THE QUANTITATIVE STUDY OF LYMPHOCYTE PRODUCTION

By J. M. YOFFEY, M.Sc., M.D. (Manch.), F.R.C.S.
Lecturer in Anatomy in the University College of South Wales and Monmouthshire, Cardiff

I. INTRODUCTION

Previous work on blood formation in fishes (1) led to a re-investigation of the known facts concerning the lymphoid tissues of Mammals, and their relation to the remainder of the haemopoietic system (2). As a result of this it became desirable to investigate the problem of lymphocyte production from a quantitative standpoint, and accordingly the present work was undertaken. It has been one of the more serious defects of haematological work in the past that it has been essentially qualitative in nature. The few observations which have been made (e.g. occasional counts of white and red cells in small quantities of blood or lymph) are insufficient for accurate quantitative study of the formed elements of the blood.

In this respect it is fortunate that the lymphocytes lend themselves to quantitative study more easily than any of the other formed elements of the blood. It is possible that some lymphocytes enter the blood directly as soon as they are formed, through the thin-walled veins found in lymphoid tissue and described in detail by Schultze (3); these lymphocytes cannot be measured. Large numbers of lymphocytes, however, first of all enter the lymph stream, and by it are finally conveyed to the blood. These can be counted with a fair degree of accuracy. It is possible, by tapping the thoracic duct immediately before it drains into the blood, to collect the major part of the body lymph and estimate its lymphocyte content.

It is rather surprising to find that in the past physiological investigation has been concentrated almost exclusively upon the study of the fluid content of lymph, without taking any note of its cellular constituents. Heidenhain, for example (4), in his classical paper on lymph formation, makes only a passing reference to the occurrence of lymphocytes in lymph. He had noticed that under certain conditions the lymph became somewhat turbid, and says (p. 228) in commenting upon this: "Unter dem Mikroskope sieht man oft, aber nicht immer, zahlreiche Leucocyten. Da das weissliche Aussehen auch dann beobachtet wird, wenn die Leucocyten sehr sparsam sind, konnen sie nicht die Ursache desselben sein."

Since Heidenhain's time numerous observers (Rous (5), Haynes and Field (6), and others) have made occasional counts of the lymphocytes both in

thoracic duct lymph and in lymph from other vessels. Only scattered observations have been made, however, with no attempt to analyse their total quantitative significance, or to correlate them with the lymphocytes of the blood.

II. MATERIAL AND TECHNIQUE

The present work was carried out on twelve dogs, ranging in weight frmo 9 to 14 kg. The animal, after 24 hours' starvation (during which, however, it was given abundant fluid in the form of water), was fed on half a pound of lard. Two hours later the dog was lightly anaesthetised with a C.E. mixture, and then through a cannula—to which was kept attached throughout the experiment a 50 c.c. burette—tied into the left femoral vein was introduced a solution of chloralose in normal saline, in quantites of 0·1 gm. of chloralose per kg. body weight. The anaesthetic effect of the chloralose would last about 5 hours, after which half the original dose would keep the animal comfortably anaesthetised for the remainder of the experimental period, though occasionally a third dose would be necessary. The experimental period usually lasted 9 hours.

The exposure of the thoracic duct may present a little difficulty. An incision about 4 in. long is made along the line of the left external jugular vein, extending down over the left pectoralis major muscle. The outer border of the left sterno-mastoid is defined, and by dissecting down in the interval between sterno-mastoid and external jugular vein one reaches the junction of the external and internal jugular veins, and a little below the further junction of the common jugular vein with the left subclavian vein. The thoracic duct is usually found on the inner side of this second junction, between the veins externally, and trachea and oesophagus internally. The direction of the duct is upwards and forwards until immediately before its opening into the vein, when it usually dilates into a small cistern and bends rather more sharply forwards.

