AN INQUIRY INTO SOME MECHANICAL FACTORS IN THE PRODUCTION OF LYMPHOCYTOSIS.[1]

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The paper that follows deals with an attempt to separate out, and study, some of the factors which produce the clinical feature of lymphocytosis. The truth of Ehrlich's (1) doctrine that an absolute lymphocytosis is due, apart from changes in the productive activity of the lymphoid tissue, to a flushing out of the cells through increase in lymph-flow, though supported by clinical evidence, has never been proved. Indeed, there have been few attempts to come directly by experiment to the forces determining lymphocytosis; this, too, despite manifold labours to plot the fluctuation of the blood-content in lymphocytes caused by divers physiological and pathological conditions.

For the experiments here detailed the cell-output by way of the thoracic duct was utilized. Of late this path to the blood for lymphocytes has been held to be of comparatively little importance. The recognition of lymphoid centers in the bone-marrow, the study of the abundant lymphadenoid tissue of the digestive tract, the observations for a direct passage of the lymphocytes into the blood-vessels, and the realization that the lymph has important functions of its own, have tended to this conclusion, as have the many assertions that the lymph of the thoracic duct carries few lymphocytes in comparison with the blood's needs. Nowadays, as Delamere (2) says, we hold that "the lymphocytes are the casual guests of the lymph." They are supposed to be formed in the lymph-glands and the lymphadenoid tissues in general, the spleen, and the bone-marrow, with direct entrance through the vessel-walls as a frequent way by which they reach the blood.

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Nevertheless, recent experimental evidence points to the thoracic duct as the chief way to the circulation for the lymphocyte. Biedl and v. Decastello (3), working on dogs, produced fistula of the thoracic duct, and found that the lymphocytes in the blood decreased between 18 per cent. and 62 per cent. in absolute number; suspecting accessory channels, they carefully ligated the lymphatics on both sides of the neck, and obtained in the one animal so treated a diminution in the lymphocytes of 79 per cent. Selinoff (4), in a study of the blood of 18 dogs with fistula of the thoracic duct, noted an even more marked decrease. Thus, for example, in two of his cases there were, respectively, 1,800 and 2,000 lymphocytes per cubic millimeter in the blood just prior to operation, and on the fifth day thereafter, in the first case, only 100 such cells, and on the seventh day, in the second case, only 200 such cells per cubic millimeter. He made certain by controls that these results could not be laid to the effects of the operation itself. Crescenzi (5) observed the blood after splenectomy and the establishment of a fistula of the thoracic duct in the same animals. He obtained a decrease in the lymphocytes of from four fifths to ten elevenths of their number. Parodi (6) following Crescenzi and Selinoff, came to the conclusion that, in dogs, fistula of the thoracic duct, with or without splenectomy, brings about a diminution in the quantity of lymphocytes in circulation. Unfortunately he omits the figures supporting this. Yet those cited above seem convincing when one considers the direct anastomoses known to exist between the lymphatics and the blood-vessels (Lippi, Boddaert, Leaf (7)), and the undoubted migration of some lymph-cells directly through tissues into the blood-stream. True, the diminution that the figures represent is transitory; but this only emphasizes the presence of a compensatory mechanism that must mask, to an extent, the full effect of the fistula.

It has been objected that the number of cells furnished to the blood through the thoracic duct is quite inadequate to maintain the percentage of lymphocytes seen. But the important element in such calculations,—the term of existence in the circulation of the individual cell,—is not known. The number coursing through the thoracic duct (from 2,000 to 7,000 in the cubic millimeter of dog's

lymph—Winternitz (8)) may be quite adequate, as Biedl and v. Decastello are at pains to show, for the needs of the circulation.

Whether or not the thoracic duct furnish the majority of the lymphocytes to the blood, as above indicated, the system of which it is the outlet,—a more or less completely "closed" system with one principal duct,—is the part of the hæmatopoietic apparatus most accessible to direct investigation as regards variation in cell-output.

The cell-content of the lymph has been comparatively little observed, and this mostly before the discovery of the bone-marrow as a blood-forming organ, and hence before the study of the blood-cells in its modern sense. Following Virchow's (9) demonstration of the identity of the "small mononuclear" with certain elements found in lymph-glands, several observers showed these cells to be more numerous in the lymph coursing from a gland than in that coming to it (Heydfelder, Brucke, Frey (10)). Löwit (11), counting the elements from the thoracic duct of the rabbit, obtained a great increase in them by the administration of substances causing blood-leucocytosis,—a result which has since drawn some criticism. Winternitz took lymph from the vessels of the dog's thigh, following the injection of turpentine into the corresponding foot, and came to the conclusion that with inflammation of a part the cell-content of the lymph coming from it is increased, and the majority of the cells becomes one of polymorphonuclear neutrophiles. Goodall and Paton (12), during an investigation on digestive leukocytosis, made counts from several points in the lymphatic system, but with very irregular results, except for evidence that pointed to a sedimentation of the cells in the receptaculum chyli. Recently Forgeot (13) has examined the lymph of ruminants as it escaped from a thoracic duct fistula, with no constant findings, however, beyond that of a greater cell-content in the fluid from young individuals. There have been no adequate researches on the cell-content of the lymph under varying physiological conditions. Yet, needless to say, the quantity of cells in the lymph represents one side of the activity of the lymphadenoid tissue; and variations in this quantity, in addition to their value as an index to the state of that tissue, have a bearing on clinical lymphocytosis and lymphopænia, and, ultimately, on the meaning of the lymphocyte.

To obtain a count that represents the average number of elements in the lymph flowing at a certain time, one should obtain a thorough mixture of a considerable quantity of it. For, by reason of the inconstancy of lymph-flow, as tending to a sedimentation of cells, and the anatomical arrangement of the lymphatics, which prevents the mingling of successive portions of the fluid, it follows that the individual drops, as they come from the vessel, must differ much in cell-quantity. Nevertheless one finds that of the few authors who have interested themselves in the cell-content of the lymph, practically all have taken their counts from the single drop,—which accounts for much of the irregularity in their results. Those above cited did this, except Forgeot who, in his work on ruminants, utilized one quarter of a cubic centimeter,—an extremely small quantity, considering the large size of the animals on which he experimented. Dastre, Henri, and Stodel (14), in an investigation of the effect of peptone on the cells, allowed the fluid to collect in the ligated end of the thoracic duct, or in the subclavian vein, there mixing it "by light, inconstant pressure." But, in addition to awkwardness, this measure gives the opportunity for large error in successive counts. I have employed, for the work here reported, a means whereby could be utilized a quantity of lymph sufficient to insure a result representing the average cell-content of the lymph at that time. Since dogs were the animals used, and the thoracic duct the point of collection, several cubic centimeters were deemed necessary for each test, owing to the large lymph-flow (64 c.c. per kilo per diem, or, in a dog of 18 kilo, 4 c.c. in every 5 minutes,—Heidenhain (15)). The following technic was adopted:

Three cubic centimeters of lymph are allowed to flow into a tube that contains an equal quantity of a 4 per cent. solution of sodium citrate in 0.8 per cent. salt solution,—a mixture suggested by Wright (16) for the preservation of blood unclotted and with its elements intact.[2] The tubes for this purpose are 9 mm. in bore, and are graduated accurately to 3 c.c. and 6 c.c. By reason of the

[2] Wright recommends the use of 1 part of the sodium citrate solution to 5 parts of human blood. So little of the solution will not keep the dog's lymph from clotting. A mixture of the two in equal parts is just sufficient to serve the purpose.

narrow caliber it is easy to control an error in volume to within the limits of a single drop, and, with care in the preliminary introduction of the sodium citrate solution, to confine this error almost entirely to the amount of lymph added. But let us suppose in the combined bulk of 6 c.c. (or about 90 drops) the largest tolerable error,—that of one drop in the quantity of sodium citrate solution added, and of one drop in the total mixture. The extremes here possible are, 44 drops sodium citrate solution to 47 drops lymph, and 46 drops sodium citrate solution to 43 drops lymph; or 47/91 of lymph in the first and 43/89 in the second mixture, a variation from the supposed ratio (45/90) of slightly over 3 per cent., or an error of 150 cells in a count of 5,000. This may be neglected.

FIG. 1.

The lymph as it falls from the cannula into the sodium citrate solution is mixed with this by means of a fine wire, and, when the 3 cubic centimeters have been obtained, a few glass beads are introduced to aid in the distribution of the cells on shaking, and the tube stoppered preliminary to this. For stoppering a piece of glass rod with a flange of rubber is used, a capillary opening through the center of the rod permitting the escape of air so that the stopper may be pushed flush with the fluid. (*Vide* sketch in cross-section.) The closed tube is shaken for 5 minutes; a portion of its contents drawn into a "melangeur" with 1/100 its bulk of a saturated aqueous solution of methyl violet (5B); this in turn shaken for three minutes, and a count made as for blood. The lymph is thus counted in about 1/2 concentration (99 parts lymph to 101 parts diluting fluid). Leukocytes take the violet stain, whereas erythrocytes do not.

