

THE EFFECTS OF ROENTGEN IRRADIATION UPON
THE CHANGES IN THE CELL CONTENT OF THE
BLOOD AND LYMPH INDUCED BY INJECTIONS OF PILOCARPINE.¹

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The work upon which this report is based, is practically a continuation of that done by Dr. F. Peyton Rous, in this laboratory, during the last two years. Rous (1) gives a preliminary review of the experimental work done in relation to the blood changes induced by pilocarpine, and also refers to the work of Lefmann and Gasis in determining the effect of Roentgen irradiation on these changes. He also appends to his report a complete bibliography of the subject, which has been very useful in reviewing the work done under this general topic. His work had to do, particularly, with the influence of pilocarpine on the cell content of the thoracic duct as ascertained by making counts and estimations based upon the output of the duct by way of an established fistula. From his experiments he was led to state that the intravenous injection of pilocarpine nitrate causes in the dog so rapid and extensive an increase in lymphocytes in the output by way of the thoracic duct as to explain a large part, if not all, of the lymphocytosis shown in the blood of the animal under treatment with this drug. He also confirms, by calculation based upon the total amount of blood in circulation and the cell content and rate of flow of the lymph from the thoracic duct, the conclusions of previous workers (Biedl and Decastello (2), Selinoff (3), Crescenzi (4), Parodi (5)), that the thoracic duct is the principal avenue by which the lymphocytes pass from the lymphocyte-forming organs to the general blood circulation. The various theories as to how this lymphocytosis might be

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brought about are considered critically in the body of his paper, and are left without any definite conclusion, but there is shown an inclination toward the idea of Harvey (10), *i. e.*, that the phenomenon is mainly a consequence of contraction of the smooth muscle of the spleen and lymph glands.

Before undertaking the experimentation immediately necessary to determine the point indicated by my topic, it was thought best to make a few preliminary tests in order to confirm some observations which have already been given out, also to fill out some small gaps existing in the efforts to arrive at the effects of pilocarpine on the blood elements and on the input of lymphocytes by way of the thoracic duct. The increase in white cells in the circulation after administration of pilocarpine has been so definitely established as to render it practically superfluous to repeat the observation (Horbaczewski (6), Ruzicka (7), Waldstein (8), Lefmann (9), Harvey (10), Gasis (11), Rous (1)).

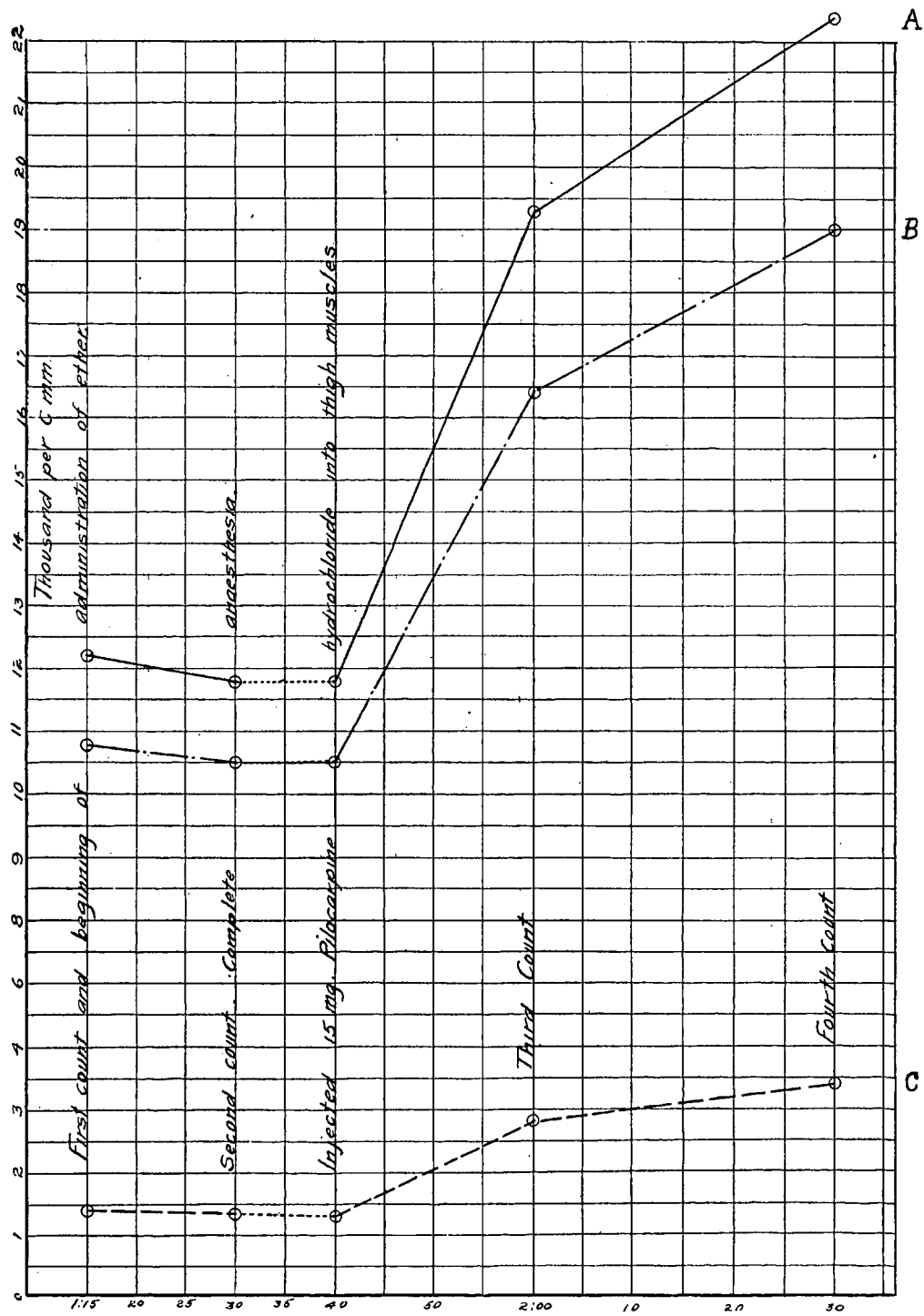
In connection with Rous's report, I may refer to an unpublished piece of work done a year ago in this laboratory by T. Dewitt Gordon. In working with pilocarpine on rabbits, he secured a great increase in white cells in the blood, characterized especially by the increase in the polymorphonuclear type of cells. As this did not seem to accord with previous tests, and especially as the rabbit can not be considered a satisfactory animal from whose blood changes any definite conclusions should be made, also because his blood counts were made with intervals of days, during which time other elements might have entered into the case so as to affect the proper changes, his work was not carried further and he made no further trials on other animals. It suggested to me, however, the fact that the "leucocytosis affecting the polymorphonuclear elements" which Rous reports as accompanying the lymphocytosis evident in his findings does not bear such a relation to the operations on the animals as to warrant attributing it at all to the surgical operations. This is clearly shown by an examination of the changes secured by Rous which I have taken the privilege to plot and describe here as Curves I, II and III.²

