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# Lymph Flow and Protein in the Normal Male Leg During Lying, Getting up, and Walking

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**Summary**

Leg lymph protein concentration and output, lymph/serum total protein and albumin ratio and lymph flow/concentration relationship were studied in four normal men with limbs immobilized in the horizontal position over two 24-hour periods, followed by a day in the upright position. A high concentration of total protein and albumin and the L/S ratio and a high protein output were found with immobilization. Assumption of the upright position after 24 hours of horizontal rest was followed by a high lymph flow with high protein concentration. This effect suggested the accumulation of large amounts of protein in the lymph space during the preceding rest period. The failure of lymph protein concentration to fall and an insignificant decrease in L/S total protein ratio render it unlikely that a sudden rise in capillary filtration occurred. Moreover, an essentially unchanging total protein and albumin L/S ratio is evidence against a sudden increase in capillary permeability. Moreover, a continuous decrease in lymph protein concentration during the day in the upright position probably occurs when "new" capillary filtrate replaces the proteins accumulated in the lymph space during the periods in the horizontal position. There is no diurnal rhythm in lymph protein concentration. The fast and large lymph flow that develops after assumption of the upright position and the continuous protein wash-out with ambulation are protective safety factors against edema formation in the leg.

Leg lymph flow and composition in normal men undergo major 24-hour variation dependent on the position of the body and type of activity (2, 8). At night lymph flow during rest is low and protein concentration reaches high levels. Getting up in the morning is accompanied by high lymph flow with high protein concentration. Fast walking in the early morning is also characterized by high lymph flow with high protein level. Later during the day, in the upright position, lymph protein level decreases to reach, before bed-time, values about 40 % lower than during the preceding night (2). Thus, two features characterize human leg lymph dynamics during a 24-hour period of normal activity. One is a lack of correlation between lymph flow and protein concentration, and the other, a steady fall in protein concentration throughout the day.

To investigate the mechanism of changing lymph flow and protein concentration, this study was designed to answer the following questions: a) Can high lymph flow and protein output upon assuming the upright position be attributed to accumulation of high amounts of protein in tissue fluid during periods in the horizontal position, and to the time required to wash this protein out of the interstitium and lymphatics (9, 10) in the upright position? b) What is the time period necessary to obtain in the leg a steady lymph flow and protein concentration level? c) Is the high lymph flow with high protein concentration observed after a shift from the horizontal to an upright position due to increased capillary permeability? d) Is there a diurnal variation in capillary protein filtration which influences the protein concentration of interstitial fluid and lymph?

## *Material and Methods*

Studies were carried out on four normal men aged 21–24 years. A superficial lymph vessel was cannulated in the leg in each individual according to a technique described previously (1). This method was successful in 5 legs. Lymph studies were started 3 days after cannulation and observations were carried out for 4 days. Days 1 and 3 were devoted to complete rest in the horizontal position. The men remained in bed for 24 hours keeping

their lower extremities motionless except for 1 minute each hour when they pedalled 30 rounds of an ergometer attached to the bottom of the bed. This method was necessary to keep lymph flowing in volumes sufficient for biochemical analysis. Days 2 and 4 were devoted to normal everyday activities. Lymph was collected continuously over 3 hourly periods, except for the morning samples after change of position of the body when the collection period was 1 hour, and the second night when collection lasted for 9 hours. Lymph was collected into sterile plastic syringes containing 2 IU of heparin in 0.05 ml saline, attached to the leg. Syringes were replaced at the end of each sampling period. The content of each tube comprising all lymph sampled during the preceding interval, was analyzed separately. Samples were frozen at $-20^{\circ}$ and stored. At the end of each sampling period 10 ml of blood was withdrawn into syringes with 5 IU of heparin in 0.5 ml saline.

The volume of collected lymph was measured and flow expressed in ml per hour. Lymph total protein and albumin concentration ratios and the relationship to lymph flow were calculated. In blood samples total protein and albumin concentration were measured in sera. Protein levels in lymph and sera were determined by biuret method while albumin concentration was measured by paper electrophoresis.

