Manning, R. Davis, J. R. Dynamics of extracellular fluid volume changes during hyperproteinemia. Am. J. Physiol. 275 (Regulatory Integrative Comp. Physiol. 44): R1878–R1884, 1998.—The dynamics of fluid volume distribution between the blood and interstitium during hyperproteinemia were studied in 12 anephric, conscious dogs during several states of hydration. After recovery from splenectomy and unilateral nephrectomy, plasma protein concentration was elevated to 8.4–8.7 g/dl by daily intravenous infusion of 330 ml of previously collected autologous plasma for 11 days. The remaining kidney was then removed, and the next day lactated Ringer solution equivalent to 10 or 20% of body weight was infused intravenously. By the end of the 25-h postinfusion period, Ringer infusion had increased circulating protein mass 20.9 ± 9.1% (mean ± SE) in the 10% group (P < 0.05) and decreased it 10.5 ± 3.3% in the 20% group (P < 0.05). The average increase in blood volume and arterial pressure during the postinfusion period was 27.4 ± 2.5 and 20.7 ± 3.7%, respectively, in the 10% group but only 17.8 ± 2.4 and 12 ± 1.6% in the 20% group (all changes significant compared with respective control). The relationship between blood volume and sodium space was similar to that found during normoproteinemia, such that elevations in sodium space of 40–50% increased blood volume but greater elevations in sodium space caused no further increases in blood volume. Overhydration during chronic hyperproteinemia causes hypervolemia and hypertension, but, in contrast to those in short-term studies, the increases in blood volume and arterial pressure are not greater than those achieved during normoproteinemia.

Although much information has accrued on the effects of hypoproteinemia on the extracellular fluid volume distribution and arterial pressure (1, 7, 10), little is known about extracellular fluid volume distribution in chronic hyperproteinemia and the resultant effects on arterial pressure, especially during conditions of sodium and water retention. A lack of understanding of factors influencing volume distribution during hyperproteinemia has contributed to the considerable controversy on whether crystalloid or colloid therapy is best during volume resuscitation (4). It is not clear whether sodium and water retention during hyperproteinemia will result in normal or extremely marked increases in intravascular volume. Large increases in blood volume could have serious hemodynamic consequences, resulting in severe hypertension, pulmonary congestion, or pulmonary edema. Therefore, the goal of this study was to examine the dynamics of distribution of extracellular fluid volume and arterial pressure during chronic hyperproteinemia and overhydration. Studies were performed in anephric, conscious dogs which, before nephrectomy, were made hyperproteinemic by daily intravenous infusion of previously collected autologous plasma for 11 days.

METHODS

Animal preparation and experimental protocol. Experiments were conducted on 12 conscious, anephric dogs with a mean body weight of 27.1 ± 1.3 kg. The project had the approval of the local Institutional Animal Committee. All dogs were splenectomized and had chronic arterial and venous catheters implanted. During the first surgical procedure, the spleen and the right kidney were removed through a midline abdominal incision. Also at this time, chronic catheters were implanted in the aorta and inferior vena cava through the femoral artery and vein. Aseptic technique was used in all surgical procedures, and atropine sulfate (1 ml of 0.4 mg/ml im; Elkins-Sinn, Cherry Hill, NJ) was administered before surgery. Anesthesia was induced with sodium thiopental (25 mg/kg iv Pentothal; Abbott, North Chicago, IL) and was maintained with a mixture of methoxyflurane (Penthrane, Abbott) and oxygen. Appropriate gas concentrations were delivered to the dogs through an endotracheal tube connected to an Ohio Medical Products anesthesia machine (Kinet-O-Meter). The catheters were tunneled subcutaneously and exited the back between the dog’s shoulders for protection. During a 10- to 14-day period of recovery after surgery, the dogs were trained to lay quietly in their cages. Water was provided ad libitum throughout the experiment.

