Changes in Interstitial Volume and Masses of Albumin and IgG in Rabbit Skin and Skeletal Muscle after Saline Volume Loading

Richard J. Mullins and Donald R. Bell
From the Department of Physiology, Neil Hellman Medical Research Building, Albany Medical College of Union University, Albany, New York

SUMMARY. This study was performed on the hindpaws of anesthetized rabbits to determine the effects of acute saline expansion (10% body weight) on the extravascular distribution of water and plasma proteins. Prenodal lymph was collected separately from skin and muscle of the hindlegs. In samples of excised heel skin and gastrocnemius muscle, the extracellular and plasma spaces were measured with $^{51}$Cr EDTA and $^{125}$I albumin, respectively. The albumin and IgG spaces were calculated from measurements of the endogenous albumin and IgG concentrations in samples of plasma, lymph, and tissue extracts, by immunochemical techniques. Four hours after expansion, lymph flow from both tissues was more than 3 times greater than control, while the interstitial volume was increased by 20% in skin and 2.3 times for muscle. The elevated lymph flow was accompanied by a decrease in the lymph:plasma concentration ratio. The extravascular mass of albumin was $9.36 \pm 0.61$ and $3.93 \pm 0.31$ mg/g dry weight for control skin and skeletal muscle, respectively. The IgG mass was $2.62 \pm 0.47$ and $0.717 \pm 0.084$ mg/g dry weight for skin and muscle. Saline expansion resulted in a 41% increase in the extravascular albumin mass in muscle and no change in albumin mass in skin. The extravascular IgG mass increased by 58% in skin and 49% in muscle. The calculated excluded volume fraction for albumin decreased in both tissues and for IgG decreased in muscle. In skin, the IgG-excluded volume fraction could not be calculated after expansion since the apparent tissue concentration was greater than lymph, indicating interstitial concentration gradients for this molecule. Acute saline expansion resulted in a shift of plasma proteins from plasma to the extravascular space of skin and skeletal muscle. (Circ Res 51: 305-313, 1982)

THE redistribution of plasma proteins between the vascular and extravascular spaces following changes in plasma volume are only partially understood. Wasserman and Mayerson (1952) showed that saline infusions, equivalent in volume to 10% body weight, resulted in a shift of plasma proteins from the vascular to extravascular spaces in dogs. In a later study (Shirley et al., 1957), they proposed that an increase in capillary permeability to proteins could cause this shift in plasma proteins. However, it has recently become apparent that changes in the hydration of the interstitial gel matrix may also affect the partition of plasma proteins between the vascular and extravascular spaces (see review: Aukland and Nicolaysen, 1981). The interstitial space of most tissues is composed of an interlinked matrix of glycosaminoglycans supported by a collagen framework (Comper and Laurent, 1978). This matrix forms a gel that restricts plasma proteins from distributing in the total intragel volume by either steric or electrostatic exclusion. In nonedematous tissues, albumin has been calculated to distribute in 50-70% of the total interstitial volume at lymph concentration in peripheral tissues (Bell et al., 1980), lung (Parker et al., 1979), and intestine (Granger et al., 1980). Thus, albumin was excluded from the other fraction of the interstitial volume.

Based upon in vitro studies, Granger and Shepherd (1979) proposed that the degree of protein exclusion would decrease as the matrix concentration decreased during edema formation. This would result in albumin distributing in a greater fraction of the intragel volume after edema formation. In the lung, the calculated excluded volume fraction for albumin decreased after an increase in the interstitial volume with Ringer's lactate expansion (Parker et al., 1979). In this study, there was also an increase in extravascular albumin mass accompanying the decreased excluded volume fraction after moderate infusions. This result suggests an alternative explanation for the increase in extravascular protein mass noted by Wasserman and Mayerson (1952) after saline expansion.

Since two-thirds of the total body interstitial fluid may be represented by skin and skeletal muscle (Aukland and Nicolaysen, 1981), we studied the effects of 10% body weight fluid volume loading on the extravascular distribution of a small and large plasma protein in these tissues. We have studied the extravascular distribution of endogenous albumin with a Stokes-Einstein radius of 36Å and γ-immunoglobulin (IgG) with a Stokes-Einstein radius of 56Å (Renkin and Curry, 1979).