To test the method counts were made at intervals from the same tube of lymph-sodium-citrate mixture.

Thus at the end of seven hours the leukocyte-count coincides, practically, with that first taken. The cells are undergoing degenerative changes at that time, yet may be easily enumerated. But it is necessary, for this, that the tube be agitated at least once per hour. Otherwise, the cells sediment, cohere, and cannot be easily distributed again.

Dog.	Tube No.	Time between Counts.	R. b. c. per cmm. Lymph.		W. b. c per cmm. Lymph.	
			At First Count.	At Second Count.	At First Count.	At Second Count.
Jl	V	2 hours	not tested		3,880	4,240
El	XII	2½ hours	440	440	11,740	12,360
Nl	VII	2½ "	39,840	22,240	8,980	10,080
Ol	VI	3 hours	3,940	1,810	not tested	
Ml	V	3½ hours	5,080	3,720	3,300	3,660
Ll	II	4 hours	18,560	10,960	11,920	11,180
Gl	III	5 "	not tested		13,120	13,440
Kl	I	5 "	1,800	1,000	2,400	2,120
Ol	I	5 "	not tested		6,760	6,900
Pl	I	5 "	3,120	1,180	2,760	2,800
Jl	III	7 "	14,720	12,800	4,060	3,800
El	X	18 "	not tested		7,040	6,440

It is different with the erythrocytes. These seem to disappear rapidly in the mixing fluid, notwithstanding the fact that, to judge from the unchanged or slightly crenated shape of such corpuscles as remain, and the absence of shadows, this destruction is not due to osmotic changes. Since the lymph of nearly all dogs contains red cells, an idea of their quantity, granting them to be due to blood-contamination, is important in work having to do with the white cells of the lymph, since it furnishes an index to the number of leukocytes also brought in from the blood. But if, on the meeting of the lymph with the sodium citrate solution, many of the red cells go immediately to pieces, this index is destroyed; and one might have in the specimen many white cells from the blood without trace of the contamination, so far as red cells are concerned. This possible source of error was tested for as follows:

Dog Cl.—Blood taken during the experiment had 8,600,000 r.b.c. and 13,800 w.b.c. per cmm., of which last 70 per cent. proved to be polymorphonuclear neutrophiles, giving thus 9,660 such cells in the cmm. of blood, or about 1 to every 890 r.b.c. The lymph at this time contained on count from the sodium citrate mixture 1,545 w.b.c. and 5,685 r.b.c. per cmm. Calculating from the ratio existing in the blood, one should have 6 polymorphonuclear neutrophiles introduced with these red cells. A differential count of the lymph obtained at the time will test this supposition, since the normal lymph of the dog contains extremely few polymorphonuclear neutrophiles of its own (Delamere, Biedl and v. Decastello). As a matter of fact, in this instance the lymph showed out of 384 cells counted 2 polymorphonuclear neutrophiles, or 8 in the 1,545 w.b.c. of a cmm. So the number of red cells found in the lymph seemed to be practically that introduced from the blood. Several controls of this type gave the same result.

Despite this proof that the sodium citrate solution is, for *prompt* enumeration, a medium wherewith can be obtained an approximate estimate of the red cells, no lymph was admitted for the white count, of which the erythrocyte content was large enough to suggest that the accompanying leukocyte contamination might influence appreciably the results. The number of polymorphonuclear neutrophiles found in the lymphs used formed, as above shown, an additional indication of the amount of this contamination.

In the work here detailed the lymph's content in white cells is alone dealt with.

The effects of muscular exertion (struggle) on the cell-content were first observed. Adult dogs were employed. They were given 0.5 centigramme of morphia sulphate per kilo of body-weight (Nolf (17)) 1 hour before the operation, and chloroform when necessary during it. For from 24 to 48 hours prior to the operation no food was allowed the animals, though they were provided with water. The thoracic duct was bared in the usual way, a cannula introduced into it just above its entrance to the vein, and the entrance tied off together with such lymphatics from the neck as joined the thoracic duct, with the result that the fluid brought by the thoracic duct proper was alone collected. During this procedure very little blood, at most 3 to 4 cubic centimeters, was lost. The cannula used was of narrow bore, as recommended by Nolf, since the rapid flow through such a tube allows little opportunity for clotting. Nevertheless, in about half the experiments a delicate clot formed within the cannula in the course of some minutes, so that the occasional use of a fine hooked wire was required to keep the bore clean. No tubes of lymph were counted in which the least clotting appeared, nor were any used regarding which it seemed possible that clots in the cannula might have altered the gross cell-number. The presence or absence of clotting is mentioned in the report of the individual experiments.

It was first necessary to observe the variations in the lymph's cell-content under the circumstances above outlined and with the animal quiet, since these circumstances do not imply an absence of changes that might affect the cell-content. The shunting of the lymph from the body, following the opening of the thoracic duct,

produces marked alterations in the body-fluids (the blood, for example, concentrating, the lymph becoming less in amount and of different character). This might affect the lymph's cell-content. Furthermore, as the experiment progresses, the effect of the morphia wears off and chloroform must be pressed into service. Other unavoidable changes might be cited. The behavior of the cell-content under these influences must be reckoned with before one can proceed.

Accordingly, in animals carefully anæsthetized to a state of quiet, though not of complete muscular relaxation, a lymph-fistula was established, and specimens of lymph collected at short intervals during the next several hours. The time required to obtain each portion of three cubic centimeters was carefully noted as indicating the rate of lymph-flow at that period. Full records were also kept of all restlessness of the animal, of the incidents of anæsthesia, etc. When important, these are included in the description of the experiments.

Experiment I.—Mongrel collie; male; wt. 13 kilo. The ainmal was given no food for 48 hours previous to experiment. Throughout the time of lymph-collection it was quiet, except for occasional tremors in the limbs. Lymph very slightly opalescent; no clotting noted in cannula or tubes. Seven tubes were taken, and immediate estimate made of their content in white cells.

The dog at autopsy proved to have been sound, except for a chronic thickening of one segment of the tricuspid valve; no evidences of functional insufficiency of this valve.

The results are best expressed in the form of a chart. (Chart 1.)

Of the three curves on this chart one represents the rate of lymph-flow, a second the number of cells per cubic millimeter of lymph, and the third (which is the resultant of these two) the total cell-output in a given period.

It will be observed that throughout the course of the experiment the lymph-flow gradually but steadily lessened in rapidity, and hence in amount voided. This is, of course, no new finding (Lassar (18), Heidenhain and others). The number of cells per cubic centimeter of lymph, the "cell-concentration," as it will henceforth be termed, remained nearly constant, sinking slightly at the last count. It follows from these findings that the total cell-output underwent a marked gradual diminution.

To test these results two similar experiments were done.

Experiment II.—Collie; male; wt. 23 kilo. The animal was given no food for 48 hours previous to operation. Lymph began to escape from the thoracic duct (which had been ligated 1 minute before opening) 15 minutes prior to the collection of the first tube for cell-estimation. It was very slightly opalescent; no clotting was observed. Counts taken in each case immediately after collection. Eight tubes were obtained at half-hour intervals. Throughout, the animal was absolutely quiet. (See Chart 2.)

Dog killed and autopsied; it proved to have been quite healthy.

Here the same lessening of the lymph-flow is noted. The cell-concentration, markedly greater than in Experiment I, fluctuated

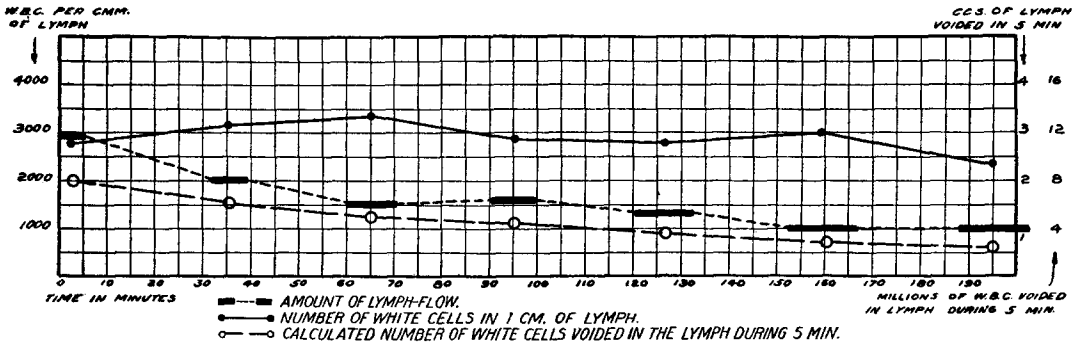

CHART I.

CHART I. 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.

proportionately, but in general remained constant during the first 2½ hours, after which it rose abruptly. The cell-output fell till toward the close of the experiment, when it regained nearly its former level.

