Lymphology 10 (1977) 198–203

Variation in Output of Leukocytes and Erythrocytes in Human Peripheral Lymph during Rest and Activity

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Summary

The output of lymphocytes, monocytes, granulocytes and erythrocytes in peripheral leg lymph from healthy volounteers has been followed during night and everyday activity, during and after venous stasis, ergometer cycling and warm foot bath and during immobilisation in horizontal position over two 24-hour periods. The output of lymphocytes exceeded the output of monocytes and erythrocytes. Granulocytes were not found except immediately after cannulation. The cell output was low during night rest and the horizontal position for 24 hours. After assumption of the upright position the output of lymphocytes increased about 80 times and the output of erythrocytes 40 times probably because of washout of cells accumulated in tissue during rest. During ergometer cycling and venous stasis the output of cells increased and even more in the one hour rest period following this experiment. During venous stasis the output dropped but increased after the stasis had been released.

The pattern of variation in lymphocyte, erythrocyte and monocyte output was the same. This indicate that the fluctuation in output depends on variation in blood flow in the tissue. The output of cells during and after heating seams to be dependent also on increased capillary permeability for cells as the output of erythrocytes exceeded the output of lymphocytes. The high erythrocyte/lymphocyte ratio in blood versus a low ratio in lymph shows that these two cell types pass through the capillary wall by different mechanisms.

Introduction

Peripheral lymph comprises 80–90 per cent of small lymphocytes. The other cells are monocytes, granulocytes and some large lymphocytes. The cells arise from blood capillaries where they migrate through the endothelium into interstitial tissue fluid and hence to the lymph. In our studies of man also a various number of erythrocytes are always found in peripheral, prenodal lymph. In contrast to the leukocytes which migrate actively through the blood capillary wall, the passage of erythrocytes to the interstitial fluid and lymph must be a passive process.

Previous studies have shown that the protein concentration and output in human peripheral lymph varies with changes in body position and physical activity (1, 2). The purpose of this study is to see if this also takes place with cells and if there might be a difference in output between motile and immotile cells. This might give us a better understanding of what regulates cell circulation kinetic in non lymphoid tissue.

Material and methods

Peripheral lymph was collected in test tubes with 20 UI heparin, continously for 5–7 days from cannulated superficial leg lymphatic according to the technique previously described (3). The test tubes were replaced at the end of the various experimental and rest periods described below. The content of each tube containing all lymph sampled during the preceding interval was analyzed separately for cell number and output. Cell counts and differential counts were performed in Bürker chamber after 1/1 dilution with Giemsa stain in saline what makes it easy to distinguish between nucleated cells and erythrocytes. All cells in both chambers were counted. Differentiation was done between lymphocytes, monocytic groups of cells (typical monocytes and phagocytes), granulocytes and erythrocytes.

Lymph studies started about 48 hrs after cannulation had been done. The study was performed on two groups (Group I and II) of

healthy volunteers taking part in two different experimental programmes. Studies of lymph flow and protein output from the same groups has previously been reported (4). Besides different programme described below, in group I Patent Blue V. was used for visualization of lymphatic. In group II cannulation was performed without any dye.

Group I

Peripheral lymph from 9 legs of 5 healthy men, age 19–27 years, was collected for 5 days to study the cell concentration and output during night rest, after getting up, after 3 hours fast walking and, after three different procedures which were designed to increase capillary filtration. These were: 1/two hours venous stasis using sphygmomanometer cuff placed on the thigh and inflated to a pressure of 50 mm Hg, 2/two hours ergometer cycling performed at a speed of 30 km/h with 2 kg load, 3/warm foot bath for two hours in water with constant temperature of 41°C. Each of these procedures was preceded and followed by one hour rest in horizontal position.

Group II

Peripheral lymph from 5 legs of 3 healthy men, age 21–24 years was collected for 4 days to study the effect on cell concentration and output of 24 hr rest in a horizontal position. Day 1 and 3 were devoted to complete rest. The men remained in bed for 24 hours keeping their lower extremities motionless except for a period of 1 minute every 3 hours when they pedalled 30 rounds of the ergometer attached to the wall at the bottom of each bed. This was necessary to keep the lymph flowing in volumes suitable for studies. Day 2 and 4 were devoted to normal everyday activities. Lymph was collected over 3 hour periods, except for the morning samples collected for 1 hour. In the end of each lymph collection period blood samples were taken from the cutibal vein for evaluation of hematocrit, white cell counts and differential counts on 500 cells.

Results

Group I

Cell output during everyday activities and procedures to increase capillary filtration.

