THE EFFECT OF PILOCARPINE ON THE OUTPUT OF LYMPHOCYTES THROUGH THE THORACIC DUCT.*

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The lymphocytosis induced in the blood by pilocarpine is a phenomenon which has been turned to the use of many theories but has had, of itself, little study. Horbaczewski (1891) (1) discovered that the drug increases the white cells. The finding helped him to work out his idea of the dependence of uric acid excretion on leukocyte destruction. Ruzicka (2) obtained a profound leukocytosis in rabbits by the intravenous injection of large doses of pilocarpine. He could not account for the rapid occurrence of this result, but thought proliferation in the hæmatopoietic tissues responsible for its continuance. Waldstein (1893) (3), assuming that an increase of the mononuclear elements in the blood would influence favorably the course of some infectious diseases, gave small amounts of pilocarpine at intervals of several days, and obtained as result a large, absolute lymphocytosis. Recently Lefmann (4) and Gasis (5) have repeated Waldstein's experiments with rabbits, in demonstration of an effect of the Roentgen rays to bring about quick disappearance of the lymphocytosis.

The evidence seems good that pilocarpine given in small doses over a considerable period of time produces a lymphocytosis absolute in type. The immediate effect of the drug is to cause (in rabbits) a general increase of the white cells, involving especially the lymphocytes. This result is often cited as an example of possible chemiotactic influence on the lymphocyte, but Harvey (6) has produced evidence to prove it due to contraction of the smooth muscle of the lymph-glands and spleen. Unfortunately his work

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is based on counts of but one hundred cells each from blood smears. There have been no other investigations on the cause of the quick change in the blood picture.

A method adopted by the author (7) for study of the cell-output through the thoracic duct has given him opportunity to note in dogs the immediate effect of pilocarpine on this source of the lymphocytes. In brief, considerable quantities of lymph (3 cubic centimeters to each specimen) are collected from the thoracic duct in specially graduated tubes containing 3 cubic centimeters of 4 per cent. sodium citrate solution in 0.8 per cent. salt solution, and the white cells per cubic millimeter estimated from the mixture after it has been thoroughly agitated. This estimation is accomplished with melangeur and counting-chamber in the ordinary manner. The mixture of lymph and sodium citrate solution is not diluted, but the addition to it of a trace of a saturated aqueous solution of methyl violet (5B) facilitates the counting. The accuracy of this method of cell-enumeration, and the slight variation in the number of cells per cubic millimeter of lymph voided from the thoracic duct during the first two hours after the establishment of a lymph-fistula in an animal quiet under morphia and chloroform, have been shown in the paper cited. The output of cells as a whole becomes gradually less during this period, as the amount of lymph voided gradually lessens.

The dogs used were given, one hour before operation, 0.5 centigram of morphia sulphate per kilo of body weight, and later chloroform to complete the anaesthesia. A cannula was introduced directly into the thoracic duct; the lymph allowed to flow free; and, after the collection of one or more specimens, a small dose of pilocarpine nitrate, dissolved in a few minims of salt solution, was injected intravenously and further collection of lymph made. In every instance the action of the drug was evident within the minute through increase in the saliva.

Since figures dealing with the effect of pilocarpine on the blood of the dog are lacking, preliminary counts were obtained on animals treated as above outlined, except that no lymph-fistula was produced, and the only operation was that necessary to give access to the left, external jugular vein, into which the injections were made.

Throughout the term of observation the animals were kept quiet under morphia and chloroform. In each instance food was withheld during the twenty-four hours previous to operation. Blood for the counts was obtained by nicking with scissors small superficial veins on the abdomen. The cover-glass preparations were stained with Wright's stain, and for each differential count (of 500–600 cells) at least two smears were used. In the table that follows the number both of large and of small mononuclear cells is given.

Experiment I.—Bull-dog, female; wt. 16.5 kilo.

