THE LYMPHOCYTE CONTENT OF RABBIT BONE MARROW

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This work has been undertaken with the primary object of studying quantitatively the lymphocyte content of bone marrow. During the progress of the work other data have been obtained, which are briefly referred to here, but which it is hoped to present more fully later on.

The literature concerning lymphocyte production has been previously reviewed (Drinker & Yoffey, 1940). In addition, mention must be made of the work of Sanders, Florey & Barnes (1940), Kindred (1940, 1942), and Erf (1940). The essential facts are that lymphocytes are daily entering the blood stream in large numbers, and disappearing from it in equally large numbers, so that the level of lymphocytes circulating in the blood remains approximately constant. How and where so many lymphocytes leave the blood is still an unsolved problem. They do not appear to be destroyed while in the blood itself (Bunting & Huston, 1921). There is no extensive circulation of lymphocytes between blood and lymph (Yoffey & Drinker, 1939). They are not excreted into the lumen of the alimentary canal. The experiments of Yoffey (1942) on this point were not conclusive, but those of Erf (1940) on rabbits appear to be so. Erf's experiments show that lymphocytes can rapidly disappear from the blood even in the absence of the spleen and the major part of the alimentary canal. There thus remains for consideration the only other destination which has so far been suggested for the blood lymphocytes, namely, the bone marrow.

The technique of sternal puncture for the examination of living bone marrow has come into widespread use since its introduction by Arinkin (1929), and has yielded valuable information concerning the qualitative composition of the marrow. But in sternal puncture there is aspirated a mixture of marrow and blood, in varying proportions, which is useless for obtaining absolute marrow counts. The literature of bone marrow deals mainly with differential counts, and contains only scattered references to absolute values.

MATERIAL AND TECHNIQUE

The basic principle of the technique employed was to place a weighed quantity of bone marrow in a known weight of plasma, and then attempt to break up the marrow so that its cells would become freely suspended in the plasma. The cell suspension thus obtained is treated exactly like blood for the purpose of obtaining absolute and differential counts.

The first method employed (Exps. 1–10) was to weigh a small watch-glass, put in some plasma, weigh again, drop in the bone marrow, and weigh a third time. The marrow is then cut up into fragments with two fine knives and the suspension transferred with a pipette to a small corked tube, which is shaken vigorously by hand for about 2 min. This method is open to the objection that some evaporation occurs while the marrow is being teased. It also suffers from the more serious disadvantage that the plasma, although heparinized, frequently clots and renders the experiment valueless. Several experiments performed with the watch-glass technique had to be discarded.

The second and better method (Exps. 11–12) was to drop the marrow into a small, clean, tightly corked glass tube (length 5 cm., internal diameter 1·0 cm.) about half filled with plasma. As with the watch-glass method three weighings are required—with the tube empty, after the addition of plasma, and again after adding the bone marrow. The corked tube, containing known weights of plasma and bone marrow, is now placed in a shaker and shaken for 5 min., making a 10 in. excursion 400 times per minute. At the end of this time most of the bone marrow cells will be found free in the plasma. If counts and smears are made immediately after the shaking is complete, clotting is not usually a source of trouble.

Adult rabbits were used throughout, presumably healthy, but of unknown age. After making a blood count, the animal was anaesthetized by intravenous nembutal, a cannula introduced into one of the carotids, and 9 c.c. of blood collected in a graduated centrifuge tube containing 1 c.c. of a 3 % heparin† solution in normal saline. The animal was then bled to death, to exsanguinate the marrow as far as possible; the heparinized blood previously obtained was centrifuged for half an hour at 3000 r.p.m., and the supernatant plasma then withdrawn.

A rib, usually the fourth or fifth, was then removed, and scraped clean down to the bone. The outer wall of the rib was cut away with fine pointed scissors to expose the marrow. With a fine needle

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† For the heparin used in these experiments we are greatly indebted to British Drug Houses Ltd.

this is worked loose, and dropped in varying amounts into the plasma-containing tube, which is then weighed and shaken as previously described.

All marrow and blood films were stained with Wright's stain. At the same time, to obtain lymphocytes for purposes of comparison, suspensions were made of cells in the glandula mesenterica magna (the single large lymph node at the root of the mesentery), a piece of which was placed in a watch-glass containing some plasma and teased into small fragments. Incidentally, some of these cell suspensions showed very convincing transition series between reticulum cells and large lymphocytes.