Because of significant variations in lymph flow and composition between individuals and from leg to leg in the same individual due to the different topography and caliber of the cannulated vessel and permeability of exchange vessels (6), the results were calculated for each leg in % of the second night value. This method also enabled comparison of the present results with those of our previous studies (2, 8).

*Results*

A) Lymph flow: Mean lymph flow was low during the 24-hour period at rest in the horizontal position but 1.2 to 4 times higher than during the second night of study taken as the reference level (Fig. 1). There were only minor variations in the flow rate, with the lowest flow recorded between 24.00 hours and 6.00 hours during deep sleep and maximum muscle relaxation. Assumption of the upright position after 24-hour rest was followed by a threefold rise in mean flow compared with the preceding rest period. This effect lasted for about 1 hour, and then a slight decrease was observed. Later, the lymph flow was maintained at a steady level, until the end of the day of normal activity in the upright position.

B) Lymph protein concentration and output: Mean protein concentration was high during the 24-hour rest period and was 30 to 82 % higher than during the second night (Fig. 2). It reached a stable level in 3–6 hours after leg immobilization in the horizontal position. Later, only minor fluctuations inverse to the lymph flow rate were observed. The protein concentration was in some samples as high as 4.0 g%. No rhythmic diurnal variations in lymph protein concentration were found. On getting up after the 24-hour rest period mean protein concentration remained essentially unchanged over the first hour. Subsequently, the protein concentration began to decrease reaching by the end of the day values as low as 1.2 g%.

Lymph/serum (L/S) total protein reached during the 24-hour rest period values of 0.55 while the L/S albumin was 0.72 (Fig. 2). Highest values were observed during the night and early morning hours. The difference between L/S concentration ratio for total protein and albumin were almost constant over the entire 24-hour period. After assuming the upright position the lymph/serum concentration ratios began to decrease reaching by the end of the day values as low as 0.25 for total protein and 0.33 for albumin.

Mean protein output during the 24-hour rest period was relatively high and 1.5 to 5 times higher than during the second night (Fig. 3). After assuming the upright position it steadily rose reaching after 1 hour values 3 times higher than during the rest period. Later, a constant fall in protein output was observed, due to a decrease in lymph protein concentration. The mean total 24-hour protein output during day 1 (rest) was about 1/3 lower than the 24-hour output during day 2 (normal activity).