This description is true of the average case, but it must be emphasised that from an anatomical point of view the termination of the duct is very irregular. Sometimes, immediately after the left cervical lymph duct has joined it, it divides into two or three small branches which open separately into the vein. There are usually one or more valves (often three or four in a row close to one another) in the duct near its opening into the vein, and these may be a source of trouble on introducing the cannula. The wall of the duct is exceedingly thin, and if the cannula cannot be coaxed past the valves the experiment often has to be abandoned. The least force tears both the valves and the vessel wall. Apart from the difficulty of introducing the cannula, the main complication to be feared is coagulation of the lymph. There is great variability in this respect. Sometimes the lymph shows no sign of clotting, at other times it keeps persistently clotting in the cannula so that the experiment may finally have to be given up. Anti-coagulants (e.g. leech extract, peptone) dare not be used, for, as pointed out by Heidenhain, they are powerful lymphagogues.

III. THORACIC DUCT LYMPH: AMOUNT FORMED AND CELL CONTENT

Amount of lymph formed

Drops of lymph from the cannula were collected in a graduated cylinder, which was emptied hourly and the quantity of lymph recorded. If the results are adjusted to a body weight of 10 kg., the average lymph flow per hour is 24 c.c. This corresponds fairly closely with the figure obtained by Heidenhain (25 c.c. per hour), and with those of other workers quoted by Heidenhain (*loc. cit.*). It is of interest to note that Heidenhain's figures were obtained with dogs which had been starved for 36–48 hours, whereas the dogs used in the present series were fed before the experiment. The quantity of lymph produced would appear therefore to be independent of the digestive processes. (For additional evidence to this effect see Heidenhain's paper.) Furthermore, in Heidenhain's experiments the entire experimental period did not last longer than 3 hours. In the present series the duration of the experimental period was usually 9 hours, during 7 of which lymph was collected.

Cell content of lymph

At the end of every hour, when the total lymph flow for that hour had been measured, three drops of lymph were taken from the cannula in a small paraffined tube, and used for a lymphocyte count. It was found that the count varied very little over a period of 1 hour, and in fact even over a period of several hours remains astonishingly constant (Table I). The lymphocyte count at the end of each hour represents with fair accuracy the lymphocyte count throughout the hour. If therefore at the end of 1 hour the lymph contained 10,000 lymphocytes per c.mm., and if the total lymph flow for that hour was 25 c.c., then the total lymphocyte output for that hour would be

$$25 \times 10{,}000 \times 1000 = 250{,}000{,}000.$$

Over a 24-hour period this would represent a lymphocyte output of 6,000,000,000. In exceptional cases (Table II), there may be a more marked difference between the hourly lymphocyte counts. In these cases the mean of the two counts was taken as the average count for that hour. As already pointed out, however, the difference between the hourly counts was not usually marked.

The total daily output of lymphocytes

Taking the average of the whole experimental series, we arrive at the following figures for the total daily lymphocyte production—worked out for a 10 kg. dog:

Lymph formed in 24 hours	= 576 c.c.
Lymphocytes per c.c. = 9040 (per c.mm.) × 1000	= 9,040,000
Total daily output of lymphocytes	= 5,207,000,000

These are average figures. The figures for each experimental animal may be worked out from a set of data of the kind given in Tables I and II.

Table I

22. iii. 32. Weight of animal 11,000 gm.

Time	Lymph collected c.c.	Lymphocyte count
1.25 p.m.	25	11,500
2.25 ,,	23	11,300
3.25 ,,	22	12,200
4.25 ,,	26	11,100
5.25 ,,	23	11,300
6.25 ,,	23	10,000
7.30 ,,	24	10,100

Lymph collected over 7-hour period = 166 c.c.
Percentage of fat in total lymph = 1·05 per cent.
Grammes of fat carried by lymph = 1·7
Total blood lymphocytes at 1.25 p.m. = 1,210,000,000
Total blood lymphocytes at 7.35 p.m. = 518,000,000
Lymphocytes collected from thoracic duct* = 1,549,200,000
Difference between blood lymphocytes at beginning and end of experimental period = 692,000,000

* Beginning from 1.25, when first blood count taken.

