VARIATION IN LYMPHOCYTE PRODUCTION

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INTRODUCTION

It has been noted, during the quantitative study of lymphocyte production, that the number of lymphocytes to be found in the thoracic duct lymph of the dog may show considerable variation. In previous work the fact that this variation may occur was not sufficiently appreciated (Yoffey, 1933). Many of the earlier experiments happened to be on animals in which the lymphocyte count remained fairly constant, thus according with the findings of Rous (1908). But even among these earlier experiments there were a few which showed marked variation. In two experiments, for example, of which details have already been given (Yoffey, 1933), one shows a series of hourly counts which undergo very little change (Table I), whereas the other shows a progressive fall in the number of lymphocytes (Table II).

Table I

Dog. 22. iii. 32

Time	Lymphocytes per c.mm. of lymph	Lymph c.c. per hour	
1.25 p.m.	11,500	25·0	(collection begun at 12.25 p.m.)
2.25 p.m.	11,300	23·0	
3.25 p.m.	12,200	22·0	
4.25 p.m.	11,100	26·0	
5.25 p.m.	11,300	23·0	
6.25 p.m.	10,000	23·0	

Table II

Dog. 26. iv. 32

Time	Lymphocytes per c.mm. of lymph	Lymph c.c. per hour	
3.12 p.m.	33,400	23·0	(collection begun at 2.12 p.m.)
4.12 p.m.	14,200	23·0	
5.12 p.m.	13,300	23·0	
6.12 p.m.	12,100	22·0	

The observation of such changes in lymphocyte count occurring during the course of a single experiment suggested the possibility of variations occurring as between one animal and another, and led to a comparison of lymphocyte production in different animals. It is clear that merely to compare lymphocyte counts per c.mm. of lymph is insufficient. What is required is a knowledge of the total lymphocyte output, i.e.

(lymphocyte concentration) × (volume of lymph produced).

The results of twenty-one experiments (all on dogs) are summarised in the

following table, and in order to facilitate comparison results have been calculated for unit time (1 hour) and unit body weight (10 kg.).

Table III. *Hourly output of thoracic duct lymphocytes (dog), in each case calculated for a body weight of* 10 *kg.*

No. of animal	Lymphocytes (in millions)	No. of animal	Lymphocytes (in millions)
4ES20	140·9	4ES34	240·4
4ES19	88·5	4ES35	209·6
4ES21	44·7	4ES36	406·1
4ES23	199·9	4ES37	194·4
4ES24	185·5	4ES38	147·7
4ES26	30·2	4ES39	875·4
4ES28	59·3	4ES40	121·5
4ES29	168·2	4ES41	149·8
4ES30	256·6	4ES42	138·7
4ES31	441·5	4ES65	221·5
4ES33	123·5		

Average hourly output (21 experiments)	=211·6 millions
Average output for 24 hours	=5078 millions
In proportion to body weight a human being weighing 70 kg. would have a total daily thoracic duct lymphocyte output of	35,546 millions

THE DAILY REPLACEMENT FACTOR

It will be observed, on examining Table III, that the thoracic duct lymphocyte output shows great variation in different animals. The question now arises: "Is this variability permanent, or not?" Does one animal normally and regularly have only 30 million lymphocytes per hour passing *via* the thoracic duct into the blood, while another has 875·5 millions, almost thirty times that amount? It seems unlikely, for a number of reasons, that this should be so. In the case, for example, of 4ES39 this would mean a lymphocyte production during the 24 hours of 21,000 millions, or 4·15 times the average figure. The significance of this may best be appreciated when it is compared with the total number of lymphocytes in the circulation.

The number of lymphocytes in the blood stream may be calculated if we know (*a*) the count per c.mm., (*b*) the blood volume. As to the *count per c.mm.*, Mayerson (1930), in sixty dogs, found the average count of "small and large mononuclears" in the blood to be 2680 per c.mm. If we assume that all these mononuclears are lymphocytes, which they almost certainly are not, then any error introduced will lead to an understatement of the position in calculations of the daily replacement of blood lymphocytes. In a series of my own (twenty-six experiments) the average count of blood lymphocytes was 3910 per c.mm. The *blood volume* may be calculated from the body weight. As is well known, there are marked differences between the results obtained by Welcker's, the carbon monoxide, and dye methods of estimation. Smith, Arnold and Whipple (1921), after a careful comparison of the various methods upon the same group of dogs, and an analysis of the possible sources of error, conclude that the

blood volume is 9·2 c.c. per 100 gm. body weight, so that a 10-kg. dog would have a blood volume of 920 c.c.