Experiment III.—Skye-terrier; male; wt. 11 kilo. The fast before operation was of 24 hours duration, yet the lymph was quite chyliform throughout the period of observation. Clotting in the cannula necessitated several times the use of the hooked wire to avert blocking of the lymph-flow. The thoracic duct was ligated one half hour, and opened 15 minutes before the collection of the first tube for cell-estimation. The contents of the tubes were submitted to count in the order of their collection, but not till 3 to 4 hours after it, that is to say

at the close of the experiment proper. Seven tubes were collected at intervals in a period of 240 minutes. (See Chart 3.)

Animal normal, to judge from findings at autopsy.

A gradual drop in the rapidity of the lymph-flow occurred, similar to that in the other experiments, except for the presence of two transient fluctuations, apparently traceable to respiratory changes. The cell-concentration remained practically constant throughout the four hours, at the end of which it differed by only 100 cells per

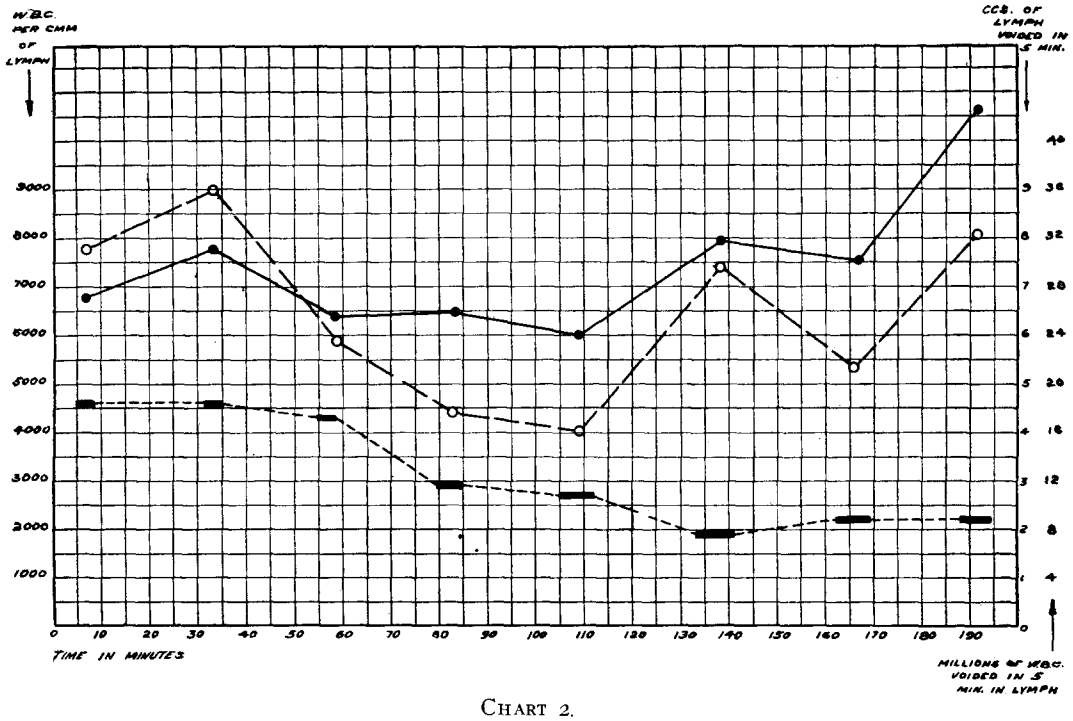

CHART 2.

cubic millimeter from that at the beginning. The total cell-output diminished, except during the fluctuations in lymph-flow above noted.

The results from the three animals form nearly a unit and are best discussed together. In all were observed:

1. A gradual decrease in the amount of lymph voided. This is no new finding.

2. A cell-concentration that varied little during the first 2½ hours of lymph-fistula. Quantitatively the variation accords with the degree of cell-concentration involved, being greatest in Experiment I, with its high cell-concentration (averaging 6,981 cells per cubic millimeter, from which there is a variation of 997 cells, or 17.6 per cent.) and least in Experiment II (in which the cell-concen-

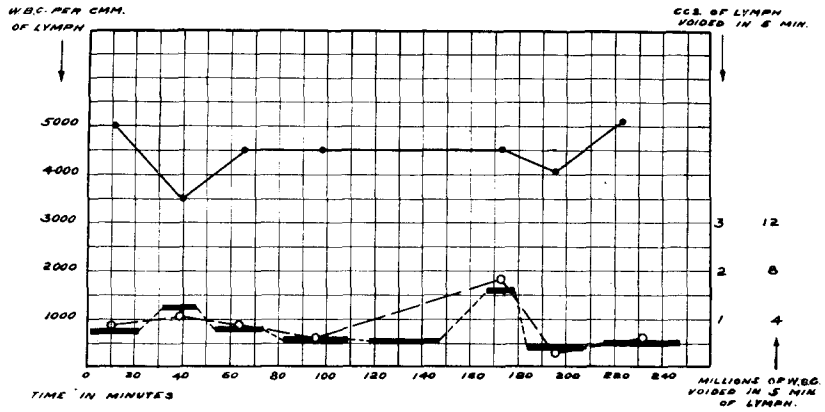

CHART 3.

tration averages 2,886 cells per cubic millimeter, and the variation is 506 cells, or 14.3 per cent.). In Experiment III the variation is 21.8 per cent.

So much for the cell-concentration during the first 2½ hours. Later, in one case it rose markedly, in another fell slightly, in the third remained unvaried. The only previous counts under conditions somewhat similar are those of Forgeot already referred to. The animals he employed (ruminants) were not anæsthetized, and his results show a variation, often of many thousand cells per cubic millimeter, in the counts from hour to hour. One understands this better on consideration of the small quantity of lymph he used in his estimations, the clotting that he frequently had to do with, and the absence of precaution to prevent struggle, which, as will later be shown, has a profound effect on the lymph's cell-concentration. Of fifteen animals on which he made observations, often over a period of more than 24 hours, the lymph in eight showed in general a tendency to lessened cell-concentration, in six, apart from large

transient variations, there was no change, and one exhibited an increase.

For our purposes it may be accepted that in the fasting dog anæsthetized with morphine and chloroform the cell-concentration of the lymph escaping from a fistula of the thoracic duct, is, during the first 2½ hours, fairly constant, when the animal is quiet and the lymph formation is suffered to take place undisturbed. The variation in cell-concentration during this period is not greater than 25 per cent.

3. The total cell-output, apart from transient fluctuations dependent on those in the lymph-output and cell-concentration, showed a decided tendency to lessen.

Here an interesting point presents itself for discussion: How is a constant cell-concentration maintained during 2½ hours under the conditions of a slowing stream of lymph, and a diminishing total cell-output? The explanation is not evident.

According to Ehrlich, the lymph-cells, on their maturation, are caught up by the lymph-stream and transported passively into the blood; or, as he puts it in another connection, "one is obliged to conclude that a lymphocytosis occurs, when, in response to an increased circulation of lymph in a greater or less extensive lymphatic region, more elements are mechanically forced from the lymph-glands." [3] Can one suppose that here the gradually lessening cell-output occurs because the lymph, through slowing, is rendered unable to force from the glands, and transport, its usual quota of cells? Or do the lymph-cells sediment along the course of the vessels in which they travel? Goodall and Paton, on the basis of counts from the receptaculum chyli, hold that some sedimentation in this reservoir is normal. Or do the lymph-glands, under the circumstances of the experiment, fail progressively in the maturation of cells? Any one of these happenings might explain the case.

Struggle, as will later be shown, increased promptly and markedly the cell-concentration of the lymph, although previously, under conditions of quiet, it had been lessening gradually. Thus it is

[3] The observations for an "active lymphocytosis" (Almkvist (19), Wolff and v. Torday (20), Proscher (21)) do not affect this conclusion, since they are concerned, not with lymphocytosis of the blood, but with the emigration of lymphocytes into the tissues and body-cavities.

shown that the glands are not lacking in cells fit for output. So the third hypothesis falls to the ground. One is left to explain in mechanical ways the constant cell-concentration in a lymph diminishing progressively in amount voided. One may suppose that the slowed current is not capable of transporting all of the many cells ready for it, and that, of those it picks up, some "sediment" on the way to the thoracic duct. The late rise in cell-content in Experiment II might, perhaps, be cited as an instance in which, despite these factors, the lymph became crowded with cells from the accumulation of those ready for it. In any event, the fact that the cell-concentration remains so long unchanged is surprising; one would expect to find immediately such variations as showed themselves only after several hours. Yet that the results are not (as might be supposed from Forgeot's work) examples of coincidence, is shown by the charts illustrating the effects of muscular exertion and of lymphagogue action. In these, despite varying cell-concentrations with varying physiological states, the same tendency to a constant cell-concentration is noted to occur hand in hand with a diminishing lymph-flow.

