

cause an increase in the rate of protein movement out of the capillaries. According to Jacobson and Kjellmer (1964) however, there are two mechanisms which may lower the protein concentration of the capillary filtrate when the filtration rate is increased. The degree of molecular sieving of substances passing through the capillary wall by restricted diffusion is increased by high rates of filtration, which results in a lower concentration of high molecular mass substances, e.g. proteins, in the filtrate (Pappenheimer et al, 1951). The rate of absorption of water at the low pressure end of the capillaries is diminished at increased capillary pressures, which also tends to decrease the protein concentration of interstitial fluid. Kjellmer (1964) had noted that direct determination of the permeability characteristics of the capillary membrane in the resting and contracting calf muscles of the dog shows that the capillary permeability does not change during exercise. This then eliminates it as a factor contributing to increased fluid and protein filtration. At the same time, the massaging action of the contracting muscle increases lymph flow from the area of activity, thus moving protein that was present within the muscle interstitial space, into the vascular volume (White et al, 1933). The initial protein content of lymph should be higher than that obtained later in the exercise period. The reason for this is that the initial increase in lymph flow flushes out protein 'trapped' in this volume whereas the protein content of later lymph would be more directly dependant upon the rate of protein movement from the vascular volume to the interstitial spaces (Senay, 1972). The initiation of exercise is therefore

responsible for the increase of the protein content within the vascular volume by the movement of protein from the interstitial spaces and by the fluid leaving the vascular volume. Further augmentation of extravascular fluid volume is prevented by balancing protein and fluid movement into and out of the vascular volume. The amount of protein retained within the vascular volume is now greater than at rest because of the initial movement of protein into the vascular volume accompanying onset of exercise. It was found by De Lanne et al (1958) that significant amounts of water left the vascular volume during the first five minutes of exercise. Continued exercise brought about no further decrease of the vascular volume. Within thirty minutes after terminating the exercise, the vascular volume had returned to its control values.

Poortmans (1971) had stated that during muscular activity the renal glomerulus becomes more permeable to protein molecules. The way this would effect the results of this study is speculated later on in this section (section 5.4.2).

Röcker et al (1976) concluded from their results that changes in plasma protein concentration are due not only to transcapillary fluid shifts, but also to a protein redistribution between the intravascular and extravascular pools.

5.4.1 Plasma

A summary of the results before and after exercise can be found in table 13. The total protein concentration increased significantly ($P < 0,05$) whereas the

concentration of albumin remained the same. The increase in total protein concentration was thus due to the significant increase in the globulin concentration ($P < 0.05$). The protein exchange between the vascular compartment and the interstitial space depends not only on the fluid transfer and capillary circulation, but also on the ability of the various molecules to go through the pores and circulate in the interstitial spaces. As a result of the factors discussed in the previous section (vasodilation and hydrostatic pressure), albumin molecules should move more easily through the capillaries because of their smaller molecular size. The globulin molecules, on the other hand, are larger and would therefore be more readily retained and concentrated in the capillaries. In spite of the fact that the albumin molecules move into the interstitial space from the vascular circulation at a faster rate, they are being returned to the vascular circulation at a faster rate by means of the lymph. The result is that the albumin concentrations before and after exercise remained static in this present study. If the movement of albumin molecules out of the capillaries did not increase with the onset of exercise, the concentration of these molecules in the vascular volume should have increased because of the higher filtration rate of the vascular fluid. On the other hand, because the intravascular albumin concentration in this study did not decrease either, it is an indication that more albumin had to be returned to the vascular circulation by the

lymph at the onset of exercise. This is possibly due to the contraction of muscles whereby more fluid and protein is initially returned to the blood (see also discussion in previous section). Contrary to the findings of this study is the report of De Lanne et al (1958) of a 2 per cent increase in the albumin concentration during muscular exercise. Poortmans (1971) found increases in both the globulin and albumin concentrations. (For further comments see section 5.4.2.)