On the basis of these data (using Mayerson's figures for the blood lymphocytes) the blood of a 10-kg. dog contains 2465 million lymphocytes. Since the blood lymphocytes remain fairly constant over a period, the same number of lymphocytes must daily be entering the blood as are leaving it (Yoffey, 1933). Lymphocyte production on a scale as high as that found in 4ES39 would be sufficient to replace the blood lymphocytes in the average dog 8·5 times daily. Actually the blood lymphocytes in 4ES39 were above the average, being 6170 per c.mm., but even at this higher level they are being replaced 3·71 times per day.

The ratio

$$\frac{\text{number of lymphocytes daily entering blood } \textit{via} \text{ thoracic duct}}{\text{blood lymphocytes at beginning of experiment}}$$

will be referred to as the daily replacement factor, or D.R.F. One selects the blood lymphocytes at the beginning of the experiment as more truly representing the average level of the blood lymphocytes over a period, as during the course of the experiment the blood lymphocytes rapidly diminish, owing to the fact that such large numbers of thoracic duct lymphocytes, which in the intact animal would have been entering the blood stream and so have maintained the level of the blood lymphocytes, are now prevented from doing so. Consequently at the end of the experiment the blood lymphocytes are much lower than they were at the commencement, when the count does not differ from what it is in the intact animal, as the only factor which might have altered it is the anaesthetic, and the effect of this has been shown by previous workers to be negligible (Goodall and Paton, 1905; Latta and Ehlers, 1931).

4ES39 is the animal with the highest lymphocyte output of the series. The average figure for the series is probably of more universal applicability, and gives a truer picture of thoracic duct lymphocyte production over a period. The average daily output of lymphocytes from the thoracic duct of a 10-kg. dog is 5078 millions, and this means that the blood lymphocytes are normally replaced 2·06 times daily (using Mayerson's figures for blood lymphocytes). The lymphocytes therefore remain in the blood for 11·65 hours.

One further point may be noted in passing. In 4ES39 the thoracic duct lymphocyte production is 4·15 times the average figure, whereas the blood lymphocytes are only 2·3 times the average. In other words the level of the blood lymphocytes gives little indication of the degree of lymphocyte production. Lymphocytes may be entering the blood in vastly increased numbers, but if they are also leaving the blood rapidly, the level of the blood lymphocytes will not show any proportionate change. This point will be dealt with in detail in a further paper.

ARE THE THORACIC DUCT LYMPHOCYTES NEWLY FORMED CELLS?

The data which have been presented in the preceding paragraphs have all been based upon the assumption that the thoracic duct lymphocytes are cells which have been newly formed in the lymphoid tissues, and are entering the lymph for the first time. There is the alternative possibility to be considered, however, that they are not newly formed cells, but that there is a continuous circulation of lymphocytes from blood to lymph and from lymph to blood back again. The fact that we are not aware of any function which such a lymphocyte circulation might subserve does not, of course, constitute proof that it does not occur. The question as to whether the thoracic duct lymphocytes are newly formed cells or not is a fundamental one in all quantitative work based on thoracic duct lymphocyte estimations, and merits detailed consideration.

Thoracic duct lymph is the efferent lymph from the lower limbs, the abdomen, the left half of the thorax, left fore-limb, and left side of the head and neck. Under the conditions of the present experiments, the left cervical lymph trunk is divided, and the cannula is inserted well down into the duct, below the point of entry of the main efferent lymph trunk from the left fore-limb. Lymph, before it reaches the thoracic duct, passes through varying numbers of lymph nodes—in the present experiments chiefly those of the lower limbs and the large lymphoid mass at the root of the mesentery.