Discussion on the difference in average cell-output of the individuals will be reserved at this point.

With these results as a control, the effects of muscular exertion were taken up. It has been long known that this greatly accelerates the flow of lymph (Genersich, Lassar, Cohnheim (*22*)), but there have been no observations of its effect on the lymph's cell-content. A priori, on the theory that an increased output of lymphocytes is due, apart from special activity of the cell-forming tissues, to the flushing action of increased lymph-flow, one should find a transient increase in cells, traceable partly to those elements washed from the lymph-glands, and partly to those caught up from the channels by the swift current. But the existence of this increase, its amount, its duration, its effect on the blood, are all matters of conjecture.

In the experiments that follow, the animals were treated as those previously, except that they were at intervals made to struggle. Since morphia sufficed for the most part as anæsthetic, this was easily accomplished by giving a strong whiff of chloroform, or by tweaking the skin, or by an abrupt noise.

Experiment IV.—Irish setter; male; wt. 20 kilo; no food for 24 hours prior to operation. The thoracic duct was ligated, and a cannula introduced into it 5 minutes before the collection of lymph-specimens began. The lymph was slightly opalescent at first, later it was clear and yellowish; no clotting in cannula or tubes. The periods of struggle are noted on the chart. The cell-counts were made in the order in which the tubes were obtained, and between three and four hours after the collection of each one.

No autopsy done.

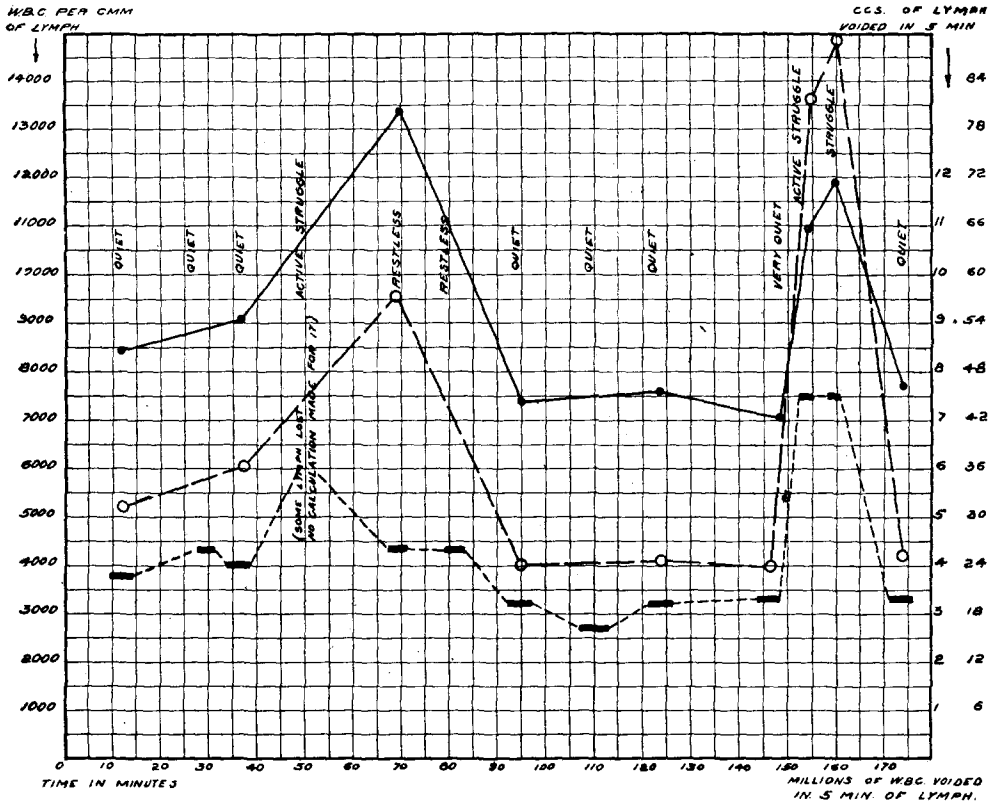

CHART 4.

In this instance struggle was twice induced. Each time the lymph-flow quickened abruptly and considerably, but with the return of quiet sank to below its former level. Each time, too, the cell-concentration became much greater. Thus the cell-output as a whole was multiplied. Indeed, it was necessary in this, and in the succeeding charts, to flatten the curve representing the cell-output.

With the return of quiet the cell-concentration and cell-output sank to slightly below their level previous to the exertion.

The next experiment was similar, but the lymph-specimens were collected at very short intervals.

Experiment V.—Bull-terrier; male; wt. 18 kilo; no food for 24 hours before operation. The duct was ligated, and opened, 4 minutes before the beginning of lymph-collection. Lymph at first slightly opalescent, yellowish and clear toward close; no clotting in tubes or cannula. The cell-estimations were made in the order of tube-collection, and 2 to 3 hours following this.

Animal at autopsy proved to have been sound. A tape-worm and several round-worms were found in the intestine.

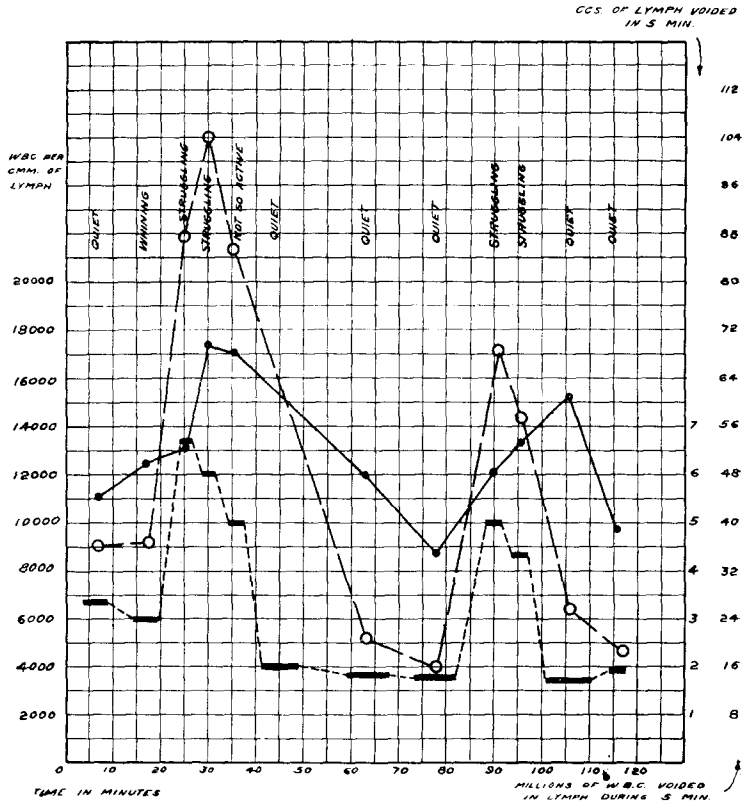

CHART 5.

The findings corroborate those of Experiment IV. Further, it is apparent that the greatest cell-concentration was not coincident

with the beginning of struggle, nor with the greatest lymph-flow, but came later. Indeed, that induced by the second struggle appeared after exertion had ceased. This second struggle did not bring out such a flow of lymph or mass of cells as did the first (which was similar in intensity); and, following both, the lymph-output, cell-concentration, and cell-output, all sank to below their level previous to the exertion.

In the next experiment the struggle was purposely made very long.

Experiment VI.—Spaniel; male; wt. 11 kilo; no food for 24 hours before operation. The duct was opened 10 minutes previous to the experiment proper, after it had undergone 5 minutes ligation. With whiffs of chloroform and tweakings of the skin a continuous struggle lasting 35 minutes was maintained. The lymph was slightly chyliform throughout; no clotting in tubes or cannula. Cell-counts were made in the order of tube-collection and between 2½ and 4 hours after this. (See Chart 6.)

At autopsy several tape-worms were found in the intestines.

This chart, while in general like the others, shows that the increase in cell-output during struggle is neither transient nor small. It endured so that at the end of the 35 minutes exertion there were being emptied from the thoracic duct 1½ times as many cells in each 5 minutes as during the preceding quiet. In the 35 minutes of muscular activity 48 cubic centimeters of lymph, containing an average of 5,100 white cells per cubic millimeter, were voided, as compared with 21 cubic centimeters of lymph, containing 3,100 white cells per cubic millimeter, in the 35 minutes just previous, or slightly over twice as much lymph, and, in sum, nearly four times as many cells as when the animal was quiet. Immediately following struggle there was a great fall in the total cell-output, and during the next 50 minutes it held to a low level.