Time.	Procedure.	Total wbc. per cmm.	Small mns. per cmm.	Large mns. per cmm.	Total mns. per cmm.	Rbc. per cmm.
A. M. 9:50	Operation.					
10:15	First count.	18,600	986	484	1,490	6,672,000
10:45	Second count.	18,200	746	528	1,274	
10:50	Pil. nit. 20 mg. intravenously.					
11:22	Third count.	24,900	1,619	896	2,515	6,960,000

Experiment II.—Fox-terrier, male; wt. 9 kilo.

Time.	Procedure.	Total wbc. per cmm.	Small mns. per cmm.	Large mns. per cmm.	Total mns. per cmm.
A. M. 9:40	Operation.				
9:48	First count.	6,400	749	179	928
10:23	Second count.	9,720	846	136	982
10:26	Pil. nit. 10 mg. intravenously.				
11:13	Third count.	15,200	1,870	274	2,144

Experiment III.—Pointer, male; wt. 22 kilo.

Time.	Procedure.	Total wbc. per cmm.	Small mns. per cmm.	Large mns. per cmm.	Total mns. per cmm.
A. M. 9:30	Operation.				
10:30	First count.	13,000	959	429	1,388
10:35	Pil. nit. 10 mg. intravenously.				
11:05	Second count.	19,200	2,170	499	2,669
11:30	Third count.	22,800	2,280	684	2,964

Generalization from these few observations is hardly warranted; yet there seems ground to suppose that pilocarpine, intravenously given, produces a prompt, moderate increase in the mononuclear cells, especially the lymphocytes, of the blood of the dog. Certainly no such extreme lymphocytosis takes place as Harvey noted

in the rabbit. A leukocytosis affecting the polymorphonuclear elements also made its appearance, but one cannot rule out the operation itself as sole cause of this. On the other hand, an absolute increase in the mononuclear cells, such as occurs here, is not a characteristic of the well-known leukocytosis due to operation. The dependence of the lymphocytosis on pilocarpine injection is indicated, furthermore, by those two instances in which repeated counts were made after the animal had been operated upon, but before the administration of the drug. In these counts the number of lymphocytes was found to be practically unvarying.

The direct cell-output by way of the lymph was now studied according to the method already described. It may be remarked in passing that lymphocytes are alone present in the dog's lymph in large number. (Delamere (8), Biedl and v. Decastello (9).)

Experiment IV.—Mongrel, male; wt. 8.2 kilo. Food was withheld from the animal for 18 hours previous to experiment. The duct was opened, and the lymph allowed to flow for 15 minutes before the first specimen was collected. It was pinkish, slightly opalescent, and at no time clotted in the cannula. When one specimen had been obtained 12 milligrams of pilocarpine nitrate were injected into the left, external jugular vein. The respirations became dyspnœic for about one minute, after which they resumed their previous rhythm. The lymph flowed faster and was nearly colorless. Five specimens of it were collected, then a second injection of pilocarpine (11 milligrams) given, and two more specimens obtained. The cell-counts were made from the tubes in the order of their collection and 2 to 3 hours following it. (See Chart 1.)

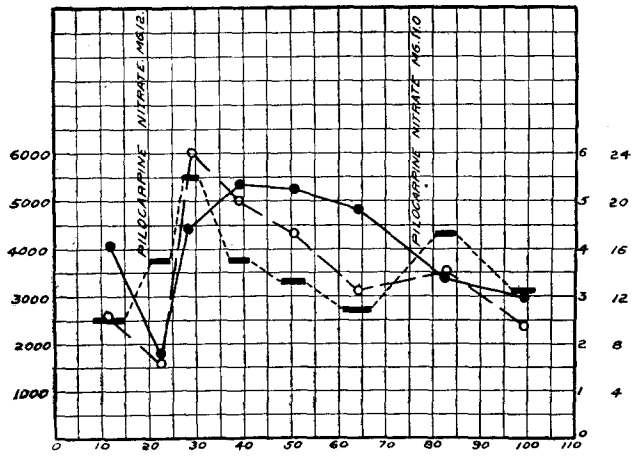

CHART 1.