Now it has repeatedly been shown that whereas lymph which has not yet passed through any lymph nodes contains few or no lymphocytes, lymph which has passed through a lymph node is usually rich in such cells. Heyfelder (1851; quoted from Poirier, Cuneo and Delamere, 1903) seems to have been the first who "stated that the white corpuscles were much more numerous in the efferent than the afferent lymphatics". Brucke (1853) observed in cats and dogs which had been fed on a fat-free diet and killed while digestion was in progress, that the lymph in the lacteals (mesenteric lymphatics afferent to the large lymphoid mass at the root of the mesentery, and known as the Pancreas of Aselli or the glandula mesenterica magna) was quite clear, while the efferent lymph was turbid and full of corpuscles. Goodall and Paton (1905) found in the lacteals of the dog (i.e. afferents to the glandula mesenterica magna) an average count of 2160 lymphocytes per c.mm. (maximum 2600, minimum 1600), whereas in the efferent lymph they found an average count of 10,600. In the cat they found the count in the afferent lymphatics to be 1680, whereas it was 52,570 in the efferent lymph (collected from the receptaculum, into which the large efferent lymphatic of the glandula mesenterica magna opens directly). Davis and Carlson (1909) found that the lymphocyte content of efferent lymph was greatly increased by massage of the lymphoid tissues. In the cervical nodes they found the count was raised from 16,530 per c.mm. before massage to 51,700 after (average of four experiments), and

in the case of the thoracic duct lymph the lymphocyte count rose from 26,500 before massage of the abdomen to 73,330 after (average of two experiments). Florey (1927), in observations on the contractility of lacteals, noted only "the presence of an occasional white cell" in their lymph. He describes, on the other hand, the presence of large numbers of lymphocytes in the efferent lymph of the glandula mesenterica magna, and showed that splanchnic nerve stimulation still further increased their numbers, the average count in four experiments being 16,340 before stimulation and 37,950 after stimulation. Menkin and Freund (1929) found an average count of 18,000 in the efferent lymphatic vessel of one of the axillary lymph nodes of the normal rabbit. Haynes and Field (1931) found that, in the dog, lymph obtained from a vessel in front of the ankle contained an average of only 33 cells per c.mm., and they conclude: "The fact that lymph from the ankle, which has passed through no lymph nodes, contains practically no cells would indicate that normally the cells enter the lymph from the lymph nodes, and not by diapedesis from the blood nor from the tissue spaces." It is of interest to note that Drinker and Field (1933) come to a conclusion diametrically opposed to that which the figures of Haynes and Field (1931) warrant, for they say: "In our opinion, they" (i.e. the lymphocytes) "escape from the blood capillaries all over the body and re-enter the circulation *via* the lymph stream."

The available evidence, therefore, is strongly in favour of the lymphocytes in thoracic duct lymph being newly formed cells which have entered the lymph from the lymphoid tissues, on their way to the blood stream. Once the lymphocytes are in the blood stream, they do not re-enter the lymph in any appreciable numbers, for lymph contains few or no lymphocytes before it has passed through lymph nodes. The lymph in the mesenteric lymphatics may at times be an exception to this, but the exception is only apparent, for this lymph may already have passed through some lymphoid tissue in the wall of the intestine.

DISCUSSION

It must be borne in mind, in all quantitative work on thoracic duct lymphocyte output, that the figures obtained are minimal. Lymphocytes may enter the blood (*a*) from the thoracic duct, (*b*) from the right lymph duct, (*c*) possibly from the bone-marrow, (*d*) from the lymphocytopoietic tissues direct. As to (*d*), Schulze (1925) has described the existence of "stomata" in the post-capillary veins of lymphoid tissue, and believes that, through these stomata, cells from the lymphoid tissues have direct access to the blood stream. The number of lymphocytes which pass through these stomata into the blood is, of course, problematical; they cannot be directly measured. In the present experiments the only lymphocytes of which it has been found possible to obtain accurate quantitative estimations are those which enter the blood *via* the thoracic duct. If the number of lymphocytes which enter the blood from other sources are at all considerable, then the total

lymphocyte output of the body will be even greater than would appear from thoracic duct estimations alone. But even for the thoracic duct itself, evidence will be presented in another paper which seems to indicate that the figures obtained in these experiments are lower than they would be in the intact, actively moving, animal.