Plasma was collected by plasmapheresis during the next 25 days for later intravenous infusion. During the plasmapheresis procedure, 1,600 U of heparin (1.6 ml of 1,000 U/ml) were administered intravenously, and 250 ml of blood was allowed to flow unimpeded from the arterial catheter into a 300-ml transfer pack (Fenwal) into which 1,000 U of heparin had

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been initially added. Next, the blood was centrifuged for 5 min and the plasma was removed, placed in another transfer pack, and immediately frozen. A volume of lactated Ringer solution equal to the volume of plasma removed was mixed with the remaining erythrocytes, and the mixture was returned to the dogs by intravenous drip. On some days, the procedure was repeated for collection of a second volume of plasma. Over the 25-day plasmapheresis period, plasma was collected 26–28 times. After the plasmapheresis period was completed, a 17-day recovery period was allowed before the beginning of the experiment.

During the experimental period, the plasma protein concentration of the dogs was elevated over a 12-day period by infusing by intravenous drip, in 1 h, ~330 ml of previously collected autologous plasma. On day 11 of the plasma infusion, the dogs were anesthetized as before and the remaining left kidney was removed through a separate flank incision. The dogs recovered rapidly from the surgery, because thiopental and methoxyflurane anesthesia were used. During the morning after nephrectomy, a number of control measurements were taken for 3 h and ~330 ml of autologous plasma was intravenously infused. At the end of the 3-h period, lactated Ringer solution, warmed to body temperature, was intravenously infused in the amount of 100 ml/kg in the 10% group and 200 ml/kg in the 20% group at an average rate of 29.6 ± 0.6 ml/min. The anephric control group received no infusion.

During the plasma collection period, sodium intake was maintained at ~75 meq/day by feeding the dogs 894 g/day of P/D prescription diet dog food (Hills Pet Products, Topeka, KS) to which 45 meq of sodium chloride (5 M NaCl) was added. During the 12-day plasma infusion period, sodium intake was maintained at 75 meq/day by intravenous infusion of ~330 ml/day of previously collected autologous plasma, which contained ~45 meq of sodium, and the dogs were fed 894 g/day of P/D prescription diet dog food (Hills Pet Products) without any additional sodium added to the food.

Experimental measurements and instrumentation. The dogs were housed in metabolic cages and were fitted with a Statham P23 AC or P23 ID transducer at the level of the heart. An infusion tube and the transducer wires exited the dog pens through protective tubing. The infusion tube was connected to a Sage model 375 A pump, which was used to infuse the lactated Ringer solution. The transducer wires were connected to a Grass model 7D recorder that was connected in turn to a digital computer. Every minute throughout the day, the computer sampled arterial pressure 500 times in a 3-s period and the average was stored on a computer disk (11). Central venous pressure was measured with a Statham P23 BC transducer that was connected to the Grass recorder. Venous pressures were determined while the dogs lay quietly in their cages.

Blood volume and sodium space were measured using the dilution principle. Blood volume was measured by dilution of $^{51}$Cr-tagged red blood cells (NEN, Boston, MA) (7, 8, 13). The red blood cells of a 20-ml sample were tagged the day before the infusion day with 100 µCi of $^{51}$Cr (7, 8, 14). A 7-ml blood sample was withdrawn through the arterial catheter for background radioactivity determination. Then 10 ml of chromated red blood cells and 2 ml (10 µCi) of $^{22}$Na (NEN) were injected through the venous catheter. Blood samples of 7 ml were drawn through the arterial catheter at 5, 20, and 40 min and 1, 2, 3, 4, and 5 h after the infusion to determine the dilution volumes. It was assumed that no chromated red blood cells were lost from the circulation during the control period in the first 5 h of the experimental period. The validity of this assumption was confirmed by the stability of the blood volume of the control group. Control values of blood volume and sodium space were determined from the sample withdrawn 3 h after injection (7, 8, 14). Overhydration was then produced by infusing the lactated Ringer solution as previously stated. Two hours after the conclusion of the infusion, 20 ml of blood was withdrawn and the red blood cells were labeled with 200 µCi of $^{51}$Cr. Just after the 24-h postinfusion sample, 10 ml of these cells were intravenously injected, and 20 min and 1 h later, blood samples were withdrawn for determination of blood volume. Regression lines relating blood and sodium space were determined using the curve-fitting algorithm from Sigma Plot (Jandel Scientific, Corte Madera, CA).