²In view of the detailed descriptions of Curves I, II and III these curves have been omitted.

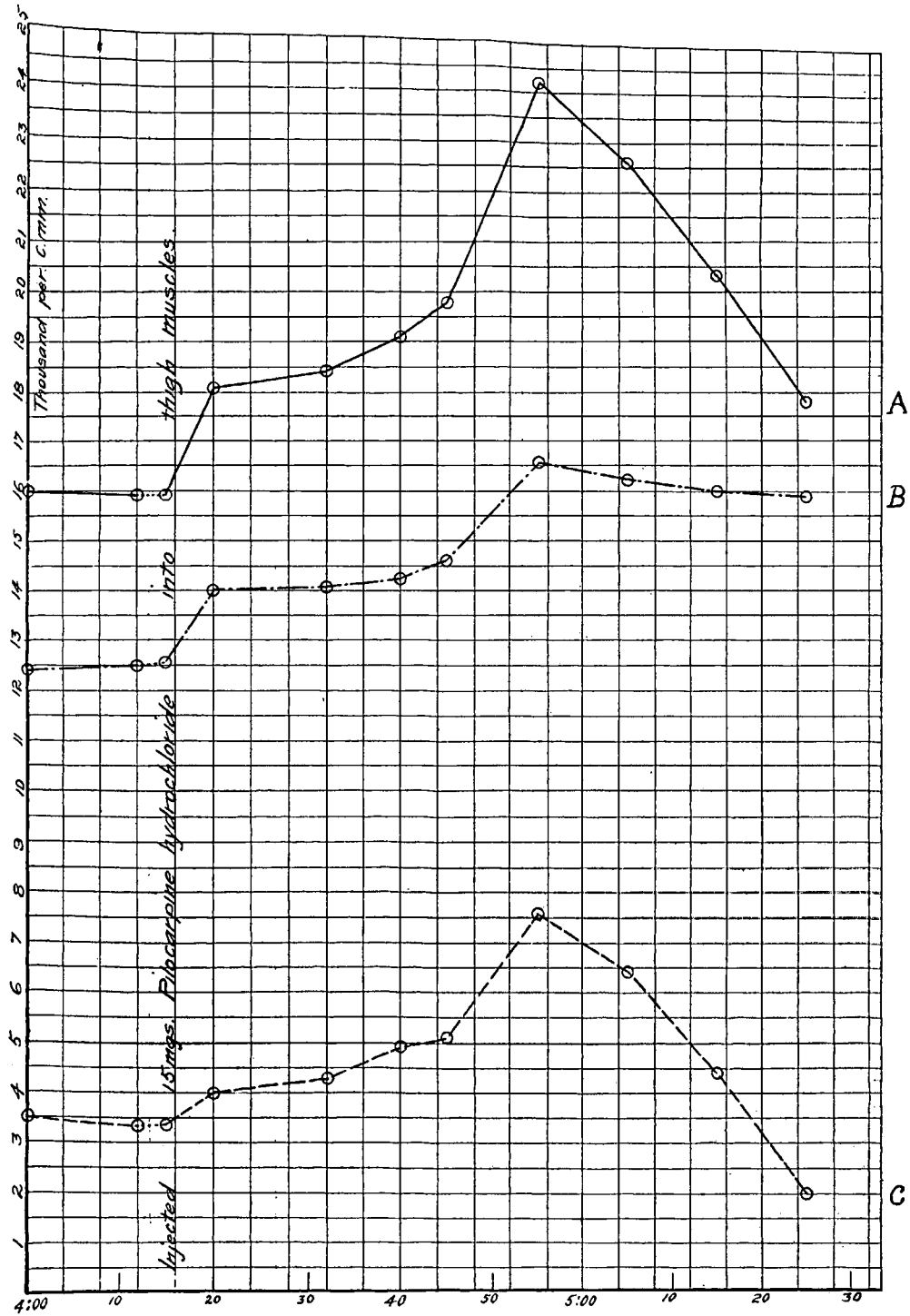
Curve I.—From this it is seen that the operation—exposure of the left external jugular vein and insertion of a cannula—was done at 9:50 o'clock. The first cell count was made at 10:15, and a second count was taken at 10:45. Now during this half hour the absolute and relative numbers of the various forms remained practically constant. The figures taken literally show a slight decrease in polymorphonuclears which, however, is so small as to be negligible. Then at 10:50 o'clock 20 mg. of pilocarpine nitrate were injected into the vein, and at the end of the next half hour the third count was made. This count showed a marked increase in number of white blood cells. Out of a total increase of 6,700 white cells per c.mm. of blood, over 5,000 constituted the increase in polymorphonuclears. Now the fact that this phenomenon was delayed until after the administration of the drug, and then appeared so promptly, seemed to me to offer reason to surmise, at least, that there might be some relation between it and the pilocarpine.

