greatly distended. On section the lymph glands yielded a large amount of clear, watery fluid with numerous small, fat-like droplets floating on the surface. Although the peritoneal cavity did not contain any excess of fluid, nevertheless the loose retroperitoneal tissue was edematous. No edema of the extremities or of the subcutaneous tissues was observed.

Examination of the histological material showed definite changes, primarily in the abdominal lymph glands and in the kidney. The most striking feature of the lymph gland was the enormous dilatation of the lymph channels, particularly in the medullary portion of the gland. The distention made the so called reticulo-endothelium appear as a stretched network of cells whose processes communicated with one another, forming meshes within which the injection mass was found. Possibly in a vitally stained animal the distention of the lymph sinuses may be a method for throwing some additional light on the interesting questions relating to the nature of the cells lining these sinuses.

Of unusual interest was the appearance of the kidney. Under low magnification numerous clear spaces of various sizes were seen throughout the kidney substance, but localized chiefly in the cortex. Higher magnification showed that these clear spaces were cross-sections of thin vessels of which the endothelium was stretched and the nuclei narrow and flattened. These vessels were numerous near glomeruli and along the medullary rays, and were undoubtedly lymph vessels. Examination of the individual cells of the tubules showed that a high degree of vacuolation existed in all the cells with the exception of those of the collecting tubules. The vacuoles were small, but numerous, and although found throughout the cell they were more numerous near the nucleus. They represented fat dissolved out during the

preparation of the section. The glomeruli showed no vacuolation and Bowman's capsule was not abnormally distended; no excess of fluid was collected in the tissue spaces.

DISCUSSION.

Although the original observation of Horbaczewski in 1891 that pilocarpine produces a marked leucocytosis has been repeatedly confirmed by subsequent investigators (Harvey; Lefmann; Waldstein; Schenk; Skórczewski and Wasserberg; Bertelli, Falta, and Schweeger) nevertheless other observers (Wollenberg; Platz) who were interested primarily in the effects of the nervous system on the blood picture were unable to obtain an outspoken lymphocytosis. Certain it is that studies of the action of pilocarpine made with the aid of thoracic duct fistulæ have conclusively shown that this drug causes a marked increase in the number of lymphocytes in the thoracic duct lymph and in the blood (Rous; Dixon (1909)).

Inasmuch as all the animals in this series showed a definite lymphocytosis, it is believed that the massive dose administered was instrumental in producing the result. The comparatively small dose of 0.01 gm. given intramuscularly or even subcutaneously to patients frequently produced equivocal results, even though such general symptoms of pilocarpine activity as salivation, lacrimation, and sweating were manifest (Wollenberg). The largest doses administered were those of Ruižcka, who gave 50 mg. of pilocarpine per kilo of body weight subcutaneously to dogs and obtained a rise in the leucocytes equal to thirty-two times the original amount. The smallest doses were given by Port and Brunow, who found that even 0.0007 gm. per kilo of body weight caused a rise in the absolute number of lymphocytes in the blood of dogs.

The intraperitoneal method of injection was chosen because of its simplicity and convenience. That the absorption of the drug from the peritoneal cavity was comparatively slow was shown by the fact that the full effect on the blood picture was not observed in some cases until the end of 2 hours. It is important to correlate the method of administration with the time of blood examination, and it is needless to point out that a single count after the drug has been given (Harvey) is not a fair criterion of its action.

Even with large doses the action of the drug was selective; vomiting occurred only early, lymphocytosis was manifest late, while salivation came on quickly and was the last symptom to disappear. This sequence of symptoms suggests either that some tissues respond more quickly than others to the direct action of the drug, or that certain organs, like the adrenal, are affected and that these in turn liberate a secretion which produced pharmacological changes.

The lymphocytosis produced may have been caused in several ways. In the first instance the pilocarpine may have caused an absolute increase in the number of lymphocytes at their centers of production. But against this view must be brought the observation that the lymphocytic response to the intravenous administration of the drug took place within 10 minutes, and it is difficult to believe that a large number of lymphocytes could have been liberated in such a short space of time, all the more so because histological examination of the spleen and lymph glands failed to show a definite increase in the number of mitotic figures.

In the second place, the lymphocytosis may have been produced by the direct passage of lymphocytes into the blood stream at their centers of formation. Since this communication was not prevented by the ligation of the thoracic duct, a relatively great lymphocytosis might have been expected after the injection of pilocarpine. Although this marked lymphocytosis never occurred, the method of passage is not entirely excluded (von Schumacher).

Finally, pilocarpine through the contraction of the plain muscle it causes in various organs may have mechanically produced a lymphocytosis by a discharge of lymphocytes first into lymph vessels and secondarily into the blood stream. Harvey originally advanced this explanation, and his view has subsequently received support from the work of Rous and of Dixon (1912). The experiments here recounted also contribute to Harvey's view. For it was found that after the intraperitoneal injection of pilocarpine the same *relative* increase in the absolute number of small lymphocytes in the blood stream occurred whether the thoracic duct was ligated or not. To this may be added the observation that pilocarpine caused not only a rise in the total number of lymphocytes, but that it also caused a drop to follow the rise, and this drop not only invariably reached the level

from which the rise began, but in half the experimental animals as well as in the two controls it went even lower with the result of a typical negative phase after the positive rise. This suggests that the drug had caused a mechanical discharge of the lymphocytes from their seats of formation with result later in a temporary lack of them. It would seem that the ligation of the thoracic duct shut off the normal avenue into the blood for about 58 per cent (the mean for the six animals) of the small lymphocytes, leaving 42 per cent to be supplied by that portion of the lymph system not obstructed by the thoracic duct ligation; and it was this latter portion of said system which responded in the same relative degree to pilocarpine as did the entire intact lymph system in the control animals. The observations do not accord with Harvey's view that the spleen is the main source for the lymphocytic increase, but indicate to the contrary that the organ is no more specialized for the production of lymphocytes than is any other part of the lymphopoietic system.

As regards the other blood cells which have come under investigation it may be said that there occur changes in the number of polymorphonuclear neutrophils after pilocarpine paralleling the changes in the number of small lymphocytes. The eosinophils were practically absent following the ligation of the thoracic duct, and the administration of pilocarpine did not cause them to appear. The other cell groups remained essentially unchanged.

SUMMARY.

In a series of animals in which the thoracic duct had been tied it was found that the relative increase in the number of small lymphocytes in the circulating blood following the intraperitoneal administration of pilocarpine nitrate was the same as for the control animals. While support is brought for Harvey's view that pilocarpine causes a lymphocytosis through the contraction of plain muscle, on the other hand evidence is presented which indicates that the spleen is no more specialized in the production of small lymphocytes than any other portion of the lymphopoietic system.

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