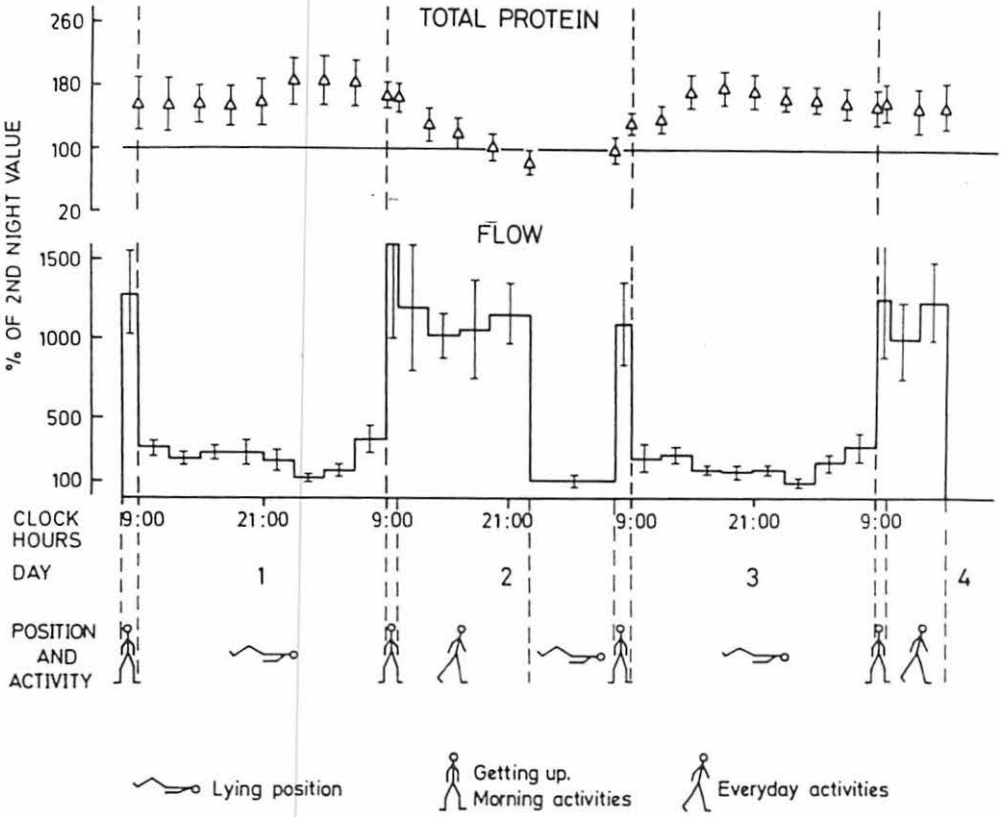


Fig. 1 Lymph protein concentration and flow in the leg during two 24-hour periods of rest in horizontal position followed by days of normal activity. Mean values from 5 legs expressed in % of the second night value ± one standard error.

C) Lymph/serum protein concentration to lymph flow relationship:

An inverse relationship between L/S total protein and albumin concentration and lymph flow was observed throughout most of the 4 day observation time, with exception of periods when position of the body changed (Fig. 4). Assumption of the upright position was accompanied by high lymph flow with high protein concentration for a period of about 1 hour; lying down after a period in upright position yielded low flow with no increase in concentration for at least the first three hours.

*Discussion*

These results indicate that placing the lower limb in the horizontal position thereby excluding the influence of gravitational forces on capillary filtration and eliminating the muscle pump, allows accumulation of tissue fluid and lymph of a high protein concentration. This conclusion is based on the findings of a high lymph/serum concentration ratio for total protein and albumin and a relatively high lymph protein output during a 24-hour period of leg immobilization, with gradual increase both in lymph protein concentration and protein volume. The mechanism of this phenomenon may be explained in the following way.

In a leg placed in the horizontal position, capillary hydrostatic pressure is low (7), especially on the venous side. Accordingly, filtration rate becomes low, but diffusion and vesicular transport of proteins through the walls of exchange vessels continues constantly tending to equilibrate the intra- and extra-