The technique described, though the best we have so far been able to devise, has some obvious defects. In our calculations, we have assumed the specific gravity of bone marrow and plasma to be 1·0, and have taken the weight in grams as equivalent to the volume in c.c. This was done in order to make comparisons with cells in blood and lymph, which are counted in numbers per unit volume. According to Mechanik (1926) the specific gravity of bone marrow is very nearly 1·0, yellow marrow being a little less, red marrow slightly more than 1·0. Costal marrow, being intermediate in cellularity between the fatty marrow of the long bones and the very red marrow of sternum and vertebrae, will therefore have a specific gravity of practically 1·0.

In the differential counts an appreciable number of cells was listed as unclassified, on account of partial damage. Some of the cells may be so fragile as to be altogether destroyed in shaking. This may apply especially to the megakaryocyte, which was never seen in our marrow smears. Granulocytes in the smears are occasionally seen to have been damaged, but not usually disintegrated to the point where they were completely unrecognizable. If there is any considerable destruction of cells, the counts will be on the low side. This effect will be accentuated in those experiments where some of the marrow fragments remain unbroken after the shaking. It is possible that the factor of cell destruction is partly responsible for the discrepancy between our results and those of Kindred (1942), who obtained a considerably higher average count than that recorded here. Kindred's average count in paraffin sections of rib marrow was 1,960,000 nucleated cells per c.mm., which compares with our average figure of 469,900. His work was done on rats, however, and there may be marked species differences in addition to technical differences.

Table 1. *Absolute counts of total nucleated cells and lymphocytes in rabbit marrow, and lymphocytes per cent of nucleated cells*

No. of animal	Weight kg.	Total nucleated cells per c.mm. of marrow	Lymphocytes per c.mm. of marrow	Lymphocytes % of total nucleated cells of marrow
1	1·7	286,000	51,420	18·0
2	2·7	217,000	12,170	5·6
3	1·8	348,000	50,500	14·5
4	1·5	434,000	70,500	16·25
5	2·6	418,000	29,980	7·17
6	2·2	828,000	144,000	17·4
7	2·2	578,000	57,760	10·0
8	1·9	380,000	66,470	17·5
9	2·2	514,000	30,840	6·0
10	2·85	583,000	105,000	18·0
*11	2·95	712,000	90,660	12·75
*12	2·35	341,000	22,580	6·6
Average	2·25	469,900	61,000	12·48

* In Exps. 11 and 12 the animals were given a subcutaneous injection of Pernaemon 2 c.c. (kindly provided by Organon Laboratories Ltd.) 1 and 2 days respectively before the marrow count. Since the counts appeared to be within normal limits, they have been included in the present series.

RESULTS

These are given in Table 1, and the cells we have designated lymphocytes are illustrated in Pl. 1.

DISCUSSION

Cell identification. 'Lymphocytes' in Table 1 include both small and medium lymphocytes, but not large lymphocytes. Most of the cells are small (Pl. 1, figs. 1–15). No large lymphocytes have been recorded in the differential counts. Cells are occasionally seen which might be interpreted as such, but they have not been classified as lymphocytes in the present work, because of possible confusion with myeloblasts and early pro-erythroblasts (Sundberg & Downey, 1942). Though medium lymphocytes (Pl. 1, figs. 16–20) have been described as well as small, the distinction between the two has been an arbitrary one. The name 'medium', though honoured by tradition, is somewhat vague;

it is retained here to emphasize some significant structural features. However, no actual measurements have been made and no size limits have been fixed for the small and medium lymphocytes, since such limits are unreliable unless one can produce films of constant thickness. Generally speaking, cells have been designated medium lymphocytes when they have been appreciably larger than the average small lymphocyte, or when they have possessed a cytoplasmic rim of fair thickness (Pl. 1, figs. 21–25, 29–30). Most of the lymphocytes in rabbit bone marrow are small (Table 2, and Pl. 1, figs. 1–15).

