transcapillary fluid balance thus demands a minimum mean capillary pressure of 3,7 mm Hg. Eliassen et al (1974), by means of isovolumetric measurement in the cat, obtained values between 12 and 15 mm Hg. Taylor et al (1973) stated that various investigators who measured the capillary pressure found this to vary between 10 and 30 mm Hg. A minimum capillary pressure of 3,7 mm Hg (see above) is not likely to be correct because of the following reason: For any reabsorption of fluid to take place at the venous end of the capillaries, the capillary pressure will have to be lower than 3,7 mm Hg. This is much lower than the average capillary pressure of 9 mm Hg used by Guyton (1971) in his calculations, even when accepting the fact that it may vary between the different regions of the body. However, the interstitial fluid pressure was recently measured in the rabbit by means of a subcutaneously implanted osmometer (Stromberg and Wiederhielm, 1976) and a value of -1,2 mm Hg was recorded. Fadness (1975) and Fadness et al (1978) obtained values of about -1 mm Hg in rats by means of the wick method. Ladegaard-Pedersen (1970) obtained an average interstitial fluid pressure of -2,74 mm Hg. If an interstitial fluid pressure of -1 mm Hg was accepted and not -6 mm Hg as reported by Guyton (1963), it would mean that the average capillary pressure in this study will have to be higher than the calculated 8,7 mm Hg before any filtration will take place at the arterial end of the capillaries or lower than this value at the venous end

for reabsorption to take place. This value, although purely speculative, is closer to the value of 9 mm Hg (venous capillary pressure) used by Guyton (1971).

A comparison between the crossed immunoelectrophoretic plates of plasma and interstitial fluid (plates 10 to 13) shows differences in the concentrations of some of the gel peaks. Other peaks appear to be absent from the interstitial fluid samples. The separation of the albumin fraction into more than one fraction was not successful. The mobility of the fractions in plasma and interstitial fluid does not appear to have altered.

5.2 Cold exposure

5.2.1 Plasma

Literature regarding certain aspects of the changes that take place in cold-induced animals are somewhat contradictory. It must however, be pointed out that few, if any, of these results were obtained under the same conditions and the same length of time or by using the same animals. Sutherland *et al* (1958) stated that the maximum response obtainable by cold exposure varies with time, temperature and the parameter studied. Hannon and Young (1959) found that there is a differential response between small and large animals. They found that the larger animals (dogs) showed an increased hematocrit value while the smaller animals (rats) showed no hematocrit changes during cold ex-

posure. The ability of an animal to acclimatize to cold conditions must have an effect on the results. The results can therefore only be used as a general guide to possible changes in the body fluids of the cold-exposed animal.

From the summary in table 8 it can be seen that the plasma protein concentration remained the same after a period of twenty one days cold exposure. Bazett et al (1940) found similar results after prolonged exposure of humans to cold although they did find an increase in the total plasma protein concentrations during adaptation to cold. Baker and Sellers (1957) found very little or no change in the total protein content of cold-adapted rats. Sutherland and Campbell (1956) reported an approximate 4 per cent increase in the total protein concentration of cold-adapted rabbits (this result, according to them, is not statistically significant). In a later study Sutherland et al (1958) found a definite increase in the plasma protein concentration of the rabbit. It is interesting to note that they found that alterations which occurred in the unclipped animals after ten weeks exposure, were relatively minor. The greatest changes occurred in the plasmas of the clipped rabbits. They concluded that the reason for this is that the unclipped animals may not have been severely stressed. It was also noticed by these authors that the onset of the response to cold exposure occurred within a week or less. Suther-

land et al (1958) deduced from their results that hemoconcentration occurred as a result of cold exposure, part of which they attributed to the increase in protein concentration. Similarly Spealman et al (1947) found that the plasma protein concentration increased on exposure to cold. Contradictory to these findings is the report by Hannon and Young (1959) who found a decrease of 7 to 9 per cent in the plasma protein levels after cold exposure of rats for approximately one month. They found that cold exposure resulted in a plasma dilution as indicated by an increase in the plasma water and a decrease in the relative density and lower protein levels of the plasma.

There seems to be a fair amount of concessus regarding certain changes in the albumin concentrations after cold exposure between the results of this present investigation and that obtained by other workers. This is in spite of the differences in results of the protein levels as discussed above. The albumin concentration (table 8) showed a significant decrease ($P<0,02$) in this study after a cold exposure period of twenty one days. Similar results were obtained by Shields et al (1960) after exposing rats to 4°C for thirty days. Waugh (1952) also reported a decrease in the albumin concentration of cold-exposed rabbits. Other decreases were reported by Sutherland and Campbell (1956) and Sutherland et al (1958).

It can be seen from table 8 that the A/G ratio of the plasma decreased significantly ($P < 0,05$). This is because of the above-mentioned decrease in the albumin content without a change in the globulin concentration. In spite of an insignificant change in the globulin concentration, the total protein concentration showed no decrease. A calculation of the data supplied by Sutherland et al (1958) also gave a decreased A/G ratio after cold exposure. These investigators reasoned that the osmotic effect of the increase in the total protein content is offset by the above reduction in the A/G ratio, so that the C.O.P. remains essentially constant. In this study however, the reduction in the albumin concentration without any apparent increase in the globulin concentration should give a lower C.O.P. after cold exposure (this was not determined).

Even though the globulin concentration remained basically the same, from the summary in table 8 it can be seen that there were significant changes in the concentrations of some of the fractions. Fractions 1 and 3 showed highly significant decreases, whereas fractions 2, 2 and 3 combined, 7 and 8 and 9 combined showed significant increases. As these fractions were not identified separately they cannot be related to any specific non-albumin protein. There is also a contradiction regarding the non-albumin fraction concentrations between different authors. Thus it was

found by Waugh (1952) that there was a reduction in beta-globulin and a less marked, but apparently significant, rise in gamma-globulin. Sutherland and Campbell (1956) and Sutherland et al (1958) on the other hand found increases in the beta-globulin as well as fibrinogen concentrations while the gamma-globulin concentration remained the same.