An autopsy proved the animal to have been sound.

Experiment V.—Mongrel hound, male; wt. 9 kilo. Food was withheld for 26 hours previous to the experiment. The lymph was opalescent. A cannula was introduced into the duct after it had been ligated 5 minutes, and 26 minutes prior to the collection of the first tube. Slight clotting in the cannula necessitated twice in the two hours the use of a hooked wire to clean the bore. In one instance the flow was momentarily interfered with. This happened in an interval when lymph was not collected, and with the return of the flow 15 minutes were allowed to pass before another specimen was taken. The injection of the 5 milligrams of pilocarpine into the left, external jugular vein did not cause dyspnœa or movement. Two tubes of lymph were collected during the hour following. Cell-counts were made in the order of collection of specimens and 1½ hours after each had been obtained. (See Chart 2.)

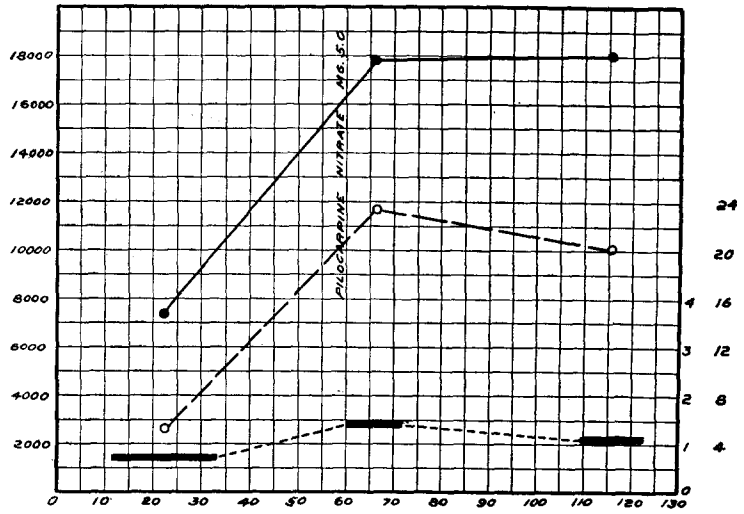

CHART 2.

The slow flow of lymph suggested the presence of an accessory thoracic duct. Accordingly, the duct proper was ligated before the animal was killed, and, with the aid of the natural injection, a search made for branches to the right side of the neck. None connected with the thoracic duct or receptaculum were found. The duct contained no clot and was patent.

Autopsy showed the animal to have been sound.

Experiment VI.—Male, collie; wt. 18.5 kilo. Food was withheld for 26 hours before operation. The thoracic duct was opened after 5 minutes ligation, and 20 minutes allowed to elapse before the collection of lymph was begun. Once in this interval the dog was partly roused by tweaking the skin, that the lymph-system might be flushed, through the quickened lymph-flow incident to struggle, of possible cell-accumulation in its channels. In the quiet following two tubes of lymph were taken, and after this 10 milligrams of pilocarpine nitrate

injected into the left, external jugular vein. The lymph, previously opalescent, became for 15 minutes quite milky, so that a fat ring developed in it on standing. The animal remained quiet and the character of its respirations did not change. Four more tubes were collected, then atropine sulphate, 0.6 milligrams, dissolved in a few minims of normal salt solution, was injected into the left subclavian vein, and two more tubes taken. The drug caused almost immediate cessation of bowel-noises, the flow of salivary secretion stopped, and the lymph slackened markedly in flow, and became clear and slightly blood-tinged. The subsequent injection of 15 milligrams of pilocarpine nitrate did not quicken its flow. There was no clotting in the cannula at any time. The tubes were counted in the order of their collection, and 1½ to 3 hours after it. (See Chart 3.)

At autopsy the animal was found to have been sound. The thoracic duct showed no branch leading to the right side of the neck.