Table 2. *Absolute counts of small and medium lymphocytes per c.mm. marrow*

No. of exp.	Small lymphocytes	Medium lymphocytes
1	45,700	5,720
2	8,700	3,470
3	44,400	6,090
4	51,000	19,500
5	25,800	4,180
7	40,460	17,300
8	61,700	4,770
11	69,300	21,360
12	17,800	4,780
Average	40,540	9,690

The nuclei of the small lymphocytes are regularly and characteristically pachychromatic (Pl. 1, figs. 1–20, 27–28). Cells of similar size and colouring, but with leptochromatic nucleus, have been regarded as micromyeloblasts. The medium lymphocytes, on the other hand (Pl. 1, figs. 21–25, 29–30), at times present a more difficult problem. Even in the lymph nodes, where the nature of the cells is much less open to doubt, a comparison of the nuclear structure of the small and medium lymphocyte shows that in the latter the nucleus tends to be more open in texture with the chromatin strands finer—in other words, leptochromatic. Pl. 1, fig. 23 shows a medium lymphocyte of this type in the bone marrow. Cells of this type, but with the nucleus still more leptochromatic and with slightly increased basophilia, could readily be interpreted as transitional forms on the way to becoming myeloblasts. They have not been included as lymphocytes in this paper.

It has not been thought necessary to make any attempt to discriminate between the small lymphocyte and the 'primitive' cell of Cunningham, Sabin & Doan (1925), since Sabin herself, in later publications (Sabin, Miller *et al.* 1936; Sabin & Miller, 1938), concluded that the morphological criteria for distinguishing primitive cell from small lymphocyte were not satisfactory.

Total lymphocyte content of bone marrow. Taking the figures of Nye (1931) for the total red marrow in the rabbit (i.e. 1·7 % of the body weight), and an average body weight in our experiments of 2·25 kg., the average volume of red marrow is 38·25 c.c. With a lymphocyte count of 61,000 per c.mm., this would mean a total marrow lymphocyte content of 2330×10^6.

Comparison of marrow and thoracic duct lymphocytes. How does this compare with the number of lymphocytes entering the blood via the thoracic duct? Sanders *et al.* (1940, Table IV), in nine rabbits with an average body weight of 2·52 kg., found an average daily lymphocyte output from the thoracic duct of 4000×10^6. Making a slight adjustment for the difference in weight in our present series, this would become 3560×10^6. It seems clear that, while these calculations can be only approximately correct, the figures are of the same order. The total lymphocyte content of bone marrow is such that, compared with the newly formed lymphocytes entering the blood via the thoracic duct, it could be derived from them. This appears to be the more probable since, as has already been pointed out, no other destination can be suggested for the blood lymphocytes, apart from those already disproved.

It is possible, of course, that the marrow itself contributes lymphocytes to the blood, and that the lymphocytes we have been counting are destined to enter the blood stream and are not derived from it. If this were so, the problem of the blood lymphocytes would be more perplexing than ever. The lymphocyte output, as measured by cannulating the thoracic duct, gives in the rabbit a daily replacement factor (D.R.F.) of 5·0 (Sanders *et al.* 1940). Estimates of lymphocyte output based on thoracic duct lymph are almost certainly minimal (Yoffey, 1936), and the true figure for lymphocyte production will therefore be in excess of this, so that in the rabbit the D.R.F. will be 5+. If one assumes that the bone marrow lymphocytes undergo mitosis once in 24 hr., a not unreasonable rate of division, and the marrow population is kept constant by the migration of excess lymphocytes into the blood, there would be on the basis of our figures a total D.R.F. of 8+. We would be faced with the extraordinary situation that lymphocytes are entering the blood in large numbers, remain there for 3 hr. or less, and then disappear without trace.

The suggested multiplication of marrow lymphocytes raises the question of the frequency with which mitoses are found in these cells. The identification even of resting marrow cells presents at times great difficulty; in the case of cells in mitosis, where finer details of nuclear structure become obscured, accurate identification becomes even more difficult unless there is present some distinguishing element in the cytoplasm such as specific granulation or haemoglobin. Ellerman's (1920) method of measuring the mitotic angle may be some help. In the absence of such measurements, and taking into account the difficulties already

mentioned, we would note for what it is worth our impression that mitoses of marrow lymphocytes are few and far between.