The significant increase in the globulin concentration is in accordance with the previously discussed theories (section 5.4). As the capillary permeability does not change during exercise (Kjellmer, 1964), the globulin molecules presumably did not leave the vascular circulation at a rate higher than normal. There is also a possibility that more globulins are transported back into the vascular circulation by means of the lymph (see earlier), than that leaving the blood. These two factors (especially the former) plus the initial increase in the volume of capillary filtrate formed, therefore cause an increase in the globulin concentration. This is in contrast to the findings of De Lanne et al (1958) who found a decrease in the globulin concentration, but it agrees with the results of Poortmans (1971) (see above). De Lanne et al (1958) explained that the increase was due to the fact that globulins (because of their bigger size) are returned to the vascular circulation at a slower rate than

albumin, with the result that they remain longer in the interstitial space. Accepting this explanation, there is a greater likelihood that the globulin concentration will increase in the vascular volume. Hemoconcentration and apparent lack of increased movement of globulins out of the vascular circulation should cause an increase in the globulin concentration (see also section 5.4.2). The increase in the globulin concentration in this present study is also reflected by a decrease in the A/G ratio. There was a change in the concentrations of some of the globulin fractions. Fraction 2 (and the combined fractions 2 and 3) and fraction 4 increased significantly while fraction 6 showed a significant decrease. The other fractions remained approximately the same. De Lanne *et al* (1958) and Poortmans (1971) found that various globulin fractions differed from one another during exercise.

The hematocrit increased significantly in agreement with the findings of Poortmans (1971). Although he did not find any correlation between the hematocrit increase and the total protein increase of the blood, the increase of these two values in this study can be taken as an indication of hemoconcentration (see also section 5.3.1).

5.4.2 Interstitial fluid

It will be noted, from the summary in table 13, that the total protein concentration shows a highly signi-

ficant decrease ($P < 0,02$). This is to be expected when taking into consideration the increase in the plasma protein concentration. This increase was due to hemoconcentration, part of which was attributed to the increased capillary filtration rate (section 5.4.1). The excess fluid moving into the interstitial spaces therefore caused a lowering of the protein concentration. The initial increase in lymph flow at the onset of exercise should also augment this decrease in the protein content of the interstitial fluid by transportation of more proteins back into the vascular circulation (section 5.4 and 5.4.1).

The albumin concentration of the interstitial fluid did not alter significantly. (For a discussion regarding the movement of albumin into and out of the vascular circulation see section 5.4.1.) From the results of this study it therefore appears that the increase in the filtration rate of fluid of the capillaries is paralleled by an increase in the movement of albumin through the capillary membranes. In other words, the concentration of albumin molecules in the capillary filtrate before and during exercise remains constant.

The significant decrease in the globulin concentration, ($P < 0,05$), is mainly responsible for the decrease in the protein concentration as the albumin concentration remained approximately the same. The decrease in the globulin concentration is to be expected (section

5.4.1). As the interstitial fluid volume increased (due to increased capillary filtration), it caused a lowering of the globulin concentration. The only fractions affected were numbers 1 and 6 (and possibly fraction 4), all of which showed significant decreases. Fraction 6 therefore showed an overall decrease in the body fluids (table 13). The reasons for the decrease in the concentration of fraction 6 in the body can only be speculated upon. The fraction is either catabolized at an increased rate because of some unknown reason, or it is excreted by the kidneys. The reason for the possible excretion of fraction 6 (and possibly albumin) by the kidneys can be explained as follows: It was mentioned in section 5.1.2 that the plasma to interstitial fluid ratio for fraction 6 is 0,63, whereas the ratio for albumin is 0,64. Fraction 6 and albumin molecules must therefore have moved through the capillary membrane at approximately the same rate. This may be taken as an indication that fraction 6 is a similar, or even a smaller size molecule than albumin (Senay and Christensen, 1968). The possible excretion of protein by the kidneys during exercise (Poortmans, 1971), as mentioned in section 5.4, will affect these two fractions first. The result is a decrease in fraction 6 and albumin. Another possible reason for the decrease of fraction 6 is the redistribution of this fraction to another area of the body.

The A/G ratio did not change significantly in spite of

the significant decrease in the globulin content whilst the albumin content decreased insignificantly. The decrease in the albumin content was however, sufficient to offset the decrease in the globulin content, thereby causing the A/G ratio to remain fairly constant.

