

4.2 Crossed immunoelectrophoresis

No photographs of the Sepadex electrophoretic gels obtained by disc electrophoresis (section 3.7.1.1 and 3.8.1) were taken for comparison with their corresponding crossed immunoelectrophoretic plates because these gels became too soft for handling after being stored.

Even though only one plate for each set of results will be shown, all the gels (polyacrylamide and Sephadex) obtained in this research work have been further analyzed by crossed immunoelectrophoresis. The individual proteins were not identified on these plates.

Plates 10 and 11 show the peaks obtained by crossed immunoelectrophoresis of normal plasma and interstitial fluid respectively. Their related polyacrylamide gels are also shown. Plates 12 and 13 are similar to these except that separation was done on Sephadex gels. From a comparison of these it would appear that the Sephadex gels gave the better separation of the two albumin peaks. This is more pronounced in the case of interstitial fluid.

Plates 14 to 17 are the gel patterns obtained after cold exposure, plates 18 to 21 are those after heat exposure and plates 22 to 25 are those after exercise. From a comparison of each of these sets of plates with those obtained under normal conditions it will be noted that some of the peaks appear to be absent, while the concentrations of some of the peaks have changed.

4.3 Immunoelectrophoresis

Normal interstitial fluid and plasma samples were separated by immunoelectrophoresis as described in section 3.7.3.

Interstitial fluid and plasma samples were each placed into a different hole on the same agarose-covered microscope slide. After electrophoresis, the trough of one slide was filled with laboratory prepared antibodies against plasma (plate 7). The trough of the second agarose-covered slide was filled with commercial antibodies prepared against plasma (plate 8) and that of the third slide with immunoglobulins prepared against interstitial fluid (plate 9). They were then left for the required length of time for diffusion to take place (section 3.7.3). The results of these are discussed in sections 4.4 and 4.5.

4.4 Comparison between laboratory prepared antibodies and commercial antibodies

Plate 3 shows the reaction of rat plasma with commercial antibodies and plate 4 that of plasma with antibodies prepared in the laboratory. Plates 5 and 6 show the reactions between interstitial fluid with commercial and laboratory prepared antibodies respectively. All of these were done using Sephadex gel for the initial separation of the proteins.

Plate 7 is an immunoelectrophoretic plate of interstitial fluid and plasma with laboratory prepared antibodies and plate 8 is a similar plate of interstitial fluid and plasma with commercial antibodies. (All these antibodies were prepared against rat plasma.)

From the comparisons of plates 3 to 6 (crossed immunoelectrophoresis) very little difference, if any, is noticeable between the reactions of the two types of antibodies. In the case of the commercial antibodies the front of the albumin peaks on these plates appear to be somewhat more sharply defined. Plates 7 and 8 (immunoelectrophoresis) show that the antibody titres in the different preparations influenced the results to some extent. It would therefore appear that there is very little difference in the reactions of the two types of antibodies.

4.5 Reaction of antibodies prepared against interstitial fluid

The reactions of interstitial fluid and plasma with antibodies prepared against interstitial fluid, are shown in plate 9 (section 3.6.2). The precipitation of proteins by antibodies prepared against interstitial fluid was an attempt to locate any foreign protein peculiar to interstitial fluid which was not found in plasma. That is, an interstitial fluid protein which will not react with the antibodies prepared against plasma. No such protein was found to be present in interstitial fluid by comparison of plates 7 and 9.

4.6 Protein concentrations after six months

Table 1 shows the protein concentrations of the same rats before and after six months under normal conditions. In this instance the concentrations of albumin and the other fractions have not been determined separately. From these results it can be seen that the protein concentrations before and after six months are approximately the same. This is an indication

that there was no deleterious effect after this period of time. The possible formation of a membrane around the capsule did not have any effect on the movement of proteins into and out of the capsule (section 2.5.3).

4.7 Normal conditions

The results for these are represented in table 3 for the plasma samples and in table 4 for the interstitial fluid samples. Typical densitometric recordings of the polyacrylamide gels for plasma and interstitial fluid, under normal conditions, are shown in figure 1. Plates 10 to 13 show the different crossed immunoelectrophoretic patterns obtained for plasma and interstitial fluid samples.

All the protein fractions found in plasma are present in interstitial fluid (tables 3 and 4), but in smaller concentrations. The concentrations of the interstitial fluid fractions represent about 40 to 60 per cent of their corresponding fractions present in plasma (section 5.1.2). A possible exception is the combined fractions 2 and 3. The ratio of the concentrations of these fractions in interstitial fluid and plasma are less when compared with that of the other fractions.

It is evident from the albumin-globulin ratios that the albumin concentration in interstitial fluid represents a higher proportion of the total protein content when compared with the albumin of plasma.

A comparison of the two densitometric recordings in figure 1 shows the similarity between the fractions of the two samples. It can be seen from figure 1 that fractions 3 and 9 are not as pronounced in the interstitial fluid sample when compared with the plasma sample (see also section 3.8.1).

The ratio between the concentrations of albumin in interstitial fluid and plasma and the ratio between the concentrations of fraction 6 in interstitial fluid and plasma are very nearly the same (section 5.1.2).

Colloid osmotic pressures (C.O.P.) and their related protein concentrations for plasma and interstitial fluid are presented in table 5. The colloid osmotic pressure of interstitial fluid is approximately 45 per cent of that found in plasma (section 5.1).

4.8 Cold exposure

Table 6 contains the results obtained for plasma after cold exposure and table 7 are those for interstitial fluid before, during and after cold exposure. Table 8 is a summary of plasma and interstitial fluid results before and after cold exposure.

Typical densitometric recordings of the polyacrylamide gels of plasma and interstitial fluid are presented in figure 2.