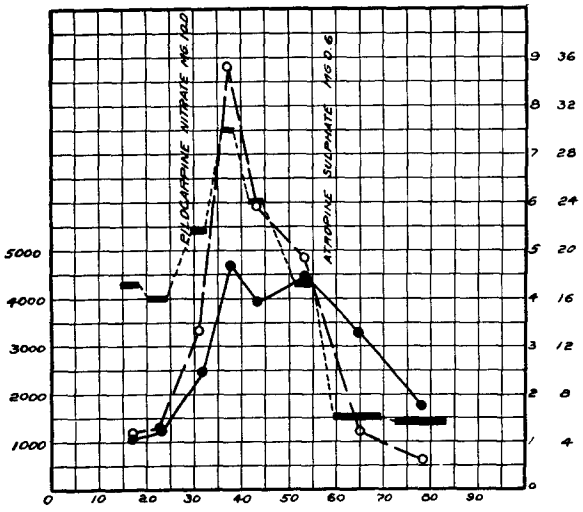

CHART 3.

The height above the base-line of the curve representing amount of lymph-flow indicates the number of cubic centimeters voided through the thoracic duct in a given time; and the black rectangles show the period required to collect the three cubic centimeters of lymph in each specimen. Thus the curve depicts in two ways the rapidity of lymph-flow.

The results of a first injection of pilocarpine in these three experiments are very similar. A well-defined increase in the number of white cells per cubic millimeter of lymph ("cell-concentration") is brought about, as also an increase in the total output of cells. The effect is fairly sustained, lasting one half to one hour. A quickening of the lymph-flow is also seen, but it is not so enduring.

One questions immediately whether the large cell-output is not a corollary to quickened lymph-flow. But comparison shows the two phenomena to lie in only a rough time-relation. Furthermore, as the following experiment demonstrates, pilocarpine will produce a profound increase of cell-output in a lymph-stream that varies little in flow. The effect of the drug is not always that of a lymphagogue (Heidenhain, Tschirwinsky (10), Spiro (11)).

Experiment VII.—Coach-dog, male; wt. 9.2 kilo. The animal was fed with lean beef 3½ hours before operation. The duct was opened after 8 minutes ligation, and the lymph allowed to run for 19 minutes before the first specimen was collected. It was milky, and showed no tendency at any time to clot in the cannula. Two tubes were taken, and then 6 milligrams of pilocarpine nitrate injected into the left, external jugular vein. The animal continued quiet, and the breathing did not change in rhythm, yet during the next 20 minutes the content in fat of the chyle was much increased, as shown by a comparison of the fat-rings that formed in the tubes after they had stood for some hours. The fluid that ran later resembled thin chalk and water. Six specimens were collected, a second injection (5 milligrams) given, and three more tubes obtained. Toward the close of the experiment the breathing was slightly labored, and rhonchi could be heard. The tubes were counted in the order of their collection, and 2½ to 4 hours after it. (See Chart 4.)