Methods

Surgical Procedures

New Zealand white rabbits of either sex, weighing between 2.2 and 3.8 kg, were anesthetized with pentobarbital
sodium (25–35 mg/kg) and given supplemental doses as required. After a tracheotomy, a heparinized cannula was inserted into the right carotid artery for blood collections and arterial blood pressure recordings, using a Statham pressure transducer connected to a Grass polygraph. Two heparin-coated cannulas were placed in branches of the right jugular vein for fluid infusions and measuring central venous pressure. A mid-line supra pubic labarotomy was used to cannulate the urinary tract. Two small flank incisions were made, and snare tourniquets were placed loosely around the renal pedicles. A small branch of the saphenous vein was cannulated to measure the venous pressure. The rectal temperature was monitored and a heating blanket was used to maintain the animal’s temperature between 38 and 40°C.

The lymphatic cannulations were done as described by Bach and Lewis (1973). For skin lymph collections, prepopliteal node lymphatics located near the calcaneal tributary vein were tied off and a single lymphatic was cannulated with Silastic tubing (0.3 mm i.d. and 0.64 mm o.d.). In preliminary studies, using subcutaneous injections of T1824, we confirmed the earlier observations of Bach and Lewis (1973) that these lymphatics drain the skin of the heel. For the muscle lymph collections, the heel skin was injected with 0.4 ml of 0.5% T1824 in saline. In each leg, the lymphatics within the femoral sheath were ligated, and a single lymphatic was cannulated distal to the inguinal node with Silastic tubing (0.3 mm i.d. and 0.64 mm o.d.). The initial lymph collected was blue from the T1824 injection. In each leg, the popliteal node was exposed, and the efferent lymph duct was ligated. Lymph flow immediately decreased after ligation of the efferent lymphatic and became clear 1–2 hours after ligation. In preliminary experiments, subcutaneous injections of T1824 after ligation of the efferent lymphatic did not result in the appearance of blue lymph. Injection of T1824 into the gastrocnemius muscle resulted in the appearance of blue in the cannulated lymphatics 15–30 minutes after injection.

Lymph collection was done with the animal in the prone position, and the hindpaws were flexed passively at 50–70 times per minute to promote lymph flow. Lymph samples were collected in preweighed heparinized vials for 30-minute intervals. Arterial blood samples, approximately 0.8 ml, were taken using heparinized syringes at the mid-point of each interval, and the hindpaw lymph was collected 10 minutes after the arterial blood sample for each interval. Blood samples were measured for the wet-dry weight ratio. Although the heel skin was carefully shaved with electric clippers at the start of the experiment, additional hair was removed from the dried samples with a razor and forceps. We then corrected all weights for the residual hair. The other set of tissue samples from each leg was homogenized in 4 ml of 1 M NaCl, using a Brinkmann Polytron homogenizer. The extract fluid contained trypsin inhibitor (Sigma), phenylmethylsulfonylfluoride (Sigma), 3,3'-diaminobenzidine tetrahydrochloride (Sigma) and sodium azide to inhibit protein digestion and bacterial growth.

Chemical Analyses and Calculations

Total protein concentrations in lymph and plasma samples were measured by the method of Lowry et al. (1951). The albumin concentration in lymph, plasma, and tissue extract samples and the IgG concentration in lymph and plasma samples were measured using rocket immunonassay (Laurell, 1972). Since the IgG concentration in the tissue extract fluid was low, we determined the concentration of IgG in these samples by single radial immunodiffusion (Mancini et al., 1965). The protein concentrations in the lymph and tissue extracts were read from a dilution curve of plasma from one rabbit. The IgG concentration in the tissue samples from each leg was determined using rabbits’ serum from the same rabbit and IgG was obtained from Cappel Laboratories. The assays were standardized using purified rabbit albumin and IgG. Albumin was purified using ammonium sulfate fractionation (Peters, 1962) and affinity chromatography (Travis and Pannell, 1973) using Cibacron Blue Sepharose (Pharmacia). IgG was purified using gel filtration on Sepharose 4B (Pharmacia) and ion exchange chromatography on DEAE-Sepacel (Pharmacia) (Fahey and Terry, 1978). The purity of the protein standards was checked using SDS polyacrylamide gel electrophoresis and gradient gel electrophoresis (Margolis and Kenrich, 1968; Weber and Osborne, 1975). For both protein standards, there was less than 5% contamination from other proteins. The total tissue distribution spaces for 51Cr and 125I were obtained by dividing the tissue activity (counts/min per g of tissue) by the plasma concentration (counts/min per ml). The total tissue distribution spaces for albumin and IgG were obtained by dividing the total tissue mass of the protein (mg/g tissue) by the plasma protein concentration (mg/ml). The extracellular spaces for 51Cr, albumin, and IgG were calculated by subtracting the 125I albumin space from the respective total spaces. The extravascular 51Cr