In this series of observations the effects of five struggles were noted, and, during the work on lymphagogue action (*q. v.*), the effects of three more. They agree in these results:

(*a*) Struggle causes the cell-concentration of the lymph to become much greater. An attempt was made to test the parallelism of this increase with that observed in the lymph-flow, by collecting specimens at short intervals of time. This was done in Experiments V, VI, and during the struggle in Experiment VIII. The

charts of these prove that the maximum cell-concentration appears after considerable struggle-lymph has been voided, and at a time when the rapidity of lymph-flow is lessening. In one instance it was present in the slowly flowing lymph obtained on the return of quiet. These facts bear on the problem of whether the

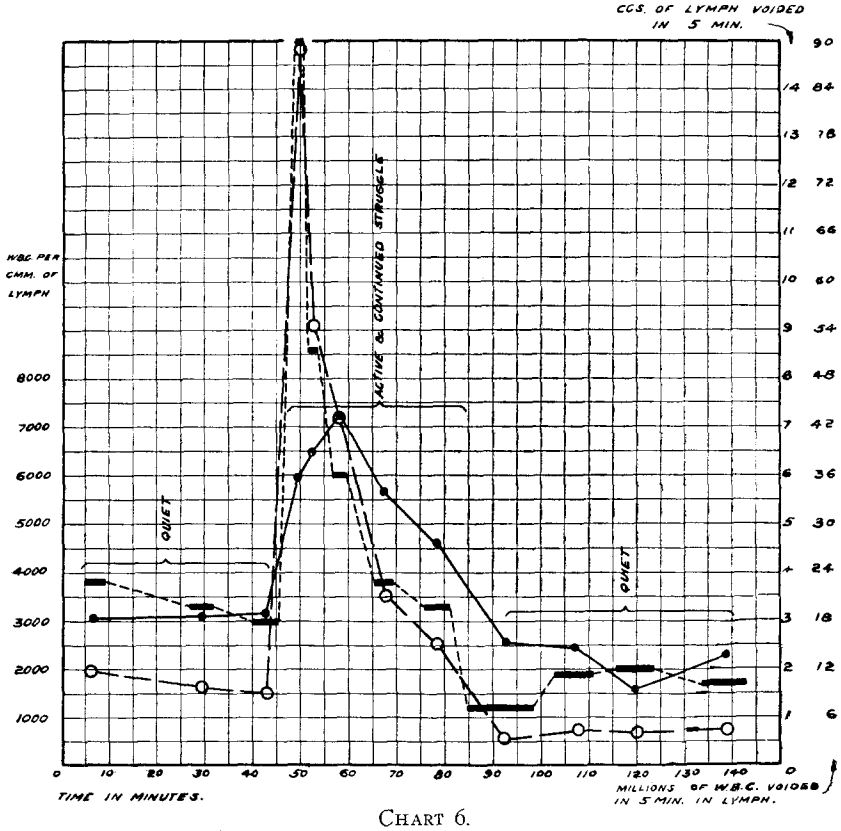

CHART 6.

cell-increase is due wholly to the flushing out of cells from the receptaculum chyli. Since the receptaculum is close to the opening of the thoracic duct, the cells flushed from it would appear, owing to the small size of the reservoir,[4] in the first few cubic centimeters

[4] I have made notes on the size of the receptaculum in several freshly killed dogs. The size varies widely, as does the shape of the reservoir, which indeed may not be present as such (Jussifow (25), and others). The walls of the receptaculum normally are held nearly apposed, so that the content is slight; but an impediment in the thoracic duct causes almost immediate dilatation with the accumulation of 1 to 4 c.c. of fluid.

voided during struggle; and, following this evacuation, the cell-increase would disappear quickly. But, in reality, the maximum cell-output was not infrequently delayed in its arrival (Experiment IV, second struggle; Experiment V, first struggle), and the maximum cell-concentration practically always was. In addition, the cell-increase is not transient (see Experiment VI) as would be the case were it traceable wholly to elements sedimented in the receptaculum.

Even granted that the first addition in cells comes from the receptaculum, whence is derived the later addition? Recent evidence (MacCallum (23), Buxton and Torrey (24)) speaks against v. Recklinghausen's view that the peritoneal cavity opens by direct channels into the lymphatics; and the quantity of free cells of this cavity is, normally, very small. The bulk of the increase is doubtless derived from the further lymphatic ramifications, in particular from the lymph-glands and other centers of lymph-cell formation. That the increased cell-concentration may persist for a short while after quiet has been restored is not surprising, since a host of cells, started from peripheral regions on the journey to the thoracic duct, need not arrive there until several minutes after the cause that got them under way had ceased to act.

(*b*) Struggle causes the cell-output by way of the lymph to become much greater. This effect persists throughout a struggle of considerable length.

In explanation of this phenomenon of increased cell-output it must be remembered that the lymph-region drained by the thoracic duct during struggle is larger than that during quiet. During the latter state, according to Starling (26), the fluid that arrives in the duct is derived, practically in toto, from the abdominal viscera. But muscular movement immediately forces from the limbs much lymph (Lassar, Cohnheim, Winternitz), as well as from the viscera; and, following this effect of direct pressure, there is a secondary increase in the flow from both sources, due to new lymph-production (Heidenhain, Starling). The territory of cell-supply opened for the first time, so to speak, by struggle, helps account for the greatly swollen cell-output.

(*c*) Following struggle, the cell-concentration and cell-output become for a time less than they would have been in the absence of muscular exertion.

This is demonstrated in the charts of Experiments IV, V and VI. The lessening in cell-output might be deemed merely such as was seen in the control animals of the first three experiments, were it not that the cell-concentration and the lymph-output (of which two the cell-output is the product) are both lower for a time than they would have been in the maintenance of quiet. One may suppose the glands to have been deprived of the majority of immediately available cells, and the slowed lymph-stream insufficient to wash with it all of those actually present.

These findings are, further on, dealt with in their clinical bearing.

Experiments in which the rapidity of the lymph's flow is varied without movement by the animal should provide increased light on the mechanism responsible for the results just described. The lymphagogue action of glucose was accordingly turned to this purpose.

The period of observation in these experiments was so short that it could not matter if the glucose acted to stimulate or retard cell-development. Other factors, though, demanded consideration. A possible effect of a lymph of high sugar-content to loosen elements from the glands, through changes in osmotic relations, could not be ruled out. Further, the lymph of struggle and of "glucosæmia" are not derived in similar proportions from the same regions.

There are three great areas of lymph-supply (Starling): The liver; the other abdominal viscera, in special the intestines, whence the lymph of the whole region may be designated "intestinal"; and the remaining portions of the body, the lymph from which may be termed "extremity-lymph." As has been said, the lymph of struggle comes from all of these sources, and in no small part from the limbs. The intravenous injection of glucose gives also increased lymph-flow from all the sources (Starling). The results of the procedures might, then, be directly compared, were the tissue forming lymph-cells equally distributed. But the liver of the dog possesses none of this tissue except that in the glands at its hilus (Ellenberger (27)), whereas the intestines and mesentery are

quite rich in it. The other body-parts possess, in proportion to their bulk, a very moderate quantity. Thus, of "mixed lymphs" from the thoracic duct, those derived most largely from the liver should be poorest in cells. So one must ask whether the lymphs of struggle and of "glucosæmia" are exactly similar in their derivation. To this only an approximate answer can be given. Starling has found that of the lymph obtained after the injection of glucose, much comes from the liver, less from the intestines, and relatively little from the other portions of the body. According to him increased blood-pressure and differences in permeability of the capillaries are responsible for the whole phenomenon. On the other hand the abrupt, initial increase in lymph-output induced by struggle is largely dependent on lymph previously present in the limbs, and now forced from them by the movements. Nothing analogous to this is caused by the glucose. The persistence of the large lymph-flow during struggle is traceable, however, to the same cause[5] as that following glucose injection, viz., increase in blood-pressure; and the resultant lymph is derived in much the same relative proportion from the three regions of production. Thus a rational basis is given to a comparison of the effects of glucose on cell-content with those observed in struggle after the initial increase in lymph-flow has subsided.

Experiment VII.—Mongrel; female; wt. 11.3 kilo; no food for 48 hours previous to experiment. The duct was ligated, and opened, 6 minutes before the beginning of lymph-collection. Lymph tinged with yellow, clear; no clotting in tubes or cannula. After three specimens had been got, 45 grammes of glucose in 72 c.c. of distilled water were injected slowly into the left subclavian vein. The animal remained absolutely quiet. The cell-counts were made in the order in which the specimens were taken, and 2½ to 3½ hours after their collection. (See Chart 7.)

At autopsy the animal proved to have been healthy; some round-worms were found in the intestines.

The results on cell-content are identical, as the chart shows, with those of struggle.

Experiment VIII.—Mongrel; female; wt. 16 kilo; no food for 48 hours previous to experiment. The duct was ligated during 10 minutes, and some stasis thus induced. On account of this, it was deemed safest to let the lymph

[5] This assertion might justly be objected to by those who oppose the theory of the mechanical formation of lymph. It stands or falls with that theory.