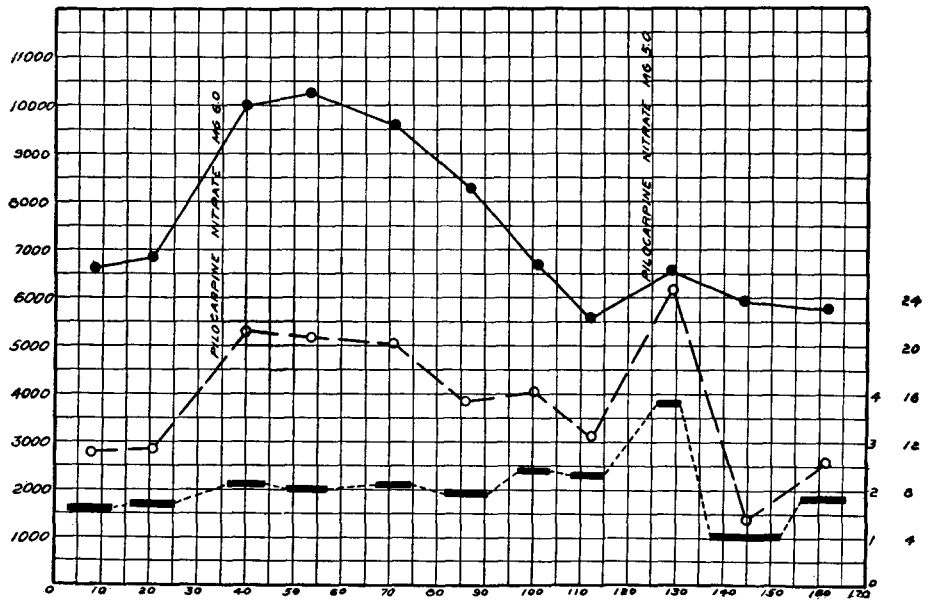

CHART 4.

The autopsy showed the animal to have been sound.

In this instance the effect on the cells of the first injection of pilocarpine was outspoken, despite the nearly constant lymph-flow. The cell-increase was prompt here, as in Experiments IV, V and VI. In all it took place in the ten minutes immediately after the injection.

The actual increase in cell-output is of interest as an indication of the extent to which contributions through the thoracic duct may be responsible for the pilocarpine lymphocytosis. The animals used for the work just presented moved voluntarily, or were induced to struggle, shortly before the experiment proper, to rule out that accumulation in the lymph-system of mature cells, which has been observed to occur in the quiet animal (Goodall and Paton (12), Rous). But the rush into the circulation of accumulated cells when pilocarpine acts on an animal previously quiet is to be reckoned with as an effect of the drug. The next experiment illustrates this.

Experiment VIII.—Collie, male; wt. 19 kilo. No food was given for 50 hours prior to the experiment. The animal was quiet for 1 hour before the collection of the first lymph-specimen which was taken 5 minutes after the opening of the thoracic duct. This had been 1 minute ligated. The slightly opalescent lymph showed no tendency to clot in the cannula. After two tubes of it had been obtained 10 milligrams of pilocarpine nitrate were injected into the left, external jugular vein. The breathing immediately became somewhat dyspnœic and remained so. Five tubes of lymph were taken, then a second injection of 10 milligrams given, and four more tubes obtained. Cell-estimations were made on the specimens in the order of their collection, and 2½ to 3½ hours after it. (See Chart 5.)

At autopsy a large mass of tape-worms was found in the small intestine. Otherwise the animal had been sound. The thoracic duct gave off no branch to the right side of the neck.

If one neglect the action of pilocarpine in changing the fluid content of the blood, a calculation is possible of the absolute increase in lymphocytes per cubic millimeter of blood which would have been caused by such an addition of cells as this.[2] The increased flow of lymph induced by the drug can hardly be supposed to act as a real diluent, since new lymph-production and active secretion from the salivary and other glands tend to drain the blood of fluid. On the basis that the dog, which weighed 19 kilo, had 7.7 per cent. of its

[2] After the administration of pilocarpine the bulk of white cells in the lymph is still one of lymphocytes.

weight in blood of a specific gravity of 1.055, one may assume 1,385 cubic centimeters as the total volume of blood. During the forty minutes following pilocarpine injection an average of 111,400,000 more white cells were given off through the thoracic duct in each five minutes than during the same period of quiet,—or a total excess over the normal outpouring of 891,000,000 in the