Plasma volume was calculated considering blood volume and large-vessel hematocrit (7, 8, 13), and total intravascular protein mass was calculated from the product of plasma volume and plasma protein concentration. Plasma protein concentration was measured with an American Optical (Buffalo, NY) refractometer.

Statistical analysis was performed by first determining overall significance with a two-way ANOVA for repeated measures (3 groups and time as the repeated measure). Second, if the two-way ANOVA showed significant changes, significance of the individual experimental times was determined with a one-way ANOVA. Post hoc analyses were performed with Dunnett’s test for multiple comparisons with a control (3). The data were considered to be statistically significant if P < 0.05. All data are expressed as means ± SE.

RESULTS

Experiments have been performed examining the distribution of extracellular fluid volume and the changes in arterial pressure during hyperproteinemia.

Plasma protein concentration responses to hyperproteinemia and overhydration. As shown in Fig. 1, after hyperproteinemia was produced by daily intravenous infusion of autologous plasma for 12 days, plasma protein concentration averaged between 8.4 and 8.7 g/dl in the anephric control group, the 10% group (which received 100 ml/kg infusion of lactated Ringer solution) and the 20% group (which received 200 ml/kg infusion of lactated Ringer solution) without any additional sodium added to the food. Overhydration was then produced by infusing the lactated Ringer solution as previously stated. Two hours after the conclusion of the infusion, 20 ml of blood was withdrawn and the red blood cells were labeled with 200 µCi of $^{51}$Cr. Just after the 24-h postinfusion sample, 10 ml of these cells were intravenously injected, and 20 min and 1 h later, blood samples were withdrawn for determination of blood volume. Regression lines relating blood and sodium space were determined using the curve-fitting algorithm from Sigma Plot (Jandel Scientific, Corte Madera, CA).

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![Fig. 1. Responses of plasma protein concentration (PPC) to intravenous infusion of lactated (Lac) Ringer solution in conscious, anephric dogs during hyperproteinemia. The 20% group received 200 ml/kg of Ringer, 10% group received 100 ml/kg of Ringer, and anephric control group received no infusion; n = 4 for each group. Data are means ± SE.](http://ajpregu.physiology.org/)

Fig. 1. Responses of plasma protein concentration (PPC) to intravenous infusion of lactated (Lac) Ringer solution in conscious, anephric dogs during hyperproteinemia. The 20% group received 200 ml/kg of Ringer, 10% group received 100 ml/kg of Ringer, and anephric control group received no infusion; n = 4 for each group. Data are means ± SE.
solution), and the 20% group (which received 200 ml/kg of lactated Ringer). The two-way repeated-measures ANOVA showed that the group × time interaction was significantly different for plasma protein concentration after the Ringer infusion (P < 0.0001). The average postinfusion plasma protein concentration decreased markedly in both the 10 and 20% groups (P < 0.05 for each group compared with control group); however, the average decrease in plasma protein concentration was greater in the 20% group than in the 10% group (P < 0.05). The minimum value of plasma protein concentration was reached at 5 min postinfusion and equaled 6.3 ± 0.1 g/dl in the 10% group (P < 0.05 compared with respective control) and 5.9 ± 0.1 g/dl in the 20% group (P < 0.05), and plasma protein concentration in the anephric control group was not significantly changed at this time.