Details of anaesthetic

Chloralose gm.	Saline c.c.	Time
1·1	100	11.30 a.m.
0·5	50	3.0 p.m.
0·5	50	4.30 ,,
0·5	50	6.40 ,,

Table II

26. iv. 32. Weight 12,500 gm.
Blood volume = 962 c.c.

Time	Lymph (c.c.)	Lymphocytes	Hour average
3.12 p.m.	23	33,400	—
4.12 ,,	23	14,200	23,800
5.12 ,,	23	13,500	13,850
6.12 ,,	22	12,100	12,800
*7.12 ,,	29	10,100	11,100

* At 6.10 eight minims of 1 : 1000 adrenalin were given intravenously. There was a slight increase in lymph flow for the following 50 min., followed by marked diminution and increased coagulability of the lymph, so that the experiment had to be abandoned.

Total blood lymphocytes A† = 4,069,000,000
Total blood lymphocytes B = 1,608,000,000
Difference between A and B = 2,461,000,000
Thoracic duct lymphocytes = 1,623,000,000

† Blood count A was at 3.0 p.m., hence thoracic duct lymphocytes counted from then.

(A = Commencement of experimental period. B = End of experimental period.)

Details of anaesthetic

11.35 a.m.	Chloralose 1·2 gm., saline 75 c.c.
3.5 p.m.	Chloralose 0·6 gm., saline 50 c.c.

The nature of the cells in thoracic duct lymph

The cells found in thoracic duct lymph are predominantly small lymphocytes, with so little cytoplasm that it does not as a rule appear to form a complete rim surrounding the nucleus. Some differential counts performed in the present series showed that on the average these small lymphocytes constitute 95 per cent. of the total cells. The remaining 5 per cent. consist of cells which are much larger. Some are large lymphocytes (8), others are macrophages.

When investigated by the dry smear technique these large cells may be interpreted either as lymphocytes or monocytes (*vide* Bloom (9), p. 278). Supravital staining by the neutral red and Janus green methods enables us to distinguish quite definitely between the two cell types. This method of examination shows that normal thoracic duct lymph contains no monocytes, and upon this fact all workers seem to be agreed (9, 10, 11, 12, 13, and my own results).

An accurate knowledge of the cell forms occurring in lymph is important for the following reason. The lymphocytes found in lymph may be either newly formed cells which have entered from the lymphoid tissues, or they may be lymphocytes which have entered the lymph from the blood, later to pass back into the blood. In other words, it is possible that there is a continuous circulation of lymphocytes, from blood to lymph, and from lymph to blood. The fact that we are unacquainted with any possible function which such a lymphocyte circulation might serve (the question of fat metabolism is considered further on) does not in itself prove that this circulation does not occur. Two considerations, however, are against it. In the first place 95 per cent. of the thoracic duct cells are small lymphocytes, and the small lymphocyte is generally believed to be a young and newly formed cell. Secondly the relative distribution of small and large lymphocytes is not the same in lymph as in blood. In lymph only 5 per cent. of the cells or even less are large lymphocytes. In blood about 20 per cent. of the lymphocytes are large, and the small lymphocytes usually have a better developed cytoplasmic rim than those of the lymph.

IV. THE LYMPHOCYTES OF THE BLOOD AND THEIR RELATION TO THOSE IN THORACIC DUCT LYMPH

The lymphocyte content of blood

The total white cell count per cubic millimetre of blood is carried out in the usual way, and then from the differential count the percentage of lymphocytes may be found. If the total blood volume is then ascertained, one may calculate the total blood lymphocytes. If l be the number of lymphocytes per c.mm. of blood, and v be the total blood volume in c.c., then the total blood lymphocytes $L = l \times v \times 1000$.