The lymph flow during night rest was in mean 0.24 ± 0.056 (SE) ml/hr. Flow increased 10–15 fold after getting up in the morning and during everyday activities. Flow was also high during venous stasis, ergometer cycling, warm foot bath and especially in the rest periods after these procedures (for details see 1 and 2).

Because of great individual variations and also variations between the two legs in the same person, the output of cells are presented as per cent of the mean night values for the same leg (Fig. 1 and 2). The mean lymphocyte output in night lymph was 81 ± 19 (SE) x 10^3/hr., erythrocyte output 20 ± 7 (SE) x 10^3/hr., monocyte output 5 ± 1.2 (SE) x 10^3/hr. Granulocytes were present in samples from the first experimental day accounting for 2–5 % of all nucleated cells. At later interval granulocytes were not found.

During everyday activity the highest output of lymphocytes, erythrocytes and monocytes was observed in the early morning sample when the mean output was 80, 40 and 43 times respectively of that of the night. During subsequent walking there was a marked drop in the output of all cell types. The three experiments to increase capillary filtration showed about the same pattern of changes: cell output during the experiment was almost the same as during walking except during venous stasis when the output of lymphocytes as well as erythrocytes and monocytes dropped and were below that of the night. In the rest period immediately after the experiment an increased output of cells was found in all experiments. The output of erythrocytes increased somewhat more than the output of lymphocytes (Fig. 1).

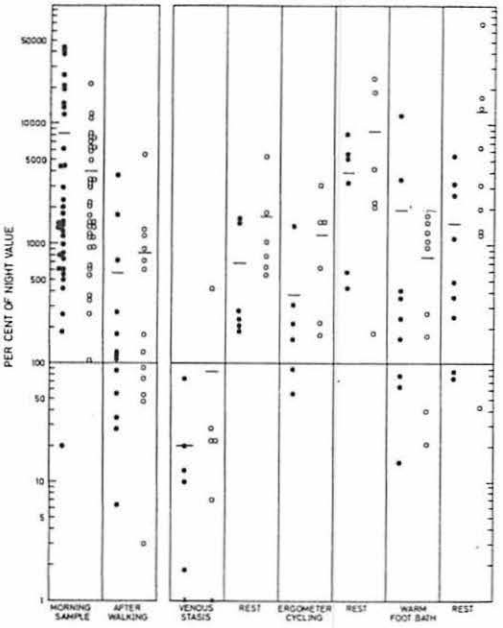

Fig. 1 Output/hr. of lymphocytes • and erythrocytes ○ in human peripheral leg lymph during 5 days of normal activity and during special procedures designed to increase capillary filtration. Values from 9 legs from 5 men expressed in per cent of the mean night value for the same leg = 100 %

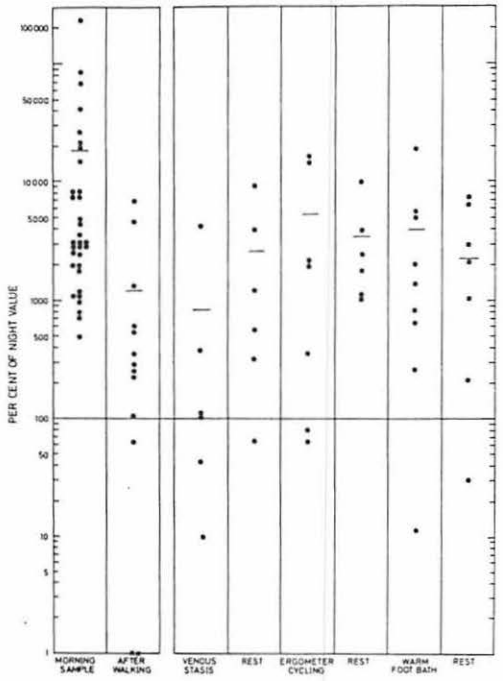

Fig. 2 Output/hr. of monocytes in human periperal lymph during the same procedures as shown in Fig. 1.

Group II

Cell output during 24 hour rest in horizontal position.

In this experiment the cell output is presented as per cent of the output on the second night from the same leg which was a night after a day of normal activity. During the second night the lymph flow was in mean 0.09 ± 0.04 SE ml/hr. During the 24 hr. rest period it was in mean 1.2–4 times higher but still low. When getting up and during everyday activities the flow increased considerably as in the first experiment (for details see ref. 4).

The mean lymphocyte output for all legs during the second night was 11.4 ± 9.2 SE x 10^3/hr., erythrocyte output 3.6 ± 9 SE x 10^3/hr and monocyte output 0.87 ± 0.4 x 10^3/hr. Figures 3 and 4 shows the output in the course of the experimental period. The variation in output for all three cell types follows the same pattern. When getting up after long periods of rest in horizontal position there is a high output of cells which is more pronounced for lymphocytes and monocytes than for erythrocytes (Fig. 3 and 4). During the two 24 hr. rest periods in horizontal position the output of cells dropped gradually and reached the lowest values at the end of these periods. During everyday activity on day 2 the output of lymphocytes, monocytes and erythrocytes was high when compared with night value.