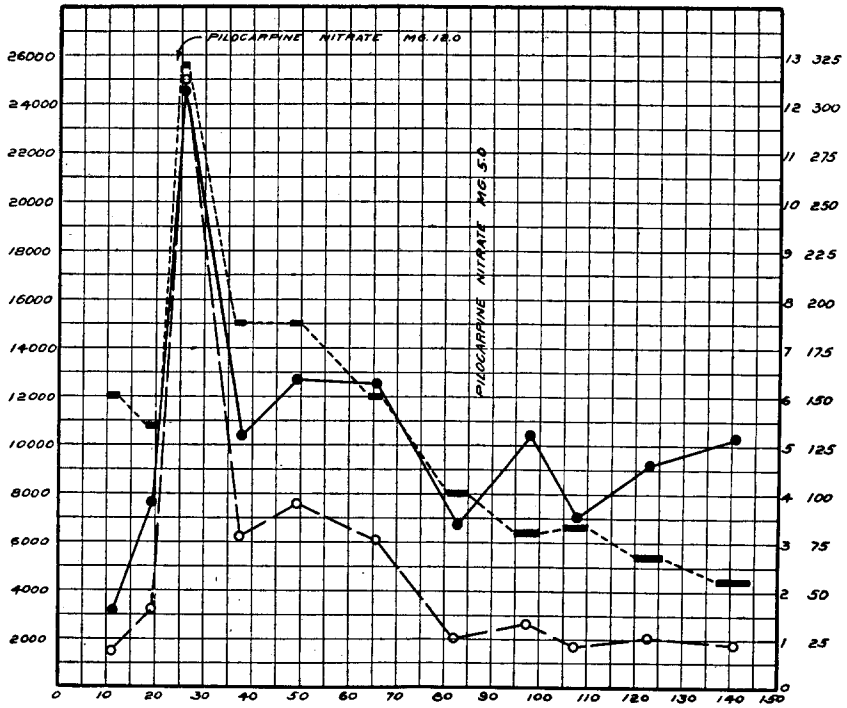

CHART 5.

forty minutes, a number sufficient to have furnished each cubic millimeter of blood with 643 lymphocytes over the normal supply.

The absolute increase in output of lymphocytes through the thoracic duct was much smaller in the other animals: enough in Experiment IV to have furnished 100 lymphocytes to each cubic millimeter of the dog's blood; in Experiment V sufficient for 382 per cubic millimeter; in Experiment VI enough in the short period of observation to give 84 extra cells per cubic millimeter; in Experiment VII enough for 146 extra cells per cubic millimeter. But

in Experiment VIII alone had the animal lain quiet as in Experiments I, II and III. In these three the increase in the circulating lymphocytes that took place in the first thirty to fifty minutes after pilocarpine injection was close to 1,000 per cubic millimeter. An output such as that obtained, despite the unfavorable condition of lymph-fistula, in Experiment VIII, would account for more than half of this lymphocytosis.

The effect of pilocarpine on cell-output through the thoracic duct, as here studied, is probably dependent on several factors:

1. *Increase in Lymph-Flow.*—Others have proved that pilocarpine often, though not always, acts as a lymphagogue. In a previous paper it has been shown that increase in lymph-flow alone,—the factor invoked by Ehrlich for the production of quickly appearing lymphocytosis,—exerts indeed a considerable influence to increase cell-output through the thoracic duct. When a condition of bodily quiet has allowed accumulation of cells in the lymph-system the number flushed out with a quickened lymph-stream may be large, as in Experiment VIII. Yet that the increased cell-output is but secondarily dependent on this factor has been made clear.

2. *Dyspnœic Breathing.*—This is frequently induced by pilocarpine (Cushny (13)). In one only of the five experiments was it marked, though in a second it was briefly present. By its pumping action on the great lymph-channels of the trunk it tends to keep their contents in motion (Starling (14)), and would hinder in this way cell-accumulation.

3. *Contraction of Smooth Muscle.*—Pilocarpine contracts the lumen of the large lymph-vessels (Heinz (15)). Obviously a result of this narrowing is a very brief increase in the amount of lymph voided through the thoracic duct, and, as this is cell-containing, the total cell-output would also be briefly increased.

Harvey believes that contraction of the smooth muscle of lymph-glands and spleen is entirely responsible for the lymphocytosis he observed to follow pilocarpine injections in rabbits. He bases his conclusion principally on the fact that atropine prevents the occurrence of this lymphocytosis, whereas it does not hinder the occurrence of that which he found barium chloride to produce.