Relation between thoracic duct and blood lymphocytes

Under normal conditions, in spite of the fact that large numbers of lymphocytes are daily entering the blood via the thoracic duct, the number of lymphocytes in the blood remains approximately constant. This must mean that while some lymphocytes are continually entering the blood, an equal number must be leaving it, or else the number of lymphocytes in the blood would be progressively increasing, instead of remaining constant. The number of lymphocytes in the blood at any given moment is therefore the result of a balance between these two processes, some entering the blood, others leaving it. "Lymphocytosis" may therefore be due to one of two fundamentally different causes. It may be a true or active lymphocytosis, due to the entry into the blood of an increased number of lymphocytes. On the other hand it may be a false or retention lymphocytosis, owing to some interference with the mechanism whereby lymphocytes leave the blood, and their consequent accumulation in it. As an example of true lymphocytosis one may mention the lymphocytosis normally occurring in childhood, where the increased number of lymphocytes in the blood can be correlated with the presence in increased amounts of active lymphoid tissue. The term lymphocytosis as used clinically is meaningless unless we can understand which of these two processes is at fault.

The lymphocyte balance

It becomes possible, by collecting thoracic duct lymph and so preventing its lymphocytes from entering the blood, to draw up what one might term a lymphocyte balance sheet. Thus, over a given period X lymphocytes enter the blood via the thoracic duct, and X other lymphocytes leave the blood in some part of the body. Since these two processes are equal, the number of lymphocytes in the blood is fairly constant. If, however, we divert the thoracic duct lymph from the blood stream, we are preventing X newly formed lymphocytes from entering the blood. We are not, however, interfering with the X lymphocytes which leave the blood during this time. Hence at the end of the experimental period the blood should contain X lymphocytes less than it did at the commencement. In other words, the difference between the blood lymphocytes at the beginning and end of the experimental period should be of the same order as the number of lymphocytes which have been collected from the thoracic duct.

A balance sheet of this kind has been drawn up in Tables I and II. It will be seen that the figures for newly formed thoracic duct lymphocytes, and lymphocytes lost from the blood, are of the same order even though they do not exactly correspond. Over a longer period the correspondence would probably be much closer; there is further the question of the experimental error involved in all haemocytometric estimations.

Average figures for blood lymphocytes

Average figures for the total daily lymphocyte production in a 10 kg. dog have already been given (p. 252). In such a dog, taking the results obtained by Mayerson (7) on sixty healthy dogs, the following figures would be obtained for the lymphocyte balance:

Blood volume (1/13h body weight)	=770 c.c.
Lymphocytes per c.c. blood	=2,680,000
Total blood lymphocytes	=2,064,000,000
Total daily lymphocyte output (p. 250)	=5,207,000,000

Comparing then the total number of lymphocytes in the circulation with the total number of lymphocytes daily formed and passing into the blood by the thoracic duct, the significant fact emerges *that the blood lymphocytes are replaced about two and a half times daily.* In addition to this it must be borne in mind that, as previously pointed out (p. 250), large numbers of lymphocytes may enter the blood stream directly through the blood vessels of lymphoid tissue, without entering the lymph and so passing through the thoracic duct. These lymphocytes we cannot measure, but even if we disregard them entirely the figures for thoracic duct lymphocytes alone are sufficiently striking.

V. THE FATE OF THE BLOOD LYMPHOCYTES

The fundamental problem of normal lymphoid tissue

The ultimate destination of the blood lymphocytes constitutes the fundamental problem which confronts us with regard to normal lymphoid tissue. Granted that enormous numbers of lymphocytes are formed in lymphoid tissue and daily enter the blood stream, granted furthermore that equally large numbers of lymphocytes daily leave the blood, for otherwise the blood lymphocytes would not remain constant, the question presents itself "In what part of the body do these lymphocytes leave the blood, and for what purpose?"

The cell status of the lymphocyte

An essential fact which must be borne in mind is that the small lymphocyte is a young and actively growing cell. Its position is in no way comparable with that, for example, of a circulating erythrocyte. The erythrocyte is really only a dead cell remnant, and is finally broken down by the mechanical wear and tear of the circulation. Fragments of broken-down erythrocytes may be found in the blood, and also ingested by special phagocytic cells in different parts of the body. This, however, is not the case with the lymphocyte. That the lymphocyte is a young and actively growing cell is shown by its response to radiation (14). "It is generally agreed that the haemopoietic organs are very sensitive to X-rays, and that the earliest and greatest destruction occurs in the lymphocytopoietic centres." This, in conjunction with the well-known fact that the more actively growing a cell is the more sensitive it is to radiation, is

very significant. Still more important, however, in this connection is the enormous powers of development which the lymphocyte reveals in tissue cultures.