There was no significant alteration in the hematocrit value after cold exposure as can be seen from table 8. In agreement with these results are those of Shields et al (1960). They could find no differences in the hematocrit readings after cold exposure for different periods of time up to forty days. Similarly Waugh (1952) found no hematocrit changes in rats after twenty days cold exposure. Hannon and Young (1959) and Bazett et al (1940) reported results similar to those obtained above for the human. Hannon et al (1958) found a slight, although not significant, decrease in the hematocrit value of cold-exposed rats. On the other hand, Sutherland and Campbell (1956) and Sutherland et al (1958) obtained increased hematocrit values for rabbits after cold exposure.

Plates 10 and 14 are the crossed immunoelectrophoretic plates of plasma (with antibodies prepared against plasma) separated initially with polyacrylamide, before and after cold exposure respectively. Plates 12 and 16 are similar to the above except that the initial

separation of the fractions were done on Sephadex. A comparison between plates 10 and 14 and between plates 12 and 16 shows that some of the fractions appear to be missing and some areas of the gel peaks seems to be different.

5.2.2 Interstitial fluid

The data obtained in this study, as summarized in table 8, showed a highly significant decrease in the total protein concentration of the interstitial fluid ($P<0,001$). This is in contrast to that of plasma which remained constant. This decrease is solely due to a highly significant decrease in the albumin concentration ($P<0,001$) since the globulin concentration remained the same. The result of this was a significant decrease in the A/G ratio ($P<0,05$). A number of the non-albumin fractions of plasma differed from one another, whereas only two of the interstitial fluid fractions showed significant changes. These are fractions 2 and 3 combined and fraction 4 which had increased after cold exposure. There appeared to be no relationship between the changes of the plasma protein fractions and the interstitial fluid protein fractions. This is in the sense that a change in any one fraction in plasma did not cause a related change in the same interstitial fluid fraction. This lack of correlation cannot be considered as an indication of a possible shortcoming of the capsular method for sampling interstitial fluid. Sufficient time was allowed for equili-

bration to take place between the plasma and capsular fluid as discussed in section 3.1. These experiments were carried out over a period of three weeks and it was noticed by Sutherland et al (1958) that the reaction to cold exposure occurred within a week. This is indeed noticeable from the interstitial fluid results in table 7. Both the total protein and albumin concentrations started decreasing after one week.

Very little research work into the characteristics of interstitial fluid during cold exposure seems to have been done. Bazett et al (1940) made some estimates of extracellular fluid by injection of sodium thiocyanate. They calculated the volume of body water of the subjects before and after cold exposure. Heroux and Gridgeman (1958) have shown that cold-exposed rats underwent a decrease in muscle mass due to a decrease in water and muscle protein. Baker and Sellers (1957) studied fluid adjustments during acclimatization in rats. Their results indicated a rise in blood volume and the extracellular water level. Shields et al (1960) undertook a study to investigate electrophoretic changes of serum and soluble muscle proteins and changes in body fluids in rats during continued exposure to cold. They homogenized samples of muscle, centrifuged the mixture and used the supernatant for an electrophoretic separation of the proteins. These authors however, did not attach any real significance to observed changes during cold exposure.

Comparisons drawn between the crossed immunoelectrophoretic plates numbers 11 and 15 (polyacrylamide gels) and between plates 13 and 17 (Sephadex gels) showed basically the same differences as those observed for plasma (section 5.2.1). Some of the peaks appear to be absent while areas of some peaks are different. The peaks that are missing are not necessarily the same as those absent in plasma. No noticeable changes in the mobilities of the fractions could be detected.

The data obtained in this study as well as other information does not really permit speculation as to the possible significance and reasons for some of the alterations that took place in the plasma and interstitial fluid. The decrease in the albumin concentration in both the plasma and interstitial fluid seems to indicate that albumin is lost from the body. A decrease in the albumin concentration can be associated with several diseases (Harper, 1971). This does not seem to be a logical conclusion in this study as none of the rats showed any outward signs of disease. Another possibility is a reduced dietary intake of protein. It had however, according to Shields et al (1960), been shown that dietary intake in the cold increased by as much as 70 per cent. Nevertheless, the metabolic rate of the animal will have to be determined before the dietary intake can be discarded. This is because the dietary intake increases mainly as a response to an increase in metabolic rate. Other factors which can

also play a role is an imbalance in the liver synthesis; catabolism of albumin and possibly diuresis. According to Bass and Henschel (1956) it is widely accepted that diuresis follows cold exposure. These authors have stated that the urine produced during cold diuresis differed from simple water diuresis in that it contains increased amounts of sodium and magnesium. Waugh (1952) found small amounts of proteins in the urine of cold-exposed animals. They found that this increased slightly after thirteen to seventeen days in the cold, after which it returned to normal. The possibility of losing proteins, especially albumin, in the urine cannot be totally excluded. One can argue that the albumin molecule, as it is smaller, is excreted more easily by the kidneys than the globulin molecules. This could explain why only the albumin concentration and not the globulin concentration, was lowered during cold exposure.

As mentioned earlier (section 5.2.1), a decrease in the plasma albumin concentration with a constant globulin concentration should lower the C.O.P. This would then cause fluid to be lost from the plasma partly into the interstitial space (section 5.1.2) and partly via the urine (osmotic diuresis). Increased fluid in the interstitial space would then lower the protein concentration. This does not explain why the globulin concentration in the interstitial space remained the same.