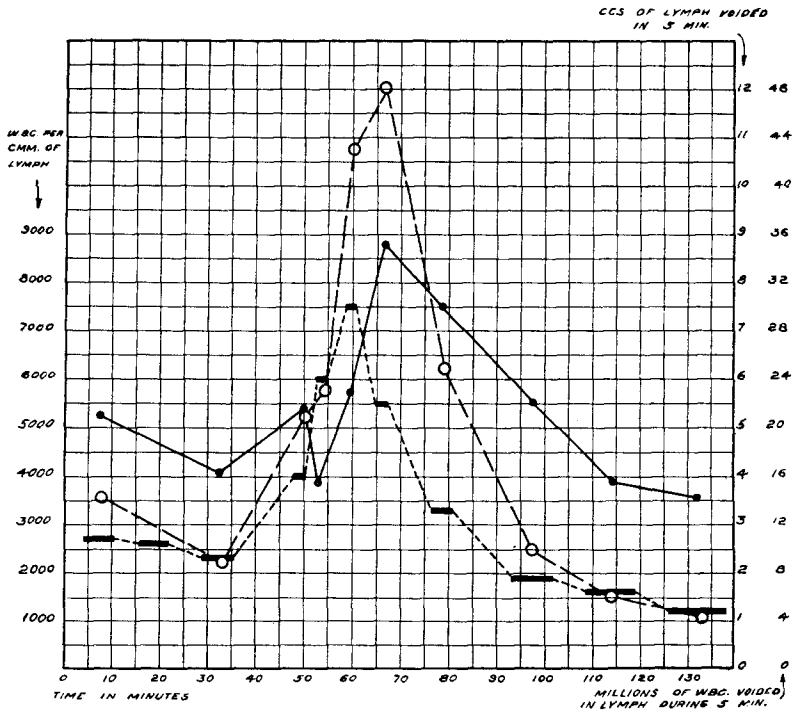

CHART 7.

escape for some minutes (12) before beginning its collection. Lymph clear; yellow-tinged; no clotting in tubes or cannula. After one specimen had been obtained, 52 grammes of glucose in 80 c.c. distilled water were slowly injected into the left subclavian vein. When the increase in lymph-flow due to this had subsided, the animal was made to struggle. Specimens were taken at frequent intervals. No chloroform was necessary, following its preliminary use to make anæsthesia complete. Cell-counts were made from the tubes in their order of collection and 2½ to 4 hours after that. (See Chart 8.)

At autopsy the animal was found to have been pregnant. There were ten embryos of an average length of 1 centimeter.

In this instance the effect of the glucose differed from that in Experiment VII. Here the cell-concentration rose, as result of the increased lymph-flow, whereas there it fell. In both cases the total cell-output became larger. In Experiment VII the curves were in all ways typical of those of struggle, whereas in Experiment VIII they were quite different, a fact which struggle in the same animal

helped to bring out. This struggle, coming after the glucose had operated, and causing an increase in the lymph but little superior to that from the glucose, was attended nevertheless by a cell-output vastly greater, and by the usual high cell-concentration.

These dissimilar results of glucose were puzzling. But the condition of the animal in Experiment VII had not been quite the

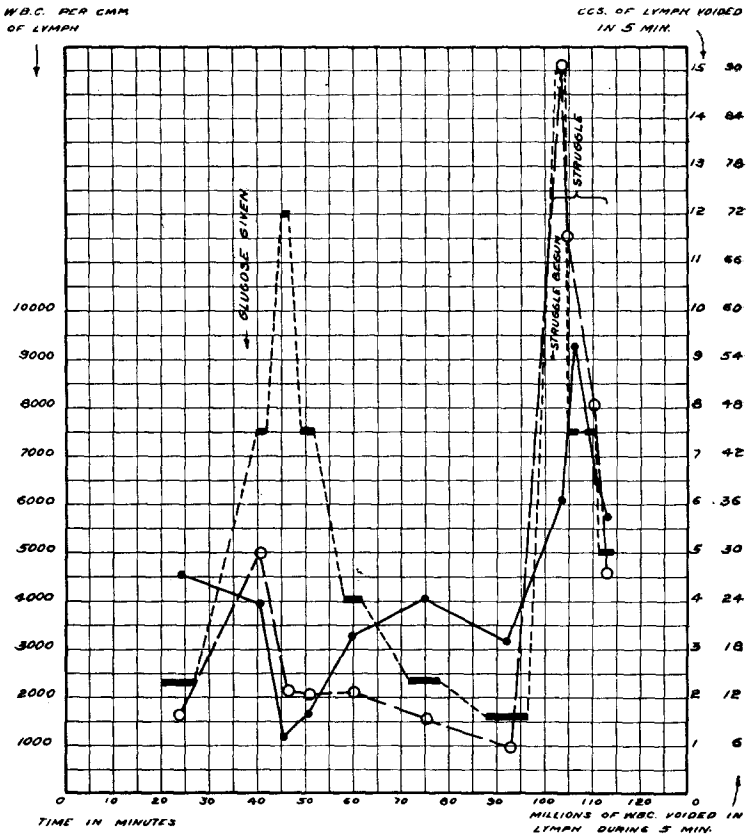

CHART 8.

same as that of the others. Owing to difficulty in the isolation of the thoracic duct, it had lain quiet under the anæsthetic 4 hours before the experiment proper began, instead of, like the others, only 1 to 2 hours. Perhaps, during this long period of preliminary quiet, the cells matured in the lymph-glands had in large part failed to be

carried away by the slow lymph-current, whence the marked appearance of them in the rush of fluid due to the glucose. On such reasoning it was determined to repeat the experiments, avoiding a long period of preliminary quiet, or flushing transiently the lymph-channels previous to the glucose injection by inducing restlessness in the animal.

Experiment IX.—Bull-dog; male; wt. 15 kilo; no food for 48 hours prior to experiment. The duct was ligated, and opened, 16 minutes before lymph-collection. The lymph was chyliform; no clotting in cannula or tubes. After one specimen had been obtained with the animal quiet, restlessness was brought

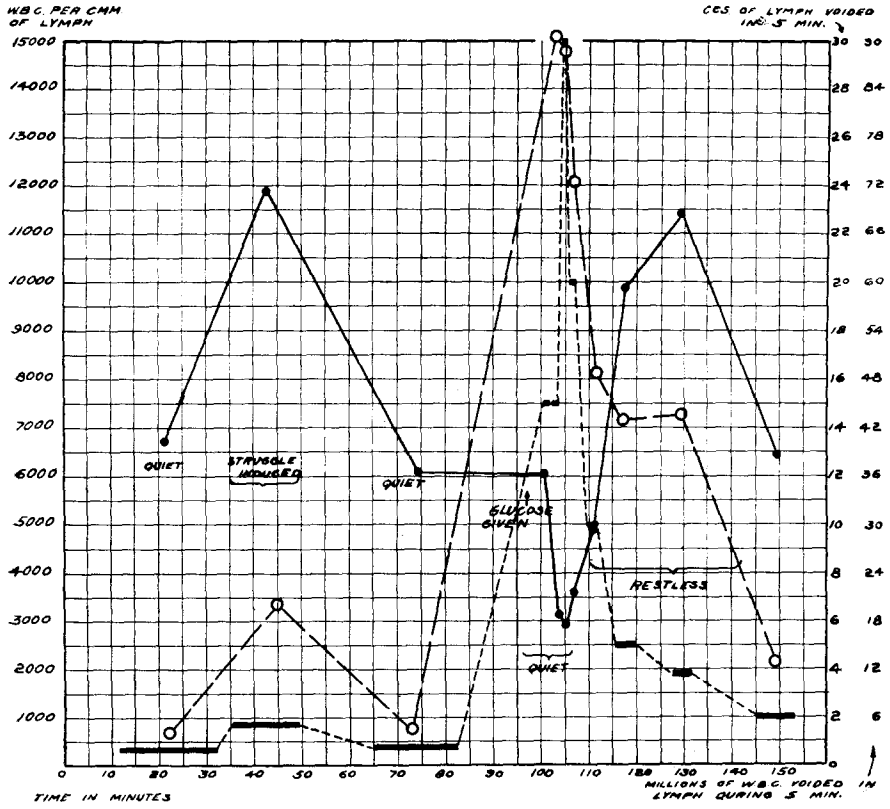

CHART 9.

about, in this case no real struggle, but a stiffening and straining, accompanied by labored respiration. The lymph became transiently more chyliform. A second specimen was now taken; on the return of quiet a third; and 50 minutes after restlessness had ceased 60 grammes glucose in 100 c.c. of distilled water were

injected slowly into the left external jugular vein. Before the lymphagogue action had disappeared the animal again became restless. Cell-counts were made in the order in which the specimens were taken, and 2½ to 3 hours thereafter.

The dog at autopsy proved to have been sound. Several large pieces of bone in the stomach accounted for the chyliform lymph. A tape-worm was found in the intestine.