Whatever may be said of the effect of barium chloride on the

blood, it is certain that Harvey's atropine-pilocarpine experiments admit of a second interpretation as regards the process taking place in the lymph-system. For it is well-known (Spiro, Tschirwinsky) that atropine slows the lymph-flow strikingly, even though comparatively large doses of pilocarpine be given. Its action is in this way antagonistic to the increase of cell-output through the thoracic duct; for no matter if some force liberated cells from the lymph-glands, the stagnant current would prove but a poor medium for their transport. In illustration the effect of atropine in Experiment VI may be pointed out. Given shortly after pilocarpine, it immediately reduced lymph-flow and cell-output to less than they had been previous to the administration of either drug. The following experiment furnishes a further illustration:

Experiment IX.—Bull-dog, male; wt. 25 kilo. No food was allowed it for 24 hours previous to the experiment. The thoracic duct was opened after 3 minutes ligation, and the lymph ran free during 17 minutes before collection was begun. It was clear and at no time clotted in the cannula. Two specimens were taken to establish the facts of output, then 1.2 milligrams of atropine sulphate in a few minims of salt solution were injected into the left,

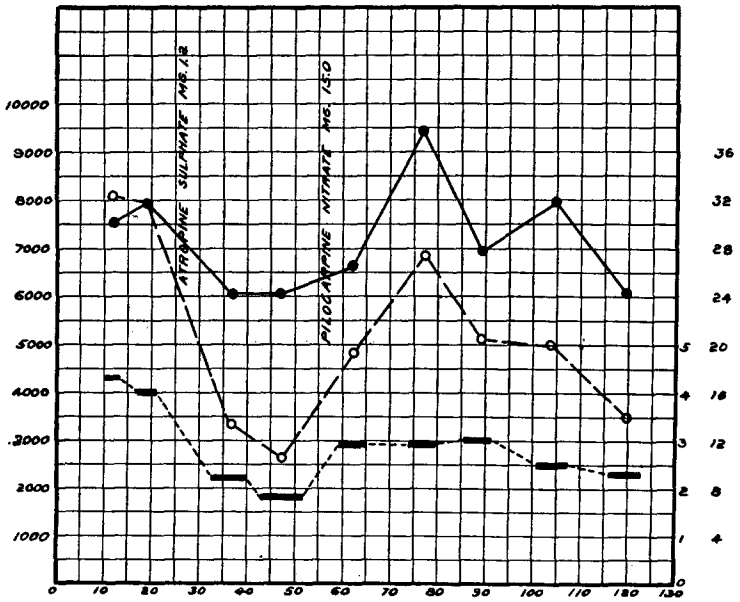

CHART 6.

external jugular vein, and, with decreased lymph-flow manifest, two more specimens obtained. The animal remained quiet. Fifteen milligrams of pilocarpine nitrate were now injected into the left, external jugular vein, causing a momentary flow of saliva, a few dyspnœic movements of the chest, and twitching of the limbs, all of which ceased within the minute. No other changes in the animal's condition were noted. Five more tubes of lymph were obtained. Cell-counts were made in the order of tube-collection, and from 2 to 3 hours after it. (See Chart 6.)

Autopsy showed the animal to have been healthy. There were many tapeworms in the large intestine.

In this instance atropine reduced the lymph-output to one third its previous quantity, and cell-output by way of the lymph to much less than half. Pilocarpine raised the lymph- and cell-output again, but not to their rate previous to atropinization. It is impossible to say that the changes in cell-output are not wholly dependent on those of lymph-flow. Similarly, the profound fall in cell-output brought about in Experiment VI by atropine may be due to nothing else than lessened lymph-flow. To seek a further factor is unnecessary.

Yet some action of pilocarpine to further cell-output, other than those of increased lymph-flow and dyspnœic breathing, is certainly present. Stimulation of the lymph-glands to productive activity cannot be responsible, since the increase in cell-output occurs practically at once. Were chemiotaxis a factor, as Gulland (16) believes, a second injection of pilocarpine ought to influence cell-output. But instances (Experiments IV, V, VI) of a second injection show it to have practically no effect. Contraction of smooth muscle must be further considered.