Jordan (1939 *a*, *b*) has consistently advocated the view that the lymphocytes are filtered out of the blood stream in the bone marrow, there to form the precursors of all the other blood cells. The data now submitted appear to lend support to the view that lymphocytes leave the blood to enter the bone marrow, though they do not afford any evidence on the fate of the lymphocytes in the marrow.

SUMMARY

A technique is described for obtaining absolute counts of the nucleated cells of the bone marrow.

The costal marrow of the rabbit (average of 12 experiments) had 469,000 nucleated cells per c.mm., of which 61,000 were lymphocytes; 75 % of these were small, 25 % medium.

The total lymphocyte content of rabbit marrow is of the same order as the number of lymphocytes which daily enter the blood through the thoracic duct.

Though the experiments on which this paper is based have been performed at Bristol, the work was begun in the Department of Anatomy, University College, Cardiff. We would like to place on record our gratitude to Prof. C. McLaren West for his kind encouragement while we were working in his Department at Cardiff.

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EXPLANATION OF PLATE

Cells from bone marrow, lymph node, and blood. Dry smears stained with Wright's Stain. Drawn with camera lucida, oil immersion ×100, Eyepiece ×12·5.

Figs. 1–15. Small lymphocytes; bone marrow.

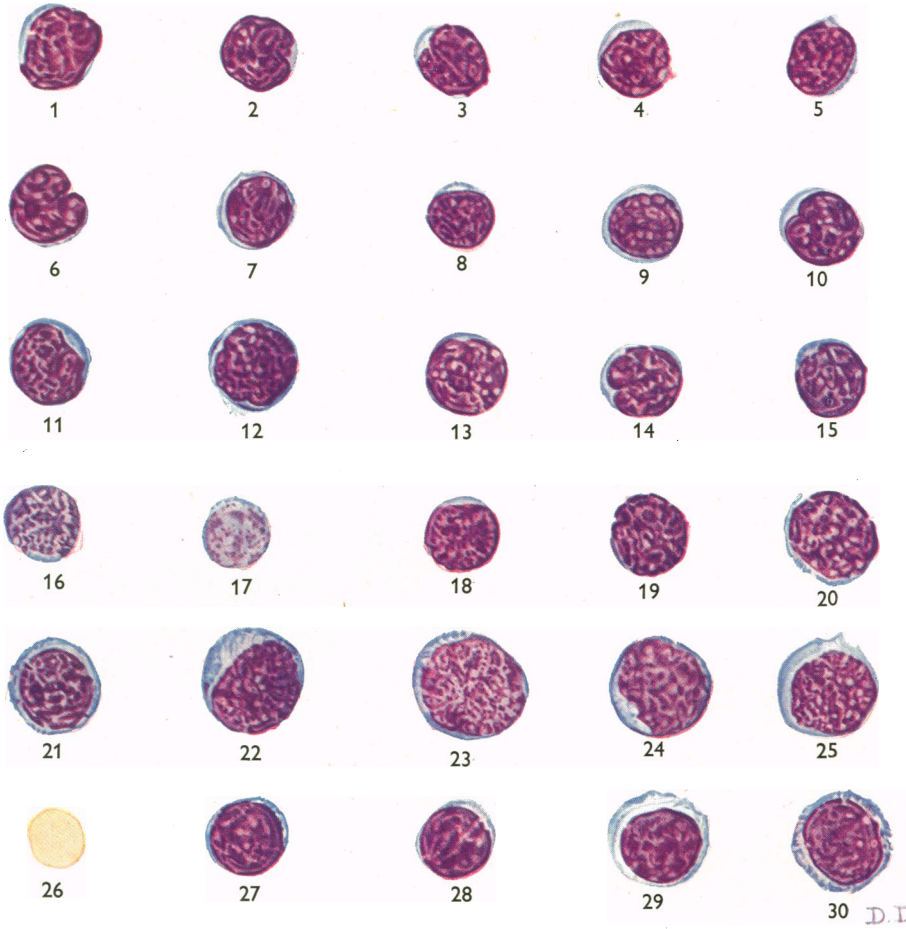
Figs. 16–20. Small lymphocytes; glandula mesenterica magna (lymph node).

Figs. 21–25. Medium lymphocytes; bone marrow

Fig. 26. Erythrocyte; blood.

Figs. 27–28. Small lymphocytes; blood.

Figs. 29–30. Medium lymphocytes; blood.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