From the chart it will be seen that struggle brought its characteristic effects on the lymph's cell-content, despite an extremely small increase in rapidity of flow. With the enormous lymph-output caused by the glucose, the concentration of the separate cubic millimeter of fluid was diminished, yet the cell-output as a whole became profoundly more.

Experiment X.—Mongrel; male; wt. 24 kilo; no food for 24 hours before experiment. The duct was opened 18 minutes previous to the beginning of lymph-collection. It had been ligated 8 minutes. After observations during quiet the animal was made to struggle during 2½ minutes, and 30 minutes later 100 grammes glucose in 100 c.c. of distilled water were injected into the left sub-subclavian vein. The lymph, which had been slightly opalescent, now became distinctly milky. There was no clotting in tubes or cannula. Except for the one struggle, the dog was quiet throughout. Counts of the tubes were made in the order of their collection and 1½ to 3 hours following that. (See Chart 10.)

At autopsy one tape-worm was found in the intestines.

No count was made of the lymph taken during struggle. The curves representing glucose action are similar to those of Experiments VIII and IX. In this instance such a rush of lymph was observed, and such a vast increase in total cell-output (despite lessened cell-concentration), as showed itself in no previous experiment. The curves representing these are much flattened.

The results justify the supposition that the high cell-concentration seen in Experiment VII is traceable to accumulation of cells during the long, preliminary quiet. Experiment VII shows that under certain conditions increased lymph-flow[6] may give results exactly similar to those of struggle.

To summarize the results with glucose:

(*a*) The increase in lymph-flow produced by the intravenous injection of a solution of glucose is accompanied by an alteration in the cell-concentration of the lymph. Usually the cell-concentration

[6] The change in osmotic relations caused by the glucose cannot be ruled out as a possible factor.

is decreased, but there is some evidence to show that, when the conditions have been such as to lead to the accumulation of cells in the lymph-system, it may be increased.

(*b*) With the increased output of lymph there goes an increase in total cell-output. This increase may be enormous. In Experiment X 5½ times as much lymph and 3½ times as many cells, were

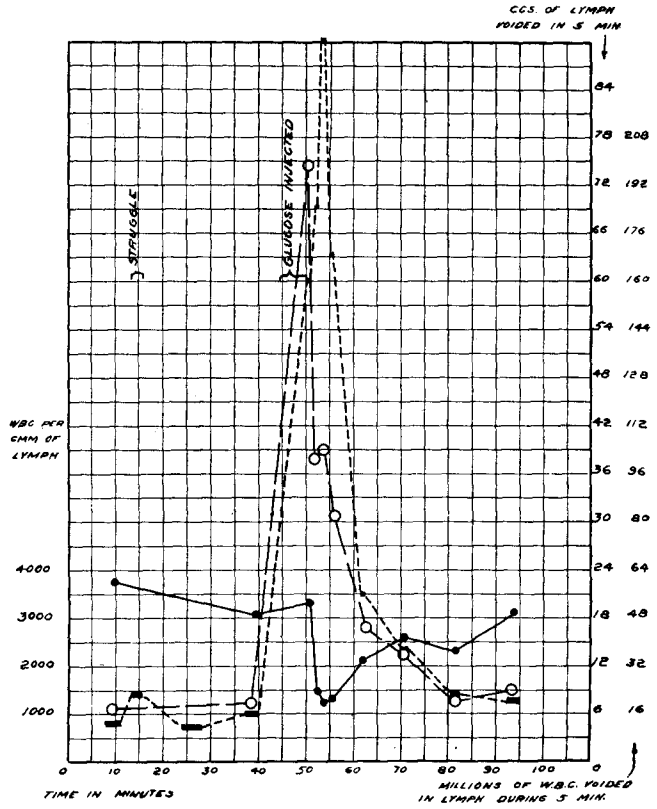

CHART 10.

voided in the half-hour immediately following the glucose administration as in the previous half-hour of quiet.

One may ask whether the results are not produced in the same way as those of struggle, and if they are not similar to them, except that the cell-concentration is rendered small by the distribution of the cells through a large amount of lymph. To put the question

directly, are the results of struggle those of simple increase in lymph-flow?

The evidence at hand is against this view. One may obtain a greatly increased cell-concentration during muscular activity in which the lymph-flow is quickened but little. Furthermore, increased lymph-flow by itself often diminishes the cell-concentration of the lymph, whereas struggle invariably heightens it. A direct comparison of the two procedures, such as Experiment VIII fortunately gave, demonstrates a difference in their results. In this experiment during the 11 minutes of muscular activity 19.25 cubic centimeters of lymph, with an average cell-content of 6877 cells per cubic millimeter, were voided; whereas, in the 11 minutes of greatest lymphagogue action, the nearly identical quantity of lymph voided (19.8 cubic centimeters) held only 2,267 cells per cubic millimeter. Differences in amount of lymph-flow alone could not be responsible for these variations of the cell-concentration in opposite directions and the differences in total cell-output.

It is true that, as already discussed, the lymphs obtained by the two methods may not be derived entirely from the same regions. The first "struggle lymph" is made up largely of that already present in the extremities, whereas that of the glucose is "liver-," "intestine-," and "extremity-lymph," in much the same relative proportions as that produced late in struggle.

It is questionable whether the cell-content of the "extremity-lymph" forced into circulation at the beginning of struggle could increase the cell-concentration of the "mixed lymph" of the thoracic duct, since it comes from a territory poor in lymphadenoid tissue, and has, indeed, been shown to hold normally fewer cells per cubic millimeter than does this "mixed lymph" (Pohl, Winternitz). But leaving this to one side, we are permitted a comparison of the effects of glucose and those that appear in struggle after the first rush of lymph has ceased. In the one instance the cell-concentration is diminished, in the other it is heightened.

One must conclude that another factor besides increase in lymph-flow per se helps during muscular activity to increase the cell-output. This is not hard to imagine. Direct pressure effects on the lymph-stream to set in motion those cells that had settled in the vessels,

and to scour the glands of mature elements, should certainly play a part. The work of Harvey (29) on the lymphocytosis caused by pilocarpine indicates that it is brought about by contraction of the smooth muscle in the capsules of the lymph-glands and spleen. Thus another possible action of struggle is suggested. Whatever the factor or factors may be, they are probably quite as influential during struggle to produce the large cell-output as is the accompanying increase in lymph-flow, which Ehrlich holds to be alone responsible for all heightened output of lymph-cells not dependent on their more abundant maturation. Nevertheless the theory that increase in lymph-flow gives increase in cell-output, is supported by the results of the lymphagogue action of glucose. The only objection to these as direct proofs of it is that an effect from the changes in osmotic relations brought about by a lymph of high glucose content cannot be ruled out.

It should be noted, in observing the charts as a whole, that the variations in cell-output are in keeping, quantitatively, with the amount of cell-output during quiet, the " cell-capital," so to speak. Succeeding to variations the cell-output tends to return close to its height previous to them. The fact that it usually becomes somewhat less than before them,—an indication of that gradual diminution in it observed in animals from which the lymph is gradually drained (Experiments I, II and III), or, in some cases (Experiments V and VI) of partial exhaustion of the supply of mature cells, —does not affect the principle. We may say that the cell-output seems " set " to maintain a stable rate during some hours at least. Healthy, adult dogs, kept, so far as possible, under the same conditions, differ widely in this rate of cell-output: in Dog Gl more than nine times as many lymph-cells per kilo of body-weight are furnished to the blood through the thoracic duct as in Dog Fl. Does this mean a difference in amount of cell-production by the tissues? The appended table helps answer this question.

From this table it is clear that, while the cell-output per kilo of body-weight does not depend on size of the individual, or on differences in length of fast,[7] it has perhaps a relation to rate of lymph-

[7] Firleiewitsch (30) has found the lymph-glands of well-fed rats to be more numerous and larger than those of starved ones; but he assigns this to a larger size of the cells making up the tissue, not to a greater number of them.