The blood leukocyte and lymphocyte counts showed the lowest values when getting up in the morning (Fig. 5). The counts increased during the day both when the individuals were resting in horizontal position and during the days of normal activity. The difference between the mean lowest and highest values were in the order of 50 %. The counts dropped during sleep between hr 24:00 and 05:00. The hematocrit values were a little higher during activity than during rest.

Discussion

The present experiments show that there is a great variation in cell output from peripheral prenodal lymph depending on the position of the body and physical activity.

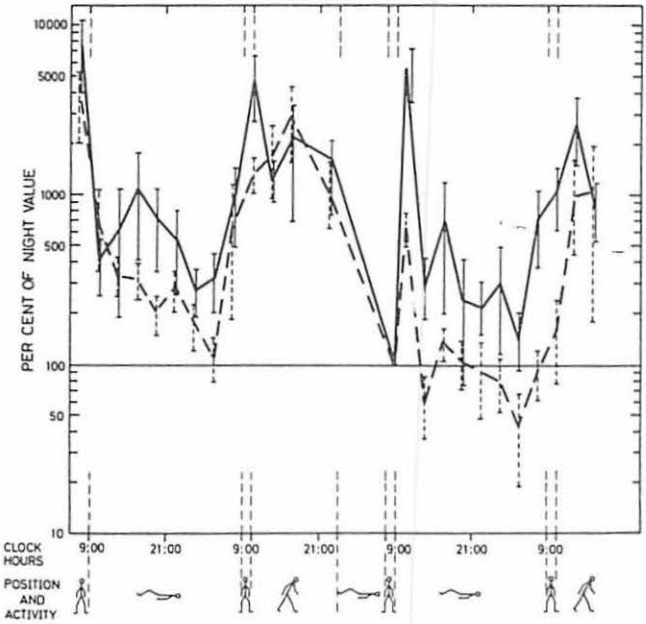

Fig. 3 Output/hr. of lymphocytes —— and erythrocytes – – – – – – in human peripheral leg lymph during 3 days including 2 days of rest in horizontal position. Values from 5 legs from 3 men expressed in per cent of the value at night 2 for the same leg = 100 %.

mean ± SE

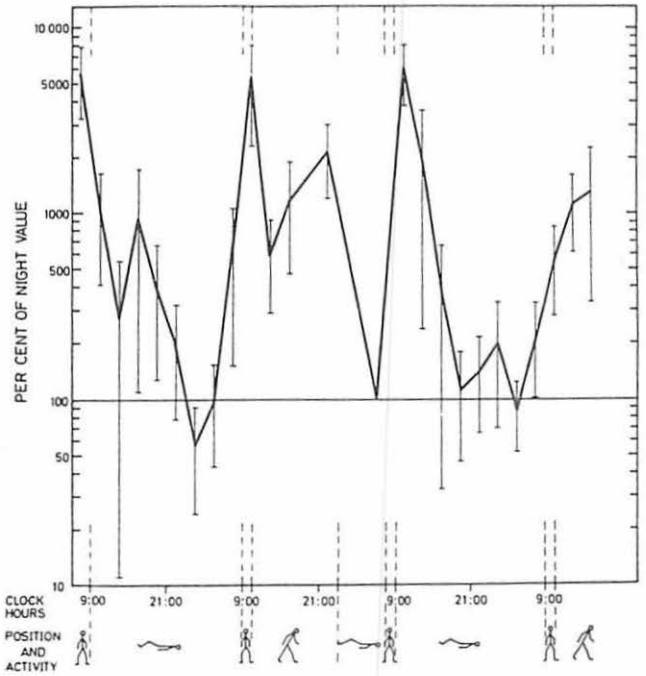

Fig. 4 Output/hr. of monocytes during the same procedures as shown in Fig. 3.