Curve Number II.—This shows the operation to have been done at 9:40, and the first count to have been made at 9:48 o'clock. A second count at 10:23 showed an increase of about 3,000 polymorphonuclears per c.mm. This, of course, had no relation to the forthcoming dose of pilocarpine which was given at 10:26 o'clock. The third count, at 11:13, gave an increase of about 1,200 mononuclears, and a further increase of over 4,000 polymorphonuclears. This gradual increase in polymorphonuclear cells from the first would appear by itself to be the consequence of the operation inasmuch as it is not materially augmented by the 10 mg. of pilocarpine.

Curve Number III.—This shows the operation to have been done at 9:30, and, unfortunately for my purpose, no count was made until the end of an hour. However, the count at the end of the hour was too small to suggest any marked increase in leucocytes which might have been instituted by the operation. A half hour later 10 mg. of pilocarpine were given intravenously, and a count made at the end of another half hour showed an increase of 6,000 white cells per c.mm. of which the increase in polymorphonuclears constituted about 5,000. Another half hour gave a further increase of only 300 mononuclear cells per c.mm., but over 2,000 polymorphonuclears per c.mm.



CURVE IV.—A.—represents total white cells per cubic millimeter of blood; B, polynuclear cells per cubic millimeter of blood; C, mononuclear cells per cubic millimeter of blood.



CURVE V.

These facts suggested to me some further experiments which I now describe briefly. First, to ascertain whether or not an operation, such as indicated above, would cause a leucocytosis so promptly and of such a degree, I exposed, after anæsthesia, an external jugular vein of a dog and inserted a cannula. A series of counts taken during the succeeding two hours showed no considerable change in total or in relative numbers of the various forms of white cells. The extreme variations were 1,200 polymorphonuclears and 250 mononuclears. This same observation was made on four other dogs subjected to other operations. There was certainly not such a leucocytosis from the operations as occurred in the animals after pilocarpine. This can be deduced from Curves VII and VIII.

The next step was to determine the effects of pilocarpine administration without any operation of consequence. The results are shown in Curves IV and V. In the first of these two cases, Curve IV, a count was made at the beginning of the anæsthetic, and another one fifteen minutes later, when anæsthesia had been established by means of chloroform followed by ether. These counts showed no considerable difference. Ten minutes later 15 mg. of pilocarpine hydrochloride were injected deep into the thigh muscles by means of an ordinary hypodermic syringe. This was followed by an immediate rise in the white blood-cell count. At the end of twenty minutes there had been an increase of 7,500 white cells per c.mm., of which the increase in polymorphonuclear cells was 6,000 per c.mm. In thirty minutes more the total increase had gone to 10,600 per c.mm. of which 8,528 were polymorphonuclear and 2,072 were mononuclear. Here we had a positive augmentation of polymorphonuclear leucocytes which can not at all be attributed to any operation, but certainly is a consequence of the pilocarpine. A determination of the percentages shows that the mononuclears increased from about 11 per cent. of the total to 15 per cent.

Curve Number V.—This represents practically the same experiment, in another dog, only prolonged somewhat, and with counts made more often. Within five minutes after the injection of pilocarpine there was a sharp increase in white cells, nearly 75 per cent. of this increase being in the polymorphonuclear type. Forty