Tissue Sampling

To measure the extracellular space in the tissue samples, 1 mCi of 51Cr labeled ethylenediaminetetraacetic acid (EDTA) from New England Nuclear was injected iv after ligation of the kidneys. The vascular space in each tissue sample was obtained by giving intravenously 125I-labeled human serum albumin (Mallinkrodt) 10 minutes before termination of the experiment. After the 10-minute mixing period, a blood sample was taken, and the rabbit was killed with an overdose of pentobarbital sodium. Two sets of duplicate samples were taken from either the thick heel skin or the gastrocnemius muscle from each leg. The samples were weighed and counted for the radioactivity of each isotope, together with plasma and lymph samples, in a Searle two-channel automatic gamma counter. After correcting for background, separation of 51Cr and 125I was done by pulse-height analysis. The albumin tracer had less than 1% inorganic label as determined by trichloroacetic acid precipitation.

After the tissue samples had been counted, one set from each leg was dried to a stable weight at 60°C for the determination of the wet-to-dry weight ratio. Although the heel skin was carefully shaved with electric clippers at the start of the experiment, additional hair was removed from the dried samples with a razor and forceps. We then corrected all weights for the residual hair. The other set of tissue samples from each leg was homogenized in 4 ml of 1 M NaCl, using a Brinkmann Polytron homogenizer. The extract fluid contained trypsin inhibitor (Sigma), phenylmethylsulfonylfluoride (Sigma), 3,3'-diaminobenzidine tetrahydrochloride (Sigma) and sodium azide to inhibit protein digestion and bacterial growth.

Chemical Analyses and Calculations

Total protein concentrations in lymph and plasma samples were measured by the method of Lowry et al. (1951). The albumin concentration in lymph, plasma, and tissue extract samples and the IgG concentration in lymph and plasma samples were measured using rocket immunonassay (Laurell, 1972). Since the IgG concentration in the tissue extract fluid was low, we determined the concentration of IgG in these samples by single radial immunodiffusion (Mancini et al., 1965). The protein concentrations in the lymph and tissue extracts were read from a dilution curve of plasma from one rabbit. The IgG concentration in the tissue samples from each leg was determined using rabbits’ serum from the same rabbit and IgG was obtained from Cappel Laboratories. The assays were standardized using purified rabbit albumin and IgG. Albumin was purified using ammonium sulfate fractionation (Peters, 1962) and affinity chromatography (Travis and Pannell, 1973) using Cibacron Blue Sepharose (Pharmacia). IgG was purified using gel filtration on Sepharose 4B (Pharmacia) and ion exchange chromatography on DEAE-Sepacel (Pharmacia) (Fahey and Terry, 1978). The purity of the protein standards was checked using SDS polyacrylamide gel electrophoresis and gradient gel electrophoresis (Margolis and Kenrich, 1968; Weber and Osborne, 1975). For both protein standards, there was less than 5% contamination from other proteins. The total tissue distribution spaces for 51Cr and 125I were obtained by dividing the tissue activity (counts/min per g of tissue) by the plasma concentration (counts/min per ml). The total tissue distribution spaces for albumin and IgG were obtained by dividing the total tissue mass of the protein (mg/g tissue) by the plasma protein concentration (mg/ml). The extracellular spaces for 51Cr, albumin, and IgG were calculated by subtracting the 125I albumin space from the respective total spaces. The extravascular 51Cr
EDTA (mol wt 362) space was used as an estimate of the interstitial volume (V). The extravascular masses of albumin and IgG were expressed as mg protein/g tissue weight by multiplying the extravascular spaces at protein concentration by the plasma concentration of the respective protein. The excluded volume fraction \( F_e \) for albumin or IgG was calculated using the equation

\[
F_e = 1 - \frac{M}{V} / C_L
\]

where \( M \) is the extravascular mass of protein (mg/g tissue wt) and \( C_L \) is the lymph protein concentration (mg/ml).

The data are expressed as arithmetic means ± SE. Significant differences in the lymph data were tested using a Mann Whitney U-test or a Wilcoxon matched pairs test, since the population of lymph flows did not appear to be normally distributed. Significant differences in the tissue data were tested using an analysis of variance (Snedecor and Cochran, 1967). Multiple comparisons were analyzed using the Bonferroni procedure.

**Results**

**Lymph Response: Skin**

Figure 1 shows the results from an experiment in which skin lymph was collected. The initial lymph flow was high due to the ligation of the lymphatic prior to cannulation. After the first 1 to 2 lymph collection periods, lymph flow became steady. Baseline values were obtained after the initial transient in lymph flow. In this experiment, saline was given over a 3-hour period until there