Responses of blood volume to hyperproteinemia and overhydration. As shown in Fig. 2, blood volume increased in the 10 and 20% groups during lactated Ringer infusion. In the anephric control group, blood volume did not change significantly during the entire postinfusion period. The two-way repeated-measures ANOVA showed that the group × time interaction was significantly different for blood volume after the Ringer infusion (P < 0.0001). In addition, blood volume significantly increased in the 10% group during the 25-h postinfusion period and averaged 127.4 ± 2.5% of control (P < 0.05 compared with control group). In the 20% group, blood volume averaged 117.8 ± 2.4% of control during the postinfusion period (P < 0.05 compared with control group). The average increase in blood volume in the 10% group was significantly greater than that in the 20% group during the postinfusion period.

Responses of sodium space to hyperproteinemia and overhydration. The two-way repeated-measures ANOVA showed that the group × time interaction was significantly different for sodium space after the Ringer infusion (P < 0.0001). Figure 3 shows that sodium space increased significantly in both the 10 and 20% groups during the entire postinfusion period (P < 0.05 compared with control group). The average postinfusion sodium space of the 20% group was significantly greater than that of the 10% group. The individual sodium spaces of the anephric control group did not change significantly compared with the respective control throughout the entire postinfusion period except for a small increase after 24 h.

Responses of mean arterial pressure to hyperproteinemia and overhydration. Figure 4 shows that the individual arterial pressures of the anephric control group did not significantly change from the respective control value throughout the entire postinfusion period. The two-way repeated-measures ANOVA showed
that the group × time interaction was significantly different for mean arterial pressure after the Ringer infusion (P < 0.02). Arterial pressure increased markedly in the 10% group after lactated Ringer infusion and averaged 120.7 ± 3.7% of control (P < 0.05 compared with control group) during the entire postinfusion period. Arterial pressure also increased in the 20% group during lactated Ringer infusion and averaged 112 ± 1.6% of control (P < 0.05 compared with control group) during the entire postinfusion period. The average difference in postinfusion arterial pressure in the 10 and 20% groups did not reach significance.

Circulating protein mass responses to hyperproteinemia and overhydration. As seen in Fig. 5, circulating protein mass in the anephric control group did not change significantly throughout the postinfusion period. The two-way repeated-measures ANOVA showed that the group × time interaction was significantly different for circulating protein mass after the Ringer infusion (P < 0.0001). The average circulating protein mass of the 10 and 20% groups did not differ significantly from the control group, but the average postinfusion decrease in intravascular protein mass was greater in the 20% group than in the 10% group (P < 0.05). Figure 5 shows that the circulating protein mass in the 10% group did not change significantly for the first 5 h of postinfusion; however, by 25 h postinfusion, the circulating protein mass increased 20.9 ± 9.1% (P < 0.05 compared with respective control). In the 20% group, by 25 h postinfusion, circulating protein mass had decreased 10.5 ± 3.3% (P < 0.05 compared with respective control).

Central venous pressure responses to hyperproteinemia and overhydration. The two-way repeated-measures ANOVA showed that the group × time interaction was significantly different for central venous pressure after the Ringer infusion (P < 0.002). Figure 6 shows that lactated Ringer infusion, the average postinfusion central venous pressure increased in the 20% group (P < 0.05 compared with control group) but not in the 10% group. The average postinfusion difference in venous pressures in the 10 and 20% groups did not reach significance. Central venous pressure of the anephric control group did not significantly change during the entire postinfusion period. However, individual central venous pressures of the 10 and 20% groups were increased significantly throughout the 25-h postinfusion period compared with their respective controls.

Distribution of extracellular fluid volume during normoproteinemia and hyperproteinemia. Figure 7 illustrates the changes in the relationship between sodium space and blood volume during normoproteinemia and when plasma protein concentration was chronically increased. Data plotted are paired sodium spaces and blood volumes during the first 5 h of the postinfusion period for groups that received 10 and 20% of their body weight of lactated Ringer solution and had either normal or high plasma protein concentration. The normal plasma protein concentration curve in Fig. 7 (9) shows that blood volume increased as sodium space increased as long as the sodium space was elevated <40–50%. Greater expansions of extracellular fluid volume resulted in no further increases in blood volume. The high plasma protein concentration curve is very similar to that of the normal plasma protein concentration curve and also shows that increases in sodium space >40–50% resulted in no further increases in blood volume. Also, for an unexplained reason, the individual sodium spaces of the high protein group, as seen in Fig. 7, lay within a narrower range than those in the normal protein group.