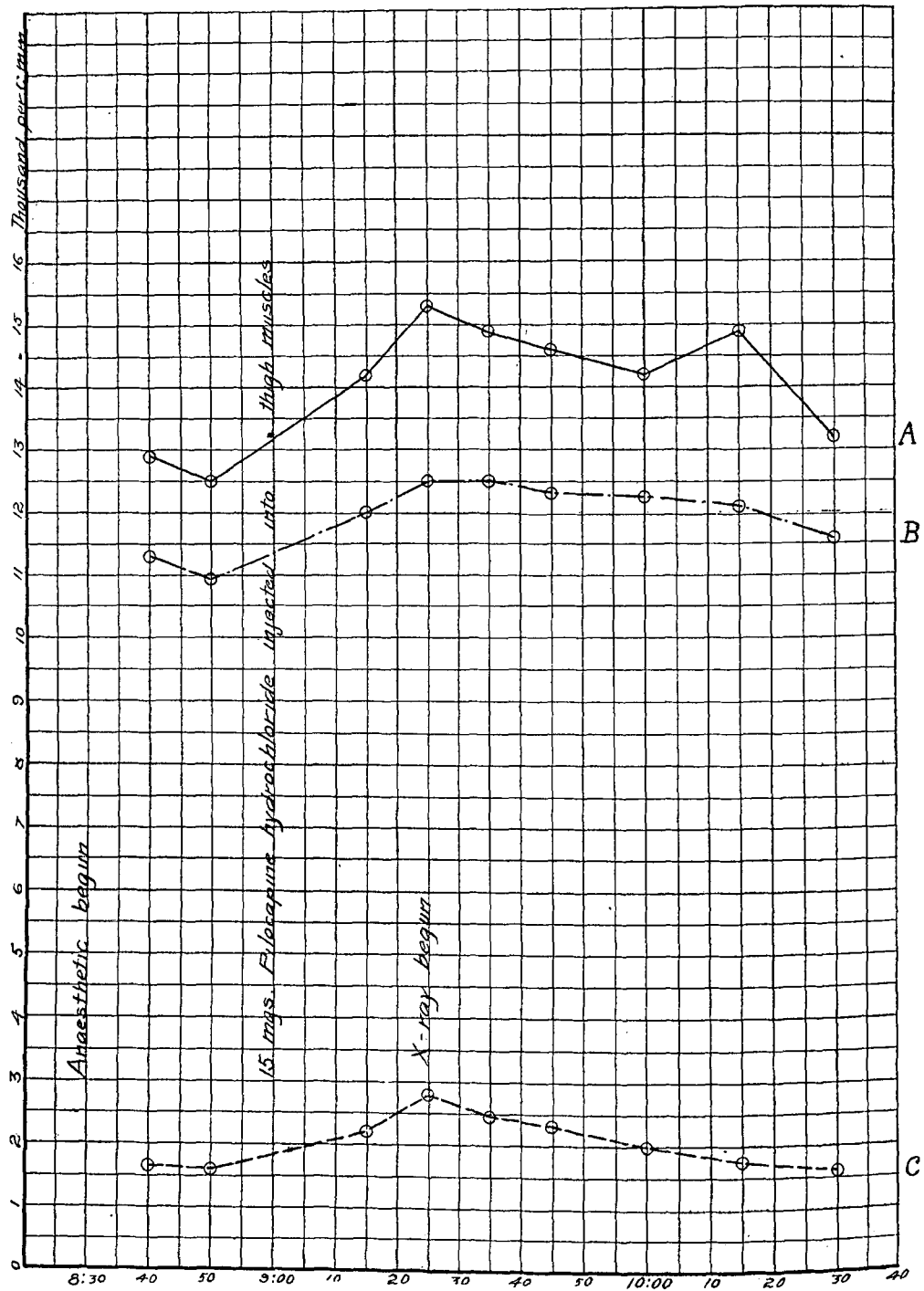
minutes after the drug was given the highest count was made. At this point the total increase consisted of 4,200 mononuclears and 4,000 polymorphonuclears per c.mm. During the succeeding thirty minutes there was a gradual decrease in the total count due almost entirely to a decrease in mononuclears. The final count showed the mononuclears to have gone 1,200 per c.mm. below the initial count, while the polymorphonuclears were still 3,200 per c.mm. above the count made at the time of beginning. This relative persistence of the polymorphonuclear increase should be given attention and will be considered further in connection with other curves showing the same feature. A determination of percentages in this case shows the mononuclears to have increased from 22 per cent. of the total to 32 per cent., and then to have gone down to 11 per cent. Such increases as are shown here, when considered on a basis of one hundred cells, would certainly attract one's attention to the lymphocytosis, as is the case in Harvey's report, but when the whole number of cells is taken into account the effect upon both types is noteworthy. The features prevailing in these experiments are also evident in the ones to be described presently, and so any conclusions which might be deduced here will be considered at the end of the next set of experiments.

The change in the lymphocyte count of the blood, produced by diverting from the blood stream the normal input by way of the thoracic duct, has been ascertained and established by several experimenters referred to above (Rous, Biedl and Decastello, Selinoff, Crescenzi, Parodi).

The following four experiments were calculated to furnish an opportunity to note the effects of Roentgen irradiation on the white blood-cells in the circulation, and on the cell content of the output from a thoracic duct fistula. The effect of the irradiation on the cell content of the circulating blood has received much attention heretofore, but, as far as I have been able to ascertain from the literature, no one has reported experiments to determine the effect on the cells in the content of the thoracic duct.

EXPERIMENT I.—Female dog, 8 Kg., Curve VI.

The dog was anesthetized by chloroform followed by ether. Two blood counts were made ten and twenty minutes, respectively, later. These counts showed about 12,900 white cells per c.mm. of which 1,600 were mono-



CURVE VI.

nuclears. At the end of a half-hour 15 mg. pilocarpine hydrochloride were injected deep into the thigh muscles of the animal. Within twenty-five minutes, the total white cell count had gone up to 15,300 per c.mm. of which 2,800 were mononuclear. At this time the animal was brought into the field of Roentgen irradiation—medium tube, eight inches from the abdomen. There began a constant decline in the number of the white cells, particularly the mononuclear type. At the end of an hour the mononuclears had been restored to the original count while the total whites were still about a thousand higher than at the beginning. This, again, shows the persistence of the leucocytosis of polymorphonuclears even in spite of the Roentgen irradiation. In this instance the interruption of the pilocarpine effect by the X-ray is evident. The picture, however, is not nearly so striking as that shown by a test reported by Lefmann (9). Starting with a lymphocyte count of about 9,000 per c.mm. he increased it by injections of pilocarpine to 17,500 per c.mm. at which time he began the Roentgen irradiation and was able to reduce the count to 3,000 per c.mm. Then he suspended the X-rays and the number of lymphocytes rapidly went up to 23,000 per c.mm. He recorded the percentage which the lymphocytes bore to the whole number of white cells. By calculation on the basis of these percentages, it can be seen that there must have been a remarkable change in the number of polymorphonuclears as well. This is shown from the following: When the lymphocytes numbered 9,000 per c.mm. their percentage was 45; when they numbered 17,500 per c.mm. their percentage was 37; and when they numbered 23,000 per c.mm. their percentage was 38. Lefmann worked with rabbits in these cases and continued the experiments over several days, which matter will be considered later.