A comparison of the crossed immunoelectrophoretic plates after exercise (plates 22 and 25) with those obtained for normal conditions (plates 10 to 13) reveals basically the same differences as that obtained for the other tests. This includes the apparent absence of some of the gel peaks whilst the areas covered by others seem to have changed.

5.5 Future Research

The following undertakings are suggested for future research work:

- 1) Labelled albumin may be used to follow the movement of albumin in the body fluids during normal and stress conditions.
- 2) A full analysis of the colloid osmotic pressures and hydrostatic pressures of the body fluids during normal and stress conditions should be carried out.
- 3) The alterations in the protein fractions of the body fluids of rats that have been acclimatized should be com-

pared with that of non-acclimatized rats during various stress conditions.

- 4) Other methods suitable for sampling interstitial fluid should be investigated.
- 5) Weekly plasma samples should be obtained during heat and cold exposures. Together with this the blood and extracellular fluid volumes could be determined during normal and stress conditions in order to obtain an improved interpretation of the present results.

The above suggestions are but a few of the numerous possibilities regarding the investigation of the function and composition of interstitial fluid.

6. CONCLUSION

6.1 Normal

The results obtained in this study show that all the major protein fractions present in the plasma are found in interstitial fluid. Furthermore, it is also obvious that interstitial fluid is not just a diluted plasma sample. This is because the ratios between the different interstitial fluid protein fractions differ from the corresponding ratios as found in the plasma. The data therefore shows that, although the plasma proteins have passed through the capillary membrane, they permeated through selectively and most probably according to molecular size. Albumin, generally accepted as having the lowest molecular mass of the plasma proteins, has the highest concentration of the different protein fractions in the interstitial fluid.

The albumin-globulin ratios of plasma and interstitial fluid are slightly lower than those obtained by some workers. In spite of this, the albumin concentration compares favourably with that obtained by other workers. This is mostly attributed to the fact that the total protein concentration of plasma and interstitial fluid is higher in this research than that obtained by most other workers. The A/G ratio for interstitial fluid is higher than the plasma A/G ratio. This is in accordance with the findings of various other authors.

The results obtained under normal conditions, show in certain cases a close correlation with those obtained by other workers

using the wick method. The wick and capsule methods are basically very different from one another, but they produce very similar results for subcutaneous fluid. It may thus be considered as substantial proof that the capsule did sample interstitial fluid.

Examination of the immunoelectrophoretic and crossed immunoelectrophoretic plates did not reveal any new protein or modified albumin molecule in the interstitial fluid. There appears to be a difference between the crossed immunoelectrophoretic plates of the samples obtained before and after the different conditions of exposure. The mobility of the fractions did not appear to have changed, but some of the peaks did appear to be missing and the areas covered by some, appeared to be different.

Attempts at separating the albumin fraction into different fractions by means of the Sephadex gels were not very successful. The mode of separation with Sephadex gels was different from that obtained when polyacrylamide gels were used.

6.2 Cold Exposure

The A/G ratios of both plasma and interstitial fluid decreased significantly. This was caused by a significant decrease in the albumin concentrations of both plasma and interstitial fluid while the globulin concentrations in these compartments did not change significantly. The decrease of the albumin concentration in the plasma did not affect the total protein concentration, as it was offset by a slight increase in the glo-

bulin concentration of the plasma. On the other hand, as the globulin concentration remained fairly constant in the interstitial fluid, the decrease in the albumin concentration caused a significant decrease of the total protein concentration of the interstitial fluid. From these results it appears as if there was a decrease in the total albumin concentration of the body during cold exposure.

The hematocrit value and globulin concentrations (which remained fairly constant during the cold exposure period) indicate a possibility that the body was maintaining its fluid volume in spite of possible diuresis.

The alterations in the various plasma fractions did not coincide with the alterations of the corresponding fractions in the interstitial fluid during cold exposure.

6.3 Heat Exposure

Albumin moved from the plasma to the interstitial space during heat exposure. The albumin concentration of interstitial fluid under these conditions, increased significantly after the first week's exposure period, but decreased again to a value just higher than normal after three weeks. The albumin concentration of plasma however, shows a highly significant decrease. It appears as if there is a possibility of a decrease in the albumin concentration of the body in spite of a possible hemoconcentration (see below).

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