The lymphocytes in connective tissue

Normal connective tissue contains a small number of lymphocytes, and it is possible that some of the lymphocytes of the blood may leave it for the connective tissues. Our knowledge of connective tissue would not, however, lead us to suppose that it requires such an enormous supply of cells, and in any case there is evidence to show that the lymphocytes of connective tissue may multiply *in situ*, constituting the so-called histiogenous lymphocytes.

Possible sites of lymphocyte utilisation

When one considers the regions of the body which might need a regular and plentiful supply of cells, the problem narrows down considerably. The various glandular organs may be ruled out; they require a regular supply of chemical substances, but not of actual cells. The only tissue in the body which regularly manufactures large quantities of cells and might therefore be in need of constant replenishment is the bone marrow. Here, however, we are faced with the difficulty that the histological interpretation of bone marrow is extraordinarily difficult, owing to the confused and crowded arrangement of the cells. In addition, therefore, to direct histological study of bone marrow, the question has to be approached from other and more indirect angles before we can arrive at some definite conclusions.

The circulatory conditions in bone marrow

One question arises immediately: Would not the filtration from the blood stream of cellular elements require special circulatory conditions? And does the bone marrow provide such conditions? The answer to both these questions is in the affirmative. It has long been recognised that the circulatory conditions prevailing in bone marrow are peculiar, and that the alternate closing down and opening up of capillaries renders possible the escape of mature cells from the bone marrow into the blood. It does not, however, appear to have been realised that these peculiar circulatory conditions may work in the opposite way also. They not only facilitate the passage of cells from bone marrow to blood, but they also render possible the movement of cells in a reverse direction, from blood to bone marrow.

Current views on blood formation

It is interesting at this stage to consider the views now prevailing on the question of blood formation. There are two schools of thought. Both the erythrocytes and the granulocytes can be traced back to a stage in which they contain neither haemoglobin nor granules in their cytoplasm, and in which the nucleus is perfectly normal in appearance, being rounded and showing no sign

of lobulation or degeneration. This cell, with large rounded nucleus and clear basophile cytoplasm, is believed by many observers, on the basis of histological examination of the blood-forming organs, to be the large lymphocyte. These observers (Maximow, Weidenreich, and others) therefore believe that the parent cell of both erythrocyte and granulocyte is the same, namely the large lymphocyte (or lymphoid haemoblast—it has been given a variety of names in the bone marrow), and this constitutes the monophyletic theory of blood formation. Other haematologists believe that although the parent cells of the erythrocytes and the granulocytes undoubtedly resemble one another very closely, there are small but definite differences between them, and this point of view constitutes the polyphyletic theory of blood formation. As already pointed out, the histological interpretation of cell changes in bone marrow is so difficult that it cannot by itself yield an absolutely decisive answer.

The evidence of tissue culture experiments

For the elaboration of a new method of approach to the problem, we are indebted to the work of Maximow and his school. Maximow first showed that under certain (experimentally produced) abnormal conditions lymphocytes could develop into myeloid tissue in the body, e.g. in the kidney of the rabbit after ligation of the renal pedicle (15). He then showed, by a series of careful tissue culture experiments, not only that mammalian lymphoid tissue could be successfully cultured outside the body (16), but that it could give rise, through its contained lymphocytes, to fibroblasts, reticulum cells, and finally, in his later work, to typical myeloid elements (17, 18). Maximow never attempted to show that this happened as a normal physiological process in the body, but his work strongly suggests that possibility.

Very important in this connection is the work of Jordan, and Latta—work whose full significance does not seem to have been as yet correctly appreciated. Jordan (19, 20), investigating the changes taking place in lymph nodes while in the body, has shown that under certain abnormal conditions lymphocytes may give rise to abortive erythrocytes, or even complete one. Similarly, Latta (21) has shown that the lymphoid tissue in the intestinal wall of the rabbit may give rise to myeloid elements which differentiate *in situ*. It is of interest to note that Jordan, as a result of his work, was led to suggest for the lymphocyte the same life history as is put forward in the present paper.