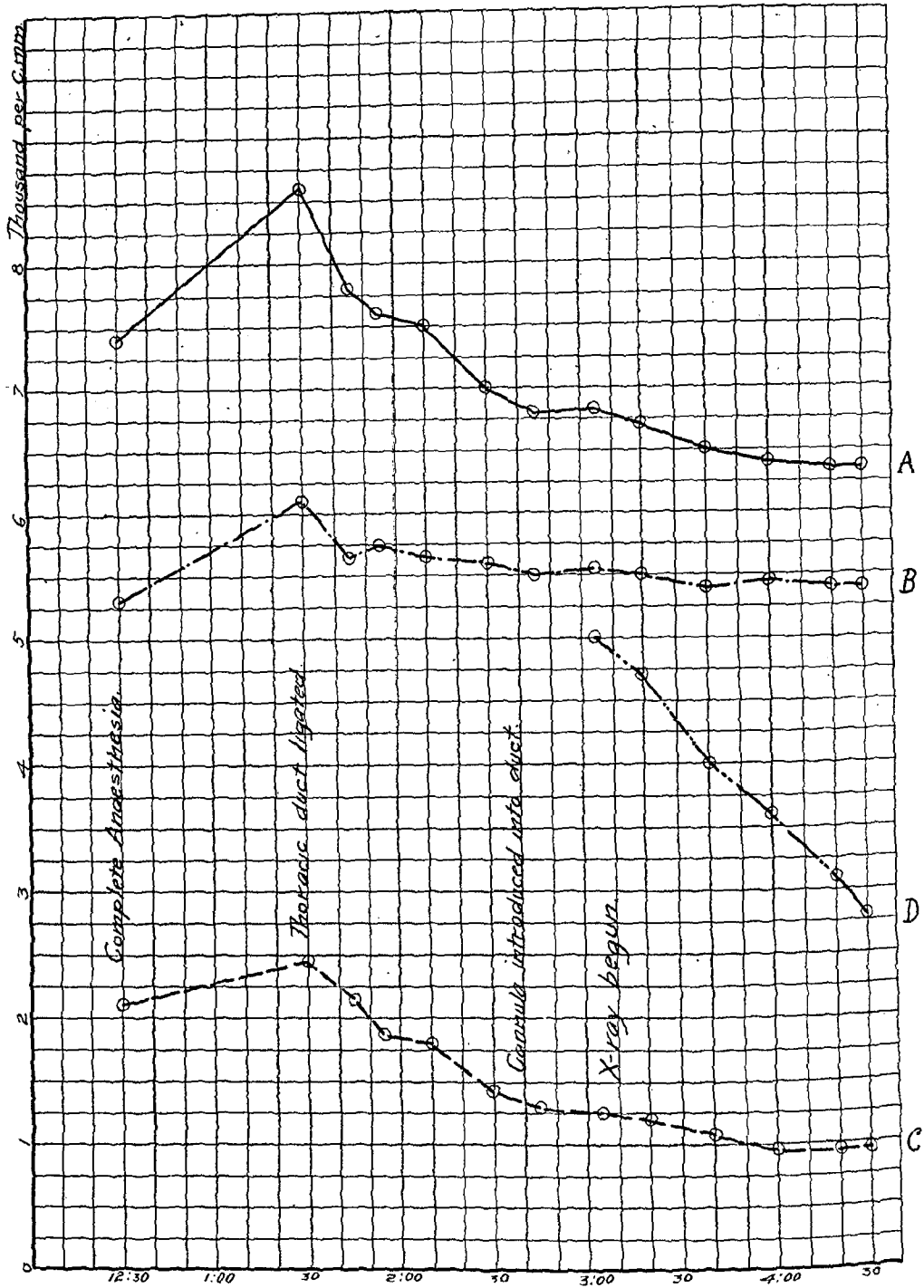
EXPERIMENT 2.—Male dog, 7Kg., Curve VII.

Anæsthesia was established by chloroform followed by ether. The initial count gave 7,400 white cells per c.mm. of blood, of which 2,100 were mononuclear. At the end of an hour the thoracic duct was ligated just before its entrance into the vein. The blood count at that time showed a rather great increase in white cells, apparently out of proportion to the operation and the time elapsed.