If it be true that the thoracic duct lymphocytes constitute only a part of the body's total lymphocyte production, it becomes all the more difficult to believe that the lymphocyte content of thoracic duct lymph could be maintained permanently at such a high level as is found in 4ES39. The alternative explanation would be that lymphocyte production does not take place at a constant and uniform rate, but is subject to marked fluctuation. If this view be correct, it would explain the great range of variation in thoracic duct lymphocyte production shown in Table III, and these data would then be interpreted as representing lymphocyte production in various phases. Over a longer period the average of Table III, namely 211·6 million lymphocytes per hour, would be a more representative figure.

Changes in lymphocyte output during the course of an experiment

That lymphocyte production is subject to fluctuation is further suggested (*a*) by the fact that evidence of change in lymphocyte production may be obtained during the course of an experiment, (*b*) by observations which have been made on the changing histological structure of lymph nodes.

The following experiment, for example, like the experiment in Table II, shows a progressive fall in the lymphocyte content of thoracic duct lymph (Table IV):

Table IV

4ES30. Dog. 13. iii. 35

Time	Lymphocytes per c.mm. lymph	Lymph in c.c. per hour
11.27 a.m.	15,000	Collection begun
12.27 p.m.	9,250	33·0
1.27 p.m.	7,100	35·0
2.27 p.m.	6,500	34·0

Table V, on the other hand, presents the results of an experiment in which the lymphocytes show a steady rise to a peak of 35,400, and then a sharp fall:

Table V

4ES39. Dog. 30. vi. 35

Time	Lymphocytes per c.mm. lymph	Lymph in c.c. per hour
1.40 p.m.	17,500	Collection begun
2.40 p.m.	24,000	15·0
3.40 p.m.	35,400	21·0
4.40 p.m.	21,500	23·0

The following experiment (Table VI) seems at first sight to show much the same phenomena as Table V.

Table VI

4 ES 75. Dog. 30. vi. 35

Time	Lymphocytes per c.mm. lymph	Lymph in c.c. per hour
12.25 p.m.	1300	Collection begun
1.25 p.m.	2500	30·0
2.25 p.m.	4600	24·0
3.25 p.m.	5000	15·0
4.25 p.m.	5200	22·0
5.25 p.m.	4300	52·0

If, however, one observes changes in lymph flow at the same time, it will be seen in Table VI that the rise in lymphocyte concentration from 1·25 to 3·25 is largely offset by the fall in the lymph flow. In the other experiments which have been quoted, on the other hand, the lymph flow has either remained constant while the changes in lymphocyte concentration were in progress, or else has increased together with the lymphocyte concentration.

The histological evidence

Finally, the conclusions of Maximow and Bloom (1930) on the histological evidence of cyclic activity in lymph nodes may here be quoted:

"The germinal centres are not constant structures. They are absent from the lymphoid tissues of the embryonic as well as of the senile organism. They appear and disappear periodically and pass through a series of cyclic changes. In the centre of an active, fully developed primary nodule there is a spherical clear area, the secondary nodule, which in man may attain a diameter of 1·0 mm. In the opposite condition, in the stage of complete rest, the germinal centre is quite inconspicuous. It consists of a small artery surrounded by a few pale nuclei of the undifferentiated syncytium in the centre of a follicle.... When a new period of activity begins—the duration of the stages is not known—the peri-arterial pale nuclei of the syncytium are mobilised and begin to divide mitotically."

SUMMARY

In twenty-one dogs a cannula was inserted into the thoracic duct, and both the flow of lymph and the concentration of lymphocytes measured.

After calculating the results for a standard body weight of 10 kg., the average hourly lymphocyte output was found to be 211·6 millions, with a maximum of 875·4 millions, and a minimum of 30·2 millions.

The entry of lymphocytes into the blood does not occur at a uniform rate, but shows marked fluctuation.

Thoracic duct lymphocytes are newly formed cells which have entered the lymph from the lymphoid tissues.

Thoracic duct lymphocytes enter the blood stream in numbers sufficient to replace the average blood lymphocytes of the dog 2·06 times daily.

The lymphocytes remain in the blood stream on the average 11·6 hours.

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