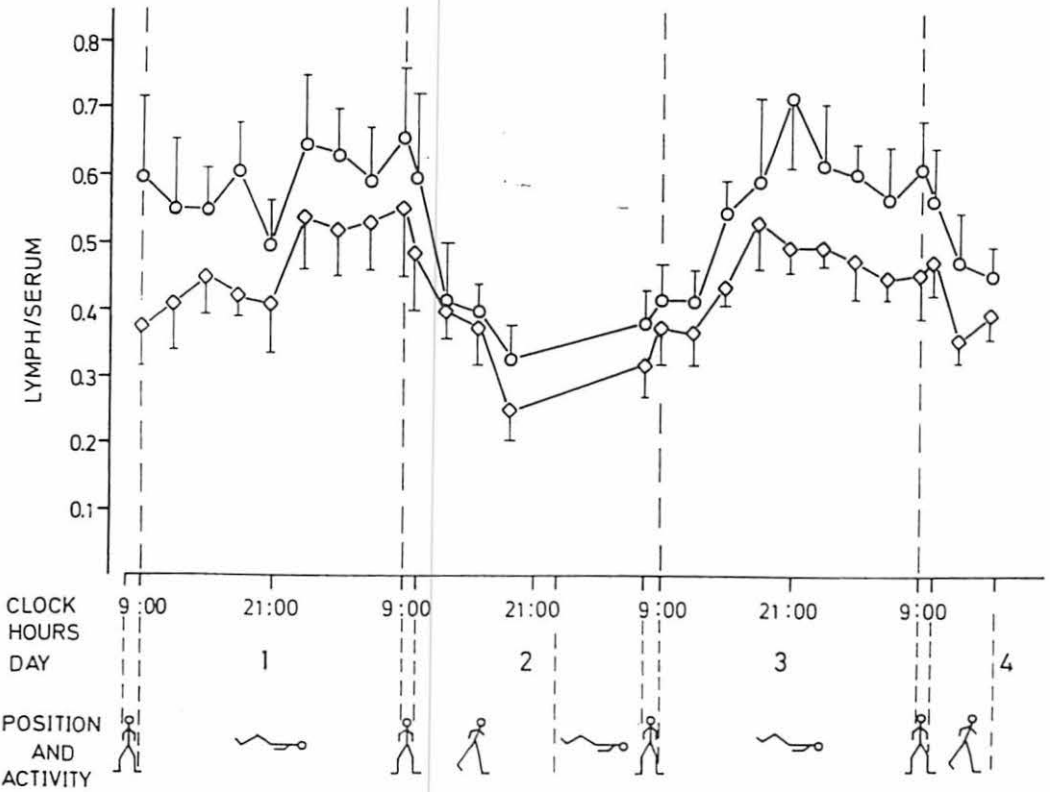


Fig. 2 Lymph/serum albumin (○) and total protein (◇) concentration ratio during the same procedures as shown in Fig. 1.

vascular protein concentrations (3). As a result, the concentration of interstitial fluid protein increases steadily. The effect is further aggravated by limited transport of proteins out of the tissue spaces because of low lymph flow with reduced muscular action (11). A rise in interstitial lymph fluid volume also occurs as the lack of muscular propulsion for optimum lymph flow allows surplus capillary filtrate to accumulate in the "lymph space" (4).

The change to an upright position was followed during the first hour by a high flow of lymph with high protein concentration, without a significant decrease in lymph/serum total protein and albumin ratio. This finding suggests that the high initial protein output was due to the "squeeze-out" of interstitial fluid and lymph by sudden venous expansion and muscular pumping rather than to increased capillary filtration. If the rise in lymph protein output was caused primarily by a sudden increase in capillary filtration, a decrease in lymph protein concentration and lymph/serum total protein concentration ratio would have been more evident. Enhanced permeability of capillaries upon assumption of upright position also does not seem likely for the increased protein output, because a decrease in ratio between lymph/serum total protein to lymph/serum albumin concentration should have occurred.

When the values of lymph/serum total protein concentration and albumin concentration were plotted against flow rates an inverse relationship between concentration and flow was found throughout most of the 4 day observation period. This finding is consistent with those of