As has been demonstrated, the effect of atropine on the lymphocytosis of pilocarpine is neither for nor against its origin by smooth muscle contraction. That direct pressure (such as this contraction would bring about) may increase the cell-output of the lymph is highly probable, since the pressure exerted by a quickened lymph-flow will increase it. In this connection it is noteworthy that pilocarpine may render briefly chylous a lymph previously opalescent, or may, during a short period, increase the fat in one already milky (Experiments VI and VII). Either the absorption of fat from the intestine is for a brief time aided by the pilocarpine, or a larger proportion of intestinal lymph in the "mixed lymph" of the tho-

racic duct causes the latter to appear more chylous. The active movements of the intestine brought about by the drug, in association with Heidenhain's observation that much lymph may be squeezed out of the lacteals by direct pressure on a loop of the gut, makes this latter supposition probable; while the fact that the increase in fat of the "mixed lymph" appears abruptly and is transient speaks against the idea of an increase in absorption. Now, as is well known, the intestines and mesentery form the area of lymph-supply richest in lymph nodes and lymphoid tissue; and pressure changes in this area, taking place through contraction of the smooth muscle, may well be supposed to increase the output of white cells through the thoracic duct.

SUMMARY.

The intravenous injection of pilocarpine nitrate causes in the dog a rapid and considerable increase in the output of lymphocytes through the thoracic duct. The corresponding lymphocytosis induced by the drug in the blood of this animal is not profound, and increased cell-output with the lymph will explain a large part if not all of it.

Quickened lymph-flow and dyspnœic breathing are accessory in the production of the large cell-output with the lymph, but it is mainly dependent on some undetermined element. The evidence points to the mechanical nature of this element. It is probably to be sought in direct pressure from contraction of smooth muscle, as suggested by Harvey, but his observation that atropine prevents the appearance of a lymphocytosis after pilocarpine cannot be quoted in proof because atropine much slows the lymph-flow, and thus decreases cell-output.

These findings are in accord with the theory that makes mechanical factors responsible for rapidly appearing lymphocytosis. They show that there are more such factors than has been supposed. Especially do they indicate that the contribution of cells through the thoracic duct may be important in the production of lymphocytosis, and is not, as is often asserted, subsidiary to direct migration into the blood of cells from spleen, bone-marrow and the lymph-glands.

BIBLIOGRAPHY.

1. Horbaczewski, J., *Sitzungsber. der k. Akad. der Wissensch., Math-Naturwissensch. Kl.,* 1890–1, xcix–c, Abt. iii, 78.

2. Ruzicka, V., *Allgemein. Wien. med. Zeitung,* 1893, xxxviii, 345.

3. Waldstein, L., *Berl. klin. Woch.,* 1895, xxxii, 368.

4. Lefmann, G., *Verhandl. d. Kong. f. innere Med.,* 1905, xxii, 149.

5. Gasis, D., *Therap. d. Gegenw.,* 1907, xlviii, 438.

6. Harvey, H., *Jour. of Physiol.,* 1906, xxxv, 115.

7. Rous, F. P., *Jour. of Exper. Med.,* 1908, x, 238.

8. Delamere, G., "The Lymphatics," by Delamere, Poirier and Cuneo, trans. by Leaf, 1904.

9. Biedl, A., and v. Decastello, A., *Arch. f. Physiol.,* 1901, lxxxvi, 259.

10. Tschirwinsky, S., *Arch. f. exp. Path. u. Pharmakol.,* 1894, xxxiii, 155.

11. Spiro, *Arch. f. exp. Path. u. Pharmakol.,* 1896–7, xxxvii, 113.

12. Goodall, A., and Paton, D. N., *Jour. of Physiol.,* 1905, xxxiii, 20.

13. Cushny, A., "A Textbook of Pharmacology and Therapeutics," 1901.

14. Starling, E., "A Text-Book of Physiology," 1898.

15. Heinz, R., "Handbuch der experimentellen Pathologie und Pharmakologie," 1904.

16. Gulland, G. L., *Fol. Haematolog.,* 1906, iii, 637.