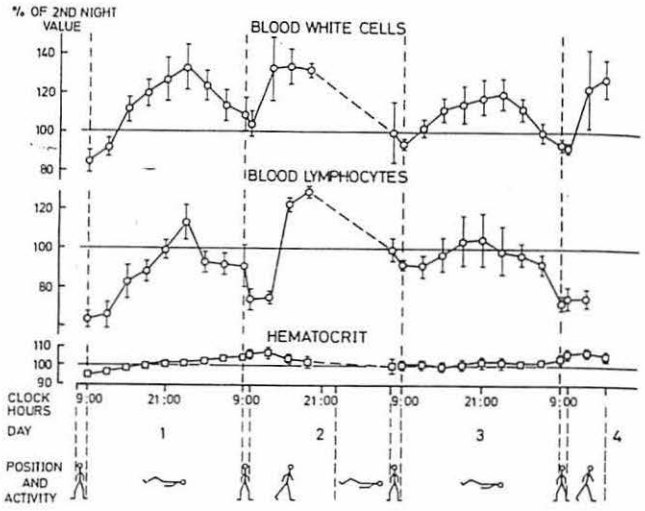

Fig. 5 Blood white cell and lymphocyte count and hematocrit during 3 days including 2 days of rest in horizontal position. Values from 3 men expressed in per cent of 2-nd night value = 100 %.

mean ± SE

The high output of cells after getting up in the morning is probably caused by a wash out of cells which have accumulated in interstitial tissue fluid and lymph capillaries during night rest, when the lymph flow is extremely low. The relatively high output also of erythrocytes in these samples shows that some erythrocytes pass through the capillary wall even during complete rest when physical traumatisation of the foot vessels during walking is eliminated. The high output of cells during and especially after the relatively short periods of venous stasis, ergometer cycling and warm foot bath shows that relatively large number of cells have passed to the interstitial fluid during this period. The high output after the experiments is considered to be caused by a wash out of fluid and cells accumulated in the tissue during the experiment in combination with protractive hyperemia.

The pattern of variation in blood leukocyte and lymphocyte counts was the same during the two days of rest. The shape of the curve is to some extent inverse of that of the cell output in lymph. Because of this the question arises if the low blood lymphocyte counts during the night could be caused by accumulation of cells in interstitial tissue, lymph vessels and nodes (5). We found this interpretation unlikely because assuming upright position was not followed by a peak in blood lymphocyte count which should be expected.

The mechanism regulating the passage of lymphocytes through the wall of the small blood capillaries into interstitial tissue fluid and peripheral lymph is probably different from the mechanism regulating erythrocytes passage to the lymph. This is clear from the fact that lymphocyte/erythrocyte ratio in blood is less than 1/3000 while the ratio in peripheral lymph is about 4/1. The difference can not be caused by peripheral flow of leucocytes in the blood vessels versus central flow of erythrocytes because the migration takes place in the narrow blood capillaries where both lymphocytes and erythrocytes have a close contact with the endothelium. It must depend on differences in cell properties such as motility and stickiness to the endothelial cell. Nevertheless, the present experiments show that the changes in output of lymphocytes, monocytes and erythrocytes follows almost the same pattern during various experimental conditions. During rest in horizontal position there is a low output, a very high output is found during the first hour after getting up and a relatively high output is also observed during and immediately after various types of physical activity. The similar fluctuation in the output of immotile

erythrocytes and motile leucocytes indicates by our opinion that these variations are not caused by changes in permeability of the capillary wall for cells. This would have caused a much greater output of erythrocytes in periods with high permeability as they are at least 1000 times more numerous in the blood than the other cells. The best explanation is that the variation in the number of cells released to the interstitial space is caused by and follows the variation in the number of open capillaries what means variation in capillary surface area. With a high blood flow more cells pass into the tissue than when the flow is low.

The highest output of cells was found after warm foot bath when the output of erythrocytes exceeded the output of lymphocytes. The highest single output observed in this period was 3 x 10^6 erythrocytes/hr which corresponds to about 0.5 μl blood. On the same leg the lymphocyte output was at the same time 7 x 10^4/hr. This shift in lymphocyte/erythrocyte ratio from around 1/1 to around 1/100 indicate increased capillary permeability for cells. It is known that skin heating dilate the local arterioles. Both arterial and venous capillary pressures rises, and capillary pulsation is obvious (6). During such conditions it might happen that "weak points" in the capillary wall, for instance at the junctions between three endothelial cells might allow erythrocytes to pass through.

The output of monocytes follow almost the same pattern as the output of lymphocytes, but the interpretation is difficult because of few cells. It is of interest that peripheral lymph contains various numbers of monocytes and phagocytes contrary to post-nodal lymph where these cells are extremely few or lacking (7, 8). This could indicate that monocyte migration is regulated by an other mechanism in the non lymphoid tissue than in lymph nodes or, that monocytes are transformed to lymphocyte like cells in the node before they enter the efferent lymphatics.

Granulocytes were found only in the first day samples of group I when Patent Blue V. had been used for localization of the lymph vessels and expresses probably an inflammatory reaction to the dye. It is of considerable interest also that the number of granulocytes in peripheral lymph is significantly lower than the number of monocytes despite they are more numerous in the blood. Both cell types are motile and can pass the junction between endothelial cells in the capillaries. We have no observation so far, that could explain why they behave differently.

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