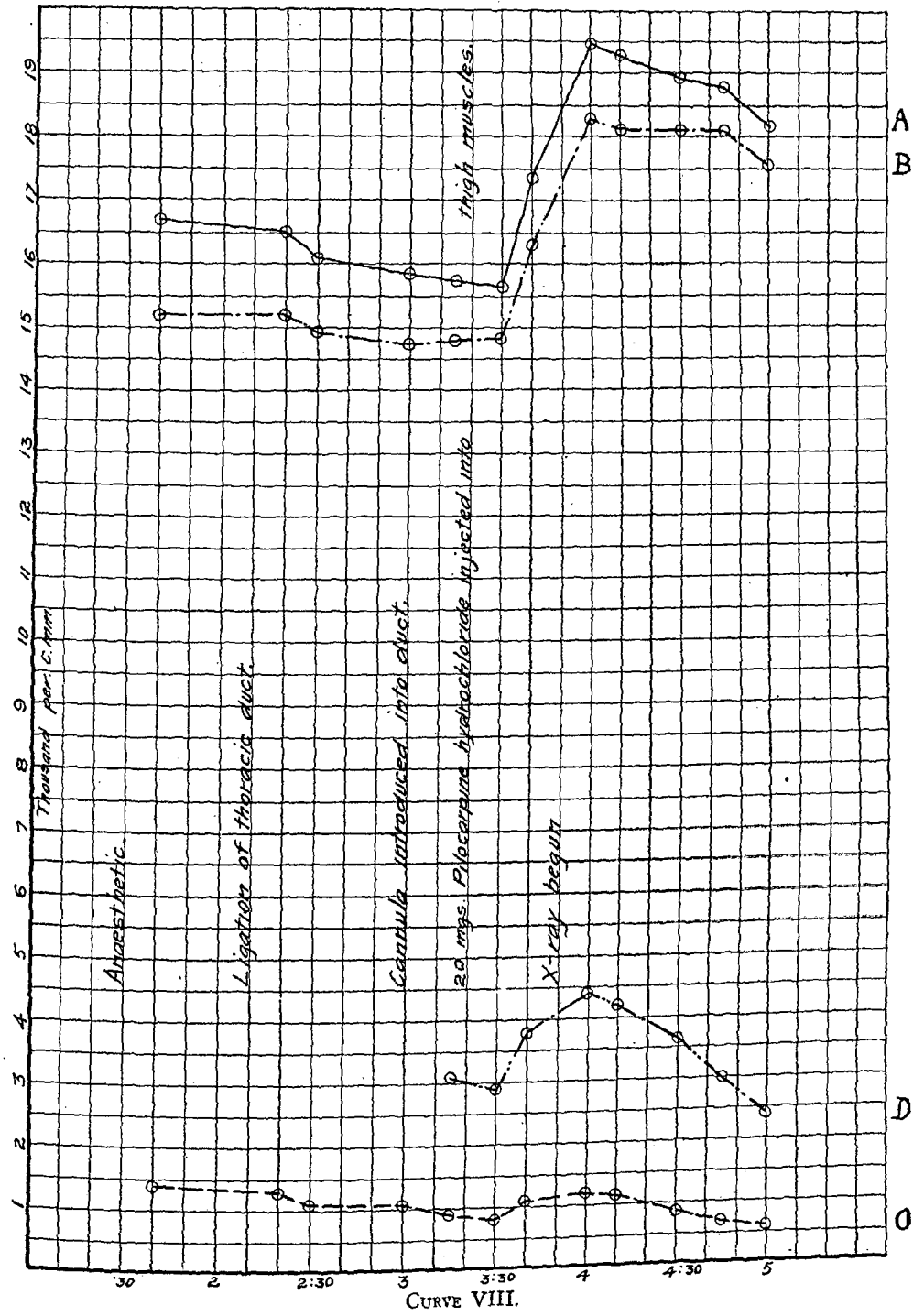
The next blood count, 15 minutes later, was near the original again, and prompts me to attribute this record of preliminary rise to error in making the count or in recording it. From then on the total whites decrease gradually, due in a great degree to a decrease in number of mononuclears. Later a cannula was introduced into the duct, and after the lymph had been allowed to flow twenty minutes, a count of the cells was made. This count gave 5,000 cells per c.mm. of lymph. Then the animal was placed in the field of Roentgen irradiation—medium tube, 8 inches from abdomen—and successive counts made of the cells in the blood, and of the cells in the outflow from the duct. In the blood the white cell number decreased generally, with some fluctuations in both types. In the duct output there occurred a definite and marked decrease in cell-content, so that at the end of an hour and a half the number of mononuclear cells had fallen from 5,000 per c.mm. to 2,800 per c.mm.

EXPERIMENT 3.—Male dog, 14 Kg., Curve VIII.

Anæsthesia was begun at 1:30 o'clock, chloroform followed by ether being used. At 1:40 the blood count showed total white cells to be 16,700 per c.mm.



CURVE VII.—D represents output of mononuclear cells per cubic millimeter from thoracic duct.