The regression of the normal plasma protein concentration curve is blood volume is $-284.7 + 7.320$ (sodium space) $- 4.404 \times 10^{-2}$ (sodium space)$^2 + 8.915 \times 10^{-5}$ (sodium space)$^3$, $r^2 = 0.68$. The high plasma protein concentration curve is blood volume is
\[ -1.217 + 28.34 \text{ (sodium space)} - 0.1977 \text{ (sodium space)}^2 + 4.544 \times 10^{-4} \text{ (sodium space)}^3, r^2 = 0.639. \]

The plateaus of the two curves were statistically compared (in terms of blood volumes) between 20 and 300 min postinfusion in the 10 and 20% groups with either normal or high plasma protein concentration. The average increase in blood volume during this time period in the normal plasma protein concentration group was 23.8 ± 0.1%, and the increase in the high plasma protein concentration group was 21.3 ± 0.1% (P not significant). Therefore, a chronic increase in plasma protein concentration did not increase the amount of volume retained in the vasculature during overhydration.

DISCUSSION

Although it is well known that short-term increases in plasma colloid osmotic pressure can cause large shifts of fluid into the vasculature (4, 12), the control of the distribution of extracellular fluid volume during chronic hyperproteineemia is poorly understood. During overhydration in the present study in hyperproteinemic dogs, blood volume increased as long as the increase in sodium space was <40–50%. However, when sodium space increased more than this, all the additional infused fluid escaped into the interstitium and there were no further increases in blood volume. Therefore, the blood volume–sodium space relationship in the present study in dogs with high plasma protein concentration was very similar to the relationship found in a previous study performed by Manning and Guyton (9) in dogs with normal plasma protein concentration, as shown in Fig. 7. The maximum increase in blood volume as described by this relationship was not significantly different in the normoproteinemic and hyperproteinenic dogs. This might be counterintuitive, because short-term increases in plasma colloid osmotic pressure can cause large increases in blood volume (4, 12). However, there are changes that occur during chronic hyperproteineemia that may prevent blood volume from changing and thus maintain the distribution of extracellular fluid volume.

To understand why the distribution of extracellular fluid volume is similar during normoproteineemia and hyperproteineemia requires an examination of the Starling capillary forces (12). These forces control the movement of fluid across the capillary membrane and thus have a major impact on fluid volume distribution. The balance of Starling capillary forces is determined by the differences between capillary and interstitial hydrostatic and colloid osmotic pressures. In the absence of other changes, an increase in plasma colloid osmotic pressure should cause an influx of fluid into the vasculature and thus increase blood volume. In short-term studies, this is certainly true (5), and intravenous infusion of colloids is an effective acute expander of vascular volume (4). However, as it has been previously shown, the long-term effects of hyperproteineemia on blood volume are quite different from the short-term effects, such that chronic hyperproteineemia causes no change in blood volume (8).