VI. THE CONTROL OF LYMPHOCYTE PRODUCTION

Of the factors which control lymphocyte production we have no precise knowledge. Taken over a short period, less than one hour, an increased flow of lymph may result in an increased number of lymphocytes being swept into the blood, even though the actual concentration of cells in the lymph may fall (*vide* Rous (22)). Ehrlich seems to have been the first to put forward the view 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" ((23), quoted from Rous).

The injection of acid into the blood stream

The type of experiment represented by Table III is significant in this connection. The injection of 10 c.c. of *N*/10 hydrochloric acid into the circulation results in slightly increased lymph flow for 2 hours, followed by a return to normal. The cell concentration of the lymph rises steadily over the 3-hour period to more than twice its original value. It is therefore possible for an increased lymphocyte output to take place without increased lymph flow. It would appear that one of the factors causing this increased production may be a lessening of the alkali reserve of the blood.

Table III

7. vi. 32. Weight of animal 8730 gm.

Time	Lymph collected c.c.	Lymphocyte count	Hour average
2.15 p.m.	13·4	6,200	—
*3.15 ,,	10·6	5,800	6,000
4.15 ,,	15·6	7,500	6,650
5.15 ,,	15·5	8,560	8,030
6.15 ,,	10·2	13,400	10,980
7.15 ,,	7·5	5,700	9,500
7.50 ,,	6·5	8,500	7,100

* At 3.15 10 c.c. of *N*/10 HCl were given intravenously. Following this there is a steady increase in lymphocyte output as shown by the steady rise in the hourly lymphocyte count till 6.15 p.m.

Total blood lymphocytes at 1.15 p.m.	= 1,972,000,000
Total blood lymphocytes at 7.55 p.m.	= 394,000,000
Lymphocytes from thoracic duct	= 604,500,000
Difference between blood lymphocytes at beginning and end of experimental period	= 1,577,800,000

From the point of view of lymphocyte balance this table is difficult to understand. It is interesting, however, in showing the increased lymphocyte production following the introduction of acid into the circulation. The effect was *not* due to increased respiratory excursions. These did not last longer than 20 min.

If the views previously propounded concerning the ultimate fate of the lymphocyte are correct, we here have a possible explanation of the increased formation of red cells which follows anaemia consequent upon blood loss, or after the relative anaemia in people changing from a low to a high altitude. The primary response would then be the lymphocytosis, and the increased red-cell formation would be secondary. Clearly, in order to solve this problem, it is desirable to investigate lymphocyte production in experimental anaemia.

VII. THE POSSIBLE RÔLE OF LYMPH AND LYMPHOCYTES IN FAT TRANSPORT

It is generally believed that lymph and lymphocytes play an important part in the transport of fat. The evidence for this is twofold. In the first place the lymph vessels leaving the intestine become full of milky white chyle, which appears to contain a large amount of fat. Secondly, in some cases of elephantiasis, a large proportion of fat administered can be recovered from the lymph.

In neither of these two cases is the evidence entirely satisfactory. In elephantiasis we are obviously dealing with a pathological condition where the distended lymphatics may be unduly permeable, and fat may enter them in much larger quantity than normal. As far as chyle is concerned the milky white appearance is very deceptive, for the percentage of fat is astonishingly low.

A few observations bearing upon this have been made in the course of the present work. The dogs were fed at 9.0 a.m. with 225 gm. (½ lb.) of lard, anaesthetised at 11.0 a.m. and lymph collected from 12.30 p.m. for 7 hours. At the end of the experimental period, although no actual chemical examination of the intestinal contents was made, the small intestine was fairly empty.

In ten cases out of twelve the lymph was still white at 7.0 p.m.—10 hours after the meal. That this is not due to delay in absorption induced by the anaesthetic is shown by some observations of Rous (22), who found that in some cases the lymph was chylous when the animal was anaesthetised even 24 hours after a meal.