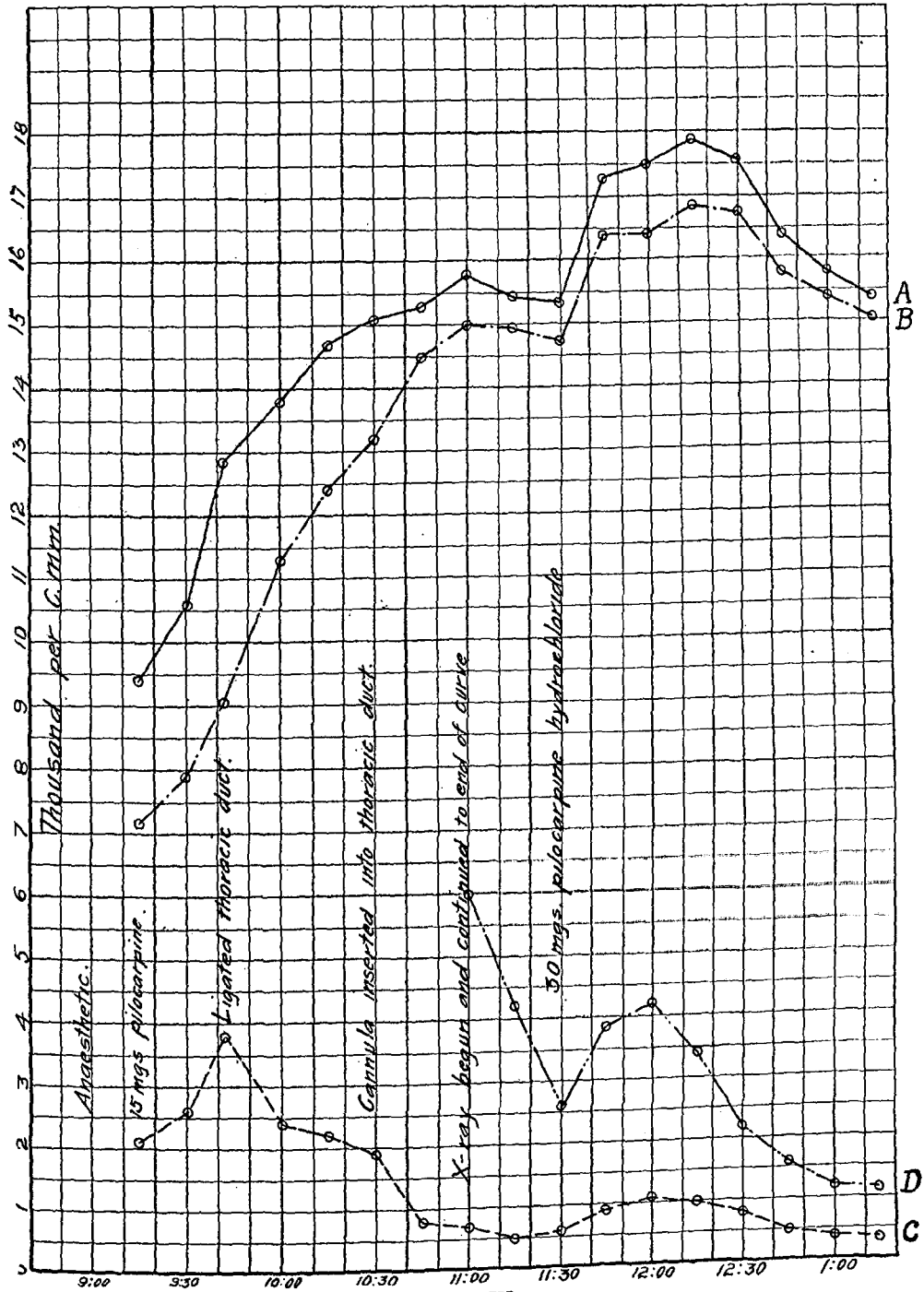
of which 1,400 were mononuclear. At 2:10 the thoracic duct was ligated, and at 3:00 a cannula had been inserted and the lymph was allowed to flow. At 3:15 o'clock the blood count showed a decrease in white cells, characterized by decrease in lymphocytes. At this time the output from the thoracic duct fistula contained 3,100 white cells. Immediately 20 mg. of pilocarpine hydrochloride were injected into the thigh muscles. The next counts at 3:30 gave still a decrease in white cells in the blood and in the duct output. At 3:40 there was an evident increase established, and the animal was exposed to Roentgen irradiation—medium tube, 8 inches from abdomen—at 3:45. The count at 4:00 o'clock gave still an evident increase of white cells in both blood and lymph, but each successive count, at intervals of about fifteen minutes, showed marked decrease in mononuclear cells in the circulation and in the lymph. At 5:00 o'clock the polymorphonuclear type remained 2,500 above the initial count, while the mononuclears were below any previous count.

This curve reaffirms the previous observations viz., (1) the increase in polymorphonuclears induced by the drug, while not so great in percentage, is remarkable in consideration of the great number of cells; (2) the persistence of the high count of polymorphonuclear cells in spite of the Roentgen rays; (3) the interruption of the lymphocytosis by Roentgen irradiation; (4) the relatively slight effect of the X-rays on the polymorphonuclear cells as compared with the effect on the mononuclear cells.

EXPERIMENT 4.—Male dog, 10 Kg., Curve IX.

Anæsthesia by chloroform followed by ether was established between 9:00 and 9:15 o'clock. Then 15 mg. of pilocarpine hydrochloride were injected deep into the thigh muscles. The white cells in the blood immediately increased. After ligation of the thoracic duct at 9:45 the mononuclear count in the blood fell, with a still increasing polymorphonuclear count. At 10:30 a cannula was inserted into the duct and the lymph allowed to flow. At 11:00 o'clock the lymph contained 6,000 mononuclear cells per c.mm., and at that time the Roentgen irradiation—medium tube, 8 inches from abdomen—was begun and continued to the end of the experiment. There followed a rapid fall in the white-cell number in the lymph, a small decrease in mononuclear cells in the circulation, and a relatively slight decrease in polymorphonuclears. I do not attribute this great fall in cell number in the lymph to be due entirely to the action of the X-rays, but in part as a consequence of the congested condition of the duct due to ligation. At 11:30, thirty milligrams of pilocarpine hydrochloride were injected. This heavy dose caused a second rise in cell counts, which, however, was soon interrupted by the continuing irradiation especially as far as concerns the mononuclear cells. At the close of the process the polymorphonuclear cells were nearly 8,000 per c.mm. above the initial count, while the mononuclear elements were very much diminished.

This curve gives reason for especial consideration, although it does not offer any features not previously shown. In the first place the action of the drug is plainly on both types of cells, with the percentage in favor of the mononuclears. Then the effect of shutting off from the circulation the usual input by way of the duct is



CURVE IX.

very striking, when we consider what the further effect of the drug would have been. Accompanying this we have the continued rise in polymorphonuclear cells, which is but slightly affected by the irradiation. Then the second and relatively large dose of pilocarpine, acting in opposition to the irradiation causes a secondary rise in all elements, which is of short duration and is succeeded by gradual and definite decline especially in the lymph.

There are some points which render it impracticable to make definite and unlimited conclusions from the foregoing sets of experiments. The variations in the thoracic duct and its tributaries render it impossible to turn completely from the circulation the great bulk of the lymph. By injecting the duct from the receptaculum upward with a colored solution various branches and irregularities become evident which could not be ascertained and taken into account in the process of establishing a thoracic duct fistula. This point might affect materially the consideration of how and where the Roentgen rays effect a diminution in lymphocytes. This will be taken up in connection with my conclusions on this point.