One reason why blood volume does not increase during chronic increases in plasma protein concentration is the accompanying increase in interstitial protein concentration (8). It has been previously shown that chronic hyperproteineemia, achieved by daily intravenous infusion of previously collected autologous plasma for 9 days, caused no change in blood volume even though plasma protein concentration increased from a control value of 6.9 ± 0.2 to 9.3 ± 0.2 g/dl and plasma colloid osmotic pressure increased 10 mmHg. During this time, prenodal lymph protein concentration, an index of interstitial protein concentration, increased from a control value of 1.6 ± 0.2 to 5.1 ± 0.1 g/dl on day 9 of plasma infusion and the calculated colloid osmotic pressure of this lymph increased 10.7 mmHg. Therefore, the transcapillary colloid osmotic pressure changed little during chronic hyperproteineemia because of extravasation of protein into the interstitial fluids. Therefore, maintenance of the transcapillary colloid osmotic pressure gradient may play a major role in preventing blood volume changes during chronic hyperproteineemia (8). In the present experiment, the value of blood volume during the control period was close to that measured in the previous study on hyperproteineemia (8). Therefore, interstitial fluid colloid osmotic pressure likely increased in the present study in a fashion comparable to that of the previous study, thus preventing hypervolemia.
As noted before, blood volume in the present study increased during overhydration as long as the increase in sodium space was <40–50%. There are several factors that could explain why further increases in sodium space caused no additional increases in blood volume. First, plasma protein concentration decreased in both the 10 and 20% groups because of hemodilution in both groups and a decrease in circulating protein mass in the 20% group. Therefore, a decrease in plasma protein concentration and plasma colloid osmotic pressure in both groups resulted (9), which would have allowed more fluid to leave the circulation (12). Second, the increase in blood volume in the 10 and 20% groups increased arterial pressure, which could have increased capillary hydrostatic pressure, which could have in turn increased transcapillary fluid flux. In addition, the increase in central venous pressure that occurred in both the 10 and 20% groups could have further increased capillary hydrostatic pressure and thus increased transcapillary fluid flux, which would limit the degree of increase in blood volume.

Blood volume increased more in the 10% group than in the 20% group after lactated Ringer infusion. This could have been responsible for the slightly greater increase in arterial pressure in the 10% group. The greater increase in blood volume in the 10% group could have been due to a decrease in circulating protein mass in the 20% group and the increase in protein mass in the 10% group. An additional factor that could have contributed to the smaller increase in arterial pressure in the 20% group compared with the 10% group is that the high central venous pressure in the 20% group could have caused the Frank-Starling relationship of the heart to operate at a nonoptimal point, thus attenuating the increase in cardiac output in this group.

The present study and previous studies on hyperproteinemia by Manning and Guyton (7, 10) indicate that the distribution of extracellular fluid volume is highly dependent on the transcapillary colloid osmotic pressure gradient, as originally predicted by Starling (12). The unique finding in the present study and in other studies performed on hyperproteinemia is that chronic elevations in plasma protein concentration result in increases in interstitial fluid protein concentration, and interstitial fluid colloid osmotic pressure and the resultant transcapillary colloid osmotic pressure gradient change little. Therefore, blood volume changes little during overhydration in hyperproteinemia and the distribution of extracellular fluid volume is unaltered from that of normoproteinemic animals.

Perspectives

Considerable controversy has recently occurred over whether crystalloid or colloid therapy is best for volume resuscitation (4). The present experiment and previous experiments from this laboratory may shed some light on this controversy.

The present experiment clearly demonstrates that chronic increases in plasma colloid osmotic pressure do not increase blood volume after infusion of an isotonic electrolyte solution. On the other hand, dogs that had their plasma protein concentration decreased to 2.5 g/dl by plasmapheresis had their kidneys removed and were overhydrated using the same protocol as in the present experiment (7). The blood volume-sodium space relationship markedly shifted down the blood volume axis (7), and, at any given sodium space, the blood volume was much lower in hypoproteinemic dogs than in either normoproteinemic or hyperproteinemic dogs. Thus a chronic decrease in plasma colloid osmotic pressure decreased blood volume and significantly impacted the blood volume-sodium space relationship. Therefore, severe chronic hypoproteinemia prevents any expansion of blood volume during intravenous infusion of a balanced electrolyte solution.

The loss of blood by hemorrhage is always accompanied by a loss of plasma proteins. If, under these conditions, plasma protein concentration decreases below 3 g/dl, infusion of an electrolyte solution will cause only a short-lived increase in blood volume. Therefore, colloid infusion (either alone or accompanied by red blood cells) would be necessary to bring blood volume back to normal. In conclusion, for successful volume resuscitation, administration of colloids is necessary in patients with severe hypoproteinemia, but increasing colloid osmotic pressure above normal does not increase blood volume except on a short-term basis.

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