Dog.	Length of Fast.	Weight in Kilos.	Average No. of w. b. c. per cmm. Lymph.	Average Flow of Lymph in 5 Minutes.	Total w. b. c. in this Amount of Lymph.	Total w. b. c. in Amount of Lymph Furnished per K. of Body-weight in 5 Minutes.	Flow of Lymph per K. of Body-weight, in 5 Minutes.
Ll, bull, male	48	15	6,700	0.7	4,690,000	312,666	0.05
Hl, terrier, male	24	11	4,960	0.75	3,720,000	338,181	0.07
Pl, collie, male	48	13	2,900	1.5	4,350,000	334,615	0.11
Al, bull, female	24	15	3,600	2.0	7,200,000	480,000	0.13
Kl, collie, male	48	16	4,510	2.3	10,373,000	648,313	0.14
Gl, bull, male	24	18	11,160	3.3	36,828,000	2,046,000	0.18
El, setter, male	24	20	8,400	3.75	31,500,000	1,575,000	0.19
Ml, setter, male	24	24	3,780	4.6	17,388,000	724,500	0.19
Ol, collie, male	48	23	6,760	4.6	31,096,000	1,352,000	0.20
Cl, mongrel, male	2	10	1,500	2.0	3,000,000	300,000	0.20
Nl, terrier, female	48	8	4,180	1.7	7,106,000	888,250	0.21
Jl, mongrel, male	48	11.3	4,640	2.5	11,600,000	1,026,540	0.22
Fl, mongrel, male	24	19	990	4.3	4,257,000	224,053	0.23
Il, spaniel, male	24	11	3,000	3.8	11,400,000	1,036,363	0.34

flow per kilo of body-weight, being, on the whole, least in those individuals in which this flow of lymph is least. It may be supposed, in the light of what has gone before, that the lymph-flow, as an agent of transportation, is here responsible for the differences in cell-output, rather than by stimulation of the cell-forming tissues; in special since, so far as we know, this rate of cell-output is constant only from hour to hour, and may not be so from day to day. Some of the wide differences may be due to the presence of an accessory thoracic duct, which is not infrequent in the dog (Biedl and v. Decastello), or to other channels conveying a share of the elements that would, normally, course through the thoracic duct. In association with the idea of actual variation in the productive activity of the cell-forming tissues, may be cited the work on ruminants of Forgeot, already quoted. He found the cell-output of young individuals to be markedly greater than that of adults. Differences in age of the animals may be at the root of some of the differences in cell-output. But the mechanical factors, just cited, will explain the larger differences, and make needless a further entrance into the dark subject of activity in the cell-forming tissues.

The cell-concentration of the lymph of the dog, as determined under the conditions of operation outlined at the beginning of this paper, has a worth in its relation to the cell-content of the blood.

Delamere, Winternitz, Goodall and Paton, Dastre, Henri and Stodel, Beidl and v. Decastello, and Ranvier give figures ranging from 1,372 to 22,729 white cells per cubic millimeter. The results in the table, as might have been expected from the technic employed, do not exhibit such wide variation. From them one may judge this "normal" cell-concentration of the "mixed lymph" of the dog to lie between 990 and 11,160 cells per cubic millimeter, with an average of 4,000 cells.

The most important outcome of this work is the discovery that the system forming the lymphocyte possesses large reserve power to increase transiently its output of cells. During muscular activity this may, for the space of a half-hour, be nearly four times what it is in quiet, as has been shown. A clinical application of the finding is not far to seek.

For this application it is necessary to know what white cells the lymph furnishes the blood. These are in the dog much the same as in man, just as the leukocytes of the dog's blood resemble in general those of man both in morphology and in relative proportion of number (Dawson (31), Tallquist and Willebrand (32), Busch and Van Bergen (33)). The bulk of cells furnished through the thoracic duct of the dog is one of lymphocytes, large and small, but a few large mononuclears, a varying, small percentage of eosinophiles, and an occasional polymorphonuclear neutrophile are also present (Delamere, Biedl and v. Decastello). The lymphocytes alone exist in sufficient quantity to be important to the blood.

Since, as has been emphasized, the thoracic duct furnishes a large, if not the greater part, of the blood-lymphocytes, an increase in the lymph's cell-concentration should produce, other factors being equal, an absolute lymphocytosis in the blood. When the amount of lymph is at the same time increased, thus multiplying the cell-output, the effect should be more profound. Thus one would expect struggle to produce, clinically, an absolute lymphocytosis.[8]

[8] But it must be assumed that the change in cell-output is not accompanied by change in cell-formula. I have made repeated counts of the lymph before and during struggle, and have found no such change.

The clinical records of blood-counts following muscular exertion indeed show the presence of an absolute lymphocytosis. Schultz (34), and later Winternitz, observed a leukocytosis following exertion, but they did not note its kind. Burrows (35) found in the normal individual, after short exercise, a distinct increase in number of all the white cells, but especially of the lymphocytes. Capps (36), previously, had studied the blood during the convulsions and apoplectic attacks of general paresis, and, here too, had observed leukocytosis, most marked in the large mononuclear elements, but affecting the lymphocytes. Burrows went further in making plain the fact that the leukocytosis associated with true convulsions in the course of paresis is invariably of the inflammatory type, the polymorphonuclear neutrophiles giving the increase. He concluded, from his findings made during muscular exertion, that in convulsions two leukocytoses are really involved, a transient "physiological," wherein occurs an increase of all the elements, most marked, as his records show, in the lymphocytes, and a more pronounced and enduring "pathological" one.

Violent and long-continued physical exertion will itself produce a profound leukocytosis. Thus Larrabee (37) observed it as an effect of a 25-mile foot-race; and he decided that, here too, a "pathological" is superimposed upon a "physiological" leukocytosis, "an increase in cells all along the line," as he puts it. Only this "physiological" leukocytosis is of interest here. Larrabee, Tileston and Emerson (38), studying the blood after a similar race, confirmed these results. Further, the lymphocytes at the end of such exertion form a very small percentage of the total, having retreated to their absolute number during quiet, or even below it. We do not know how far destruction of the lymphocytes in circulation effects this and how far it is brought about by a lessening in the supply of them. The experiments reported in this paper show that a diminution in the cell-output by way of the lymph follows prolonged struggle.

Coming to more debatable ground, the well-defined lymphocytosis that accompanies whooping-cough may be referred to. This cannot be primarily due to the mechanical effects of struggle, since it appears early in the disease; yet the fact that it is at its greatest during the period of violent coughing (Meunier (39)), is suggestive. Un-

fortunately there are no published figures dealing with the leukocyte count immediately before and after a coughing-fit.

The experiments with a lymphagogue throw a little light on the vexed subject of digestive leukocytosis, particularly on the reason that it is vexed. The frequency of this leukocytosis, and the part played in it by the mononuclear element, varies with nearly every investigator who has applied himself to the problem. The discordance in results is, to a certain extent, explicable in terms of the conditions dealt with here. Quiet previous to a meal would predispose, on increase of the lymph flow during digestion, to a cell-output such as that in Experiment VII, which would probably swell quite markedly the number of lymphocytes in the blood. Any exertion after the meal would tend to make this output greater. Exercise previous to a meal, by flushing out the reserve of mature cells, would act to prevent an increase in cell-output during digestion, and this result would be made the surer by slow lymph-flow, were but little fluid ingested with the meal. Under such circumstances the blood-content in lymphocytes would not increase.

I am aware that, considering the many factors which must enter into the determination of the blood's content in lymphocytes, this discussion is one-sided. Yet, whether the hypotheses presented above be sound or not, the work on which they are based indicates one direction in which it may be possible to simplify some of the problems connected with the leukocyte.

CONCLUSIONS.

1. The lymph of the thoracic duct furnishes to the blood a larger proportion than is usually supposed of the lymphocytes in circulation. Gross variations in its output of such cells must affect very considerably the blood picture.

2. The quantity of lymphocytes supplied through the thoracic duct of the healthy dog remains practically constant from hour to hour, if the physiological conditions are not notably changed. Transient change in physiological conditions may alter the output of cells, but with the disappearance of this change the output tends to resume its previous rate. These facts indicate that the tissues producing lymphocytes are "set" at a rate of activity definite in the individual.

3. Muscular activity (struggle) produces a prompt increase in the output of lymphocytes through the thoracic duct.

(*a*) This is assured by the presence of an increased number of cells per cubic millimeter of lymph, combined with an increase in the amount of lymph voided.

(*b*) The lymphocyte-output may be tripled or quadrupled during a long-continued struggle.

(*c*) Following prolonged struggle the output of lymphocytes is for a short time less than previous to the exertion.

4. The increased lymph-flow caused by a lymphagogue of the second class (glucose) brings with it increased output of lymphocytes through the thoracic duct.

(*a*) The individual cubic millimeters of lymph are often poor in cells, during the rapid lymph-flow, yet the total number of elements transported is large.

(*b*) The results with glucose support the theory of Ehrlich, that a rapidly appearing lymphocytosis may be produced through the flushing effect of increased lymph-flow.

5. A comparison of the effects of struggle with those of glucose demonstrates that in the former some factor besides increase in lymph-flow per se (Ehrlich) works to cause the large output of lymphocytes. The nature of this factor has not yet been determined.

6. The variations caused by muscular exertion and by increased lymph-flow in the number of lymphocytes coursing through the thoracic duct are so pronounced as to suggest that the total number of lymphocytes in circulation must be considerably influenced by them. Clinical findings by other observers indicate that this is true; and the clinical findings themselves become much simpler of interpretation.

7. The results in general prove the existence, reserved from circulation, of a large fund of lymphocytes, which is quickly yielded to the blood under certain physiological conditions.

I wish to thank Dr. Warthin for an interest in the work that has been most helpful.

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