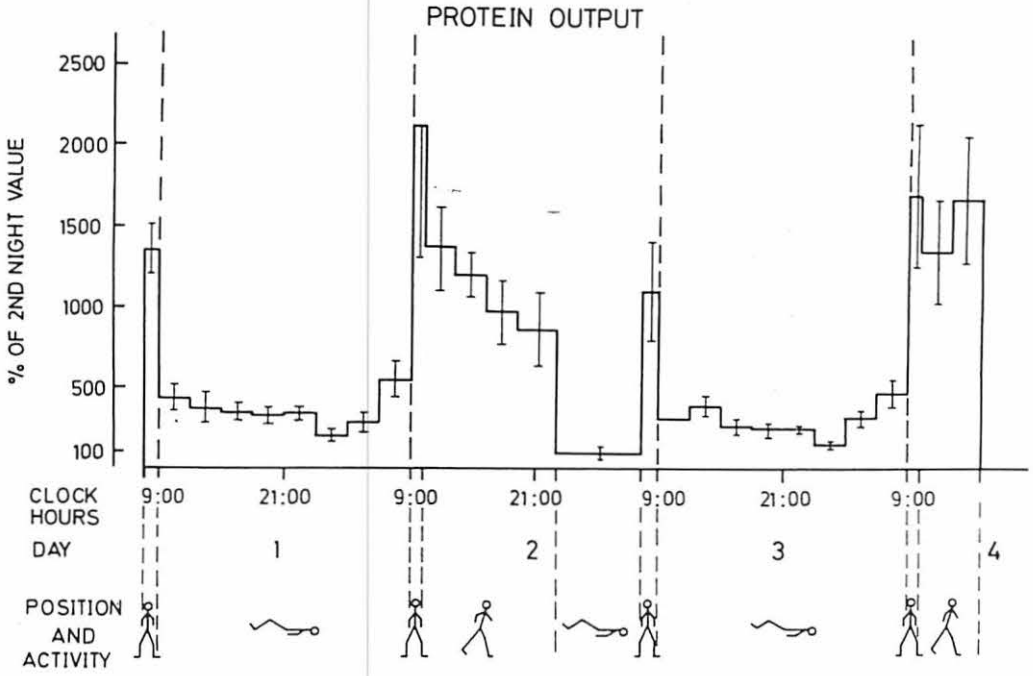


Fig. 3 Protein output in leg lymph during the same procedures as shown in Fig. 1.

*Garlick* and *Renkin* (3). However, a change in body position was preceded by a period without this inverse relationship between flow and concentration. Thus, a change from the horizontal to upright position was in each case followed by high lymph flow without a fall in lymph protein concentration. The decrease in concentration with high flows occurred after some time, probably when most of the interstitial fluid and lymph formed during the 24 hour rest period had been sufficiently squeezed out, and new capillary filtrate had refilled the lymph space during the upright position.

Lack of an inverse relationship between flow and concentration was also seen when the subjects changed from an upright to a horizontal position. In this situation an increase in protein concentration with low flows occurred at a time when the lymph space had probably been saturated with capillary filtrate formed under conditions of resting. This effect lasted longer than after changing into upright position probably due to at least 3 factors: 1) evacuation of most

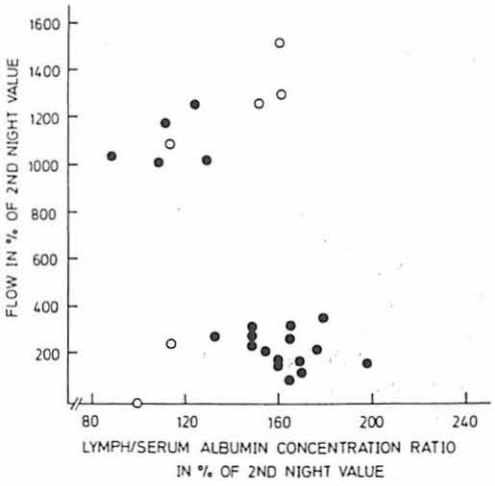


Fig. 4 Lymph flow to lymph/serum albumin concentration relationship during the same procedures as shown in Fig. 1. Black dots indicate values obtained when the men remained in the horizontal or upright position for at least 3 hours. L/S albumin concentration was inversely related to lymph flow. Open dots represent values obtained 1–3 hours after change of position of the body. Lack of correlation between L/S albumin concentration and lymph flow.

of the interstitial fluid and lymph formed during the preceding period in the upright position and walking, 2) time required for filtration and diffusion of proteins into the lymph space, and 3) high compliance of the lymph space (5).

A continuous decrease in lymph protein concentration observed while in the upright position may be explained by the wash-out of proteins accumulated in the lymph space during periods in the horizontal position. Moreover, some dilution of the interstitial and lymph proteins by the low protein capillary filtrate accumulating in the enlarged lymph space may also have occurred. Nonetheless, there was a stable lymph flow throughout the day in the upright position and since no clinically detectable edema was evident this finding would argue against an accumulation of excess low protein fluid in the lymph space. No steady state could be reached for lymph flow and protein concentration in the upright position. Lymph flow remained relatively stable while protein concentration continued to decrease. However, in the horizontal position a steady state for both flow and protein concentration could be observed in 3–6 hours following assumption of this position.

It is concluded that the rapid and increased lymph flow immediately after assuming the upright position helps to remove fluid and protein from the interstitium. Together with the continuous wash-out of protein during the day while upright provides a safety factor against formation of edema in the skin and subcutaneous tissue of the leg.

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