Another factor of consequence in calculating on the count per cubic millimeter of lymph is the variation in fluid constituent. The small variations in cell count may be only relative and not absolute. This will not affect general conclusions, but might be responsible for some of the details of a conclusion. Rous made definite note of rate of flow and absolute count of cells in the lymph and showed that practically all of the lymphocytes in the general circulation might have been supplied by this source or route.

In making the total counts and also the smears for differential counts, I made use of single drops of lymph as they appeared at the end of the cannulæ, rather than making counts from several cubic centimeters, because I considered that the latter method would not give the changes in as definite relation to time and degree on account of the time necessary to collect the bulk. The matter of struggle on the part of the animal is also of consequence (Rous (1)). In these cases the anæsthesia was maintained so completely as to preclude any struggle.

The effects of the operation must be considered. Whether or

not an operation necessary to establish a thoracic duct fistula will immediately and definitely increase the white blood-cells seems to be a question. In these cases I found no satisfactory grounds for the conclusion that such is the result.

The apparently normal variations in the relative number of the various types of cells in the dog's blood, and especially the number of cells which cannot be said positively to belong to one or another type, enter into consideration materially at times.

In making the differential counts I have discarded the cells which I could not consider with a reasonable degree of certainty to belong to a certain class.

The stability of the blood of the animal used should enter into consideration. The experiments reported as done on the rabbit should be interpreted with this in view. This is especially true when the duration of the experiments is a matter of several days, and the intervals between counts are several hours. Surely it is uncertain to attribute the great changes, even, which might then appear as consequences of the drug used or of the Roentgen rays. For that reason I have done all of these experiments on dogs and used short periods of time with heavier doses of drug and irradiation.

With these possible variations in mind, I feel justified in stating some definite conclusions from the foregoing sets of experiments.

I. *Polymorphonuclear Cells*.—1. Relatively slight operations on dogs do not cause any great degree of leucocytosis within the limits of time consumed in these experiments. The substantiation of this point has been explained under the curves calculated to give the basis for the conclusion.

2. Injection of pilocarpine hydrochloride into the muscle of a dog brings about a decided increase of polymorphonuclear cells in the circulation. This is shown in every case under consideration. The increase in percentage is usually about the same as that of the mononuclear cells, but is much greater in absolute counts.

3. The polymorphonuclear leucocytosis is more persistent than the mononuclear change. This is shown in Curve V, in which case the X-ray was not used, and in Curves VIII and IX in which cases irradiation was applied.

4. The polymorphonuclear cells are but relatively slightly re-

duced in number by the action of the Roentgen rays. This is shown in Curves VI, VII, VIII, and IX.

II. *Mononuclear Cells.*—1. In the blood—(a) The increase in number of mononuclear cells in the blood following injection of pilocarpine hydrochloride is definite. (b) This increase is interrupted by the action of the X-rays and is more susceptible to their action than that of the polymorphonuclear cells. (c) The number is materially diminished by diverting from the blood stream the normal input by way of the thoracic duct.

2. In the output from the thoracic duct—(a) The number in this output is great enough, considering rate of flow and bulk of blood and lymph, to account for practically all the lymphocytes in the blood and so gives reason for the conclusion that this is the most important route by which they reach the blood. (b) The injection of pilocarpine hydrochloride causes a marked increase in the number entering the blood by this route (Curves VIII and IX). (c) This increase is interrupted by the action of Roentgen irradiation, and the number can be reduced to below the initial count (Curves VI, VII, VIII, IX).

III. *Roentgen Irradiation.*—1. On the cells in the circulating blood there is a noticeable effect. This is shown in a diminution of all forms of white cells. In each case in which the Roentgen rays were used there is shown a much greater decrease of mononuclears than of polymorphonuclears. This is perhaps due to the fact that the parent cells of the polymorphonuclear cells were not so accessible to the action of the X-rays on account of the bone tissue enclosing them, and because the long bones of the legs were practically out of the field, while the spleen and the great numbers of lymph nodes were directly in the course of the rays and not so well protected by bone.

2. The effect of the irradiation is most plainly evident on the cell content of the lymph from the thoracic duct. In each curve this is shown. From this feature of the results it is evident that the great effect of Roentgen irradiation on the mononuclear cells in the circulating blood can be explained to a great degree, at least, by its action on these cells before they reach the circulation through the thoracic duct. The fact that not all of the lymphocytes can be diverted from the blood may be made use of to explain the changes

which apparently go on in the blood under X-ray treatment after ligation of the duct. Together with this must be considered the fact that a greater body-area than the region giving supply to the thoracic duct is being irradiated, and such changes as are produced in the blood, after ligation of the duct, may be the effects of the irradiation on these accessory regions, such as the bone marrow.

It would seem then that the theory of the production of a leucotoxin in the blood, as put forward by Linser and Helber (12) and Capps and Smith (13) is not necessary to explain the destructive action of Roentgen rays upon the white cells. As has been pointed out by Rous, there is much difficulty in classifying the cells from the thoracic duct lymph. The irregularities in the cells resulting from making the smears cause this difficulty. So in considering the effect of the pilocarpine or of the irradiation on the individual cell of the lymph, one should scarcely consider the form of the cell. From the staining reaction I could not say that the number of degenerated forms was increased after the irradiation, and should rather consider that such degenerated cells as resulted from the irradiation were retained in the blood-cell forming organs and not given into the circulation. These organs in these animals were not studied to ascertain their condition in this particular.

In reporting this investigation I am under especial obligations to Professor Warthin, who has given the work much of his attention. I also wish to thank Mr. Willey and Mr. Hill, of the Roentgenology Department, for much assistance and the use of their laboratory.

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