Plates 14 to 17 show the different crossed immunoelectrophoretic patterns obtained for plasma and interstitial fluid.

4.8.1 Plasma

From the summary in table 8 it can be seen that the concentrations of albumin, fractions 1 and 3 decreased significantly after cold exposure, whereas fractions 2, 2 and 3 combined, 7 and 8 and 9 combined increased significantly. The total protein and globulin concentrations remained however, the same. The decrease in the albumin concentration is reflected in a lowering of the albumin-globulin ratio ($P < 0,05$). The hematocrit value remained the same.

4.8.2 Interstitial fluid

In contrast to the protein content of the plasma, there was a highly significant decrease ($P < 0,001$) in the interstitial fluid protein content (table 8). This was due to the highly significant decrease in the albumin concentration ($P < 0,001$) while the globulin concentration remained the same. The albumin-globulin ratio decreased significantly ($P < 0,05$) because of this. The only other alteration was an increase in the combined fractions 2 and 3 ($P < 0,01$).

A summary of the results for plasma and interstitial fluid (table 8) therefore shows that the albumin concentrations in both plasma and interstitial fluid had decreased significantly, but only the total protein content of the interstitial fluid showed a corresponding decrease. The only fractions affected in both cases were the combined fractions 2 and 3 which showed

an increase in the case of plasma and a corresponding decrease in the case of interstitial fluid.

4.9 Heat exposure

Results for plasma and interstitial fluid are contained in tables 9 and 10 respectively. A summary of the results before and after heat exposure is given in table 11. Plates 18 to 21 show the crossed immunoelectrophoretic patterns for plasma and interstitial fluid. Plates 18 and 19 are those obtained after the initial separation of plasma and interstitial fluid proteins by polyacrylamide gels respectively, whereas plates 20 and 21 are those where the proteins have been separated by Sephadex gels. Figure 3 shows typical densitometric recordings of the polyacrylamide gels of plasma and interstitial fluid.

4.9.1 Plasma

The summary for the plasma results (table 11) shows a highly significant decrease in the albumin concentration ($P < 0,001$). This was accompanied by a significant increase in the globulin concentration ($P < 0,01$). The net result of this was a total protein concentration which remained the same, but the albumin-globulin ratio was lowered ($P < 0,001$). The globulin fractions affected were numbers 1, 2 and 2 and 3 combined which had increased significantly. The hematocrit value increased significantly ($P < 0,05$).

4.9.2 Interstitial fluid

The total protein content increased significantly ($P < 0,05$) as can be seen from a summary of the results in table 11. The increase in albumin and globulin concentrations which brought this about, were however, not significant. The albumin-globulin ratio had nevertheless decreased significantly ($P < 0,02$). The combined fractions 2 and 3 increased significantly ($P < 0,02$). The other fractions were not altered.

A summary of all the results therefore shows that the plasma protein concentration remained constant even though the albumin and globulin concentrations changed significantly. On the other hand, the interstitial fluid total protein concentration increased considerably although this was not due to a great increase by any specific fraction.

4.10 Exercise

It was necessary to give the rats some initial training before the commencement of the actual test. (For the training program see section 3.9.4) This was unavoidable because initially the rats refused to carry on running after a few minutes due to exhaustion. They also had to be familiarized with the treadmill. Interstitial fluid samples before exercise could therefore not be taken from these rats as these would have been different from that of unfit rats. The results for the interstitial fluid samples after exercise are compared with that obtained for normal rats as represented in table 4. The results for the plasma and interstitial fluid after exercise

are represented in table 12. Table 13 is a summary of the results before and after exercise. Figure 4 shows typical densitometric recordings for plasma and interstitial fluid after exercise. Plates 22 to 25 are the various crossed immunoelectrophoretic plates for these samples.

4.10.1 Plasma

The total protein concentration increased significantly ($P < 0,05$) (table 13). This was solely due to an increase in the globulin concentration because the albumin concentration remained the same. This led to a decrease in the albumin-globulin ratio ($P < 0,05$). Fractions 2, 2 and 3 combined and 4 showed an increase whereas fraction 6 decreased. The hematocrit value increased significantly ($P < 0,05$).

4.10.2 Interstitial fluid

The total protein concentration decreased significantly ($P < 0,02$) as can be seen from table 13. This was due to a significant decrease in the globulin concentration ($P < 0,05$) while the albumin concentration remained the same. The albumin-globulin ratio however, remained constant. The decrease in the globulin concentration was due to a significant decrease in the concentration of fraction 1 ($P < 0,02$).

A comparison of the results for plasma and interstitial fluid therefore shows that the increase in the plasma protein concentration is reflected by an increase in

the interstitial fluid total protein concentration. In the case of the plasma this was due to an increase in the globulin concentration whereas in the case of the interstitial fluid this was due to a decrease in the globulin concentration. The albumin concentration in both instances remained the same.

4.11 Summary

From the above results the following has thus been found:

- 1) There was very little difference between the reactions of laboratory prepared antiserum and commercial antiserum with plasma and interstitial fluid.
- 2) The effectiveness of the capsule was not affected after it had been in the rat for a period of six months.
- 3) There was a difference in the mode of separation of the protein fractions between polyacrylamide and Sephadex gels.
- 4) All the proteins found in plasma were present in interstitial fluid although they were in smaller quantities.
- 5) Variations in some of the protein fractions occurred during the different tests. The total protein concentrations were altered in some of the cases due to variations in either the albumin or globulin concentrations, or both. In some cases this caused a change in the A/G ratios. There was also a change in the hematocrit values after

heat exposure and exercise.

- 6) Attempts at separation of the albumin fraction into more than one fraction was not very successful.
- 7) There was no evidence of a protein found exclusively in interstitial fluid.
- 8) The presence of a modified albumin molecule could not be demonstrated.

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