The total fat percentage in the three lots of lymph varied from 1·05 to 3·1 per cent. Over the 7-hour period, assuming the lymph flow to be 200 c.c.—actually it would be about 170 c.c.—6 gm. of fat would pass via the lymph into the blood.

It is possible that most of the fat had been absorbed in the 3½ hours before the collection of lymph began. This would mean that 220 gm. of fat would have to be carried by about 90 c.c. of lymph. For as shown by Colin ((24), quoted by Heidenhain) the quantity of lymph flowing from the thoracic duct is very little affected by eating or drinking.

Some unpublished figures kindly sent me by Prof. H. S. Raper show results of the same order. In a dog fed at 9.30 a.m., chyle collected from 12.45 to 3.45 p.m. contained only 1 gm. of oil, whereas intestinal analyses showed that 18·4 out of 28 gm. had been absorbed.

Still more important from the point of view of the present work is the fact that the fat is carried in the fluid portion of the lymph, not in the cells. This is what one would expect, for the small lymphocyte, with the barest minimum of cytoplasm, is not an ideal cell for the transport of any cytoplasmic inclusion. One may therefore state quite definitely that the cells of the lymph are not concerned with the transport of fat.

VIII. THE "BARRIER" FUNCTION OF LYMPHOID TISSUE

In conclusion, it would perhaps not be out of place to consider briefly the prevailing conception of lymphoid tissue. For this conception the clinician and the pathologist are mainly responsible. The clinician and the pathologist have repeatedly observed that the lymphoid tissues are often the seat of secondary inflammation or malignant deposits. They have therefore assumed that lymphoid tissue exists for that very purpose, serving as a barrier which holds up—for a time, at any rate—the spread of injurious matter. Yet even a brief consideration will serve to show that there are serious flaws in this interpretation.

It is undoubtedly a striking phenomenon to observe a mass of lymph glands enlarged as a result of inflammation of malignant deposits. But while it is true that the lymph glands act as temporary barriers against the spread of disease, it is equally true that the lymphatic vessels greatly facilitate this spread. Thus, quite a small epithelioma on the back of the hand may cause metastases in the axillary lymph nodes; once in the lymph nodes the metastases are then held in check, albeit only for a time. Had it not been for the lymphatic vessels, however, affording these metastases an easy and rapid pathway, they would never have been able to travel as far as the axilla, but would have remained strictly localised to the hand. The gain resulting from any barrier action of the lymph nodes is more than offset by the dangerously easy dissemination made possible by the lymphatic vessels. Similar considerations apply, *mutatis mutandis*, to the lymphatic spread of inflammation.

There are other reasons which render the barrier theory untenable. Perhaps the most cogent argument against it is that the majority of people live the greater part of their lives without either acute inflammation or malignant disease. Are we then to assume that in most people the lymphoid tissues are functionless, merely marking time, as it were, until they are needed? One need merely propound this question to realise what the answer is likely to be. It is this "silent" lymphoid tissue, the lymphoid tissue occurring in the healthy organism, which we must first understand before we can begin to appreciate the meaning of pathological changes.

In the normal body the one function which we can definitely assign to lymphoid tissues is the formation of lymphocytes. It is therefore the life history of the lymphocyte which must form the starting-point of any investigation into healthy lymphoid tissue.

SUMMARY

1. Quantitative estimations have been made:
   (*a*) of the newly formed lymphocytes passing via the thoracic duct into the blood stream;
   (*b*) of the lymphocytes present in the blood.

2. From a comparison of these figures it appears that the lymphocytes of the blood are replaced on an average two and a half times daily.

3. Since the blood lymphocytes remain constant, the same number of lymphocytes must daily leave the blood as enter it.

4. Considerations are brought forward which point very strongly to the view that the lymphocytes leave the blood mainly in the bone marrow, where they develop into erythrocytes and granulocytes.

5. The factors controlling lymphocyte production are at present unknown. The results of further experiments bearing upon this will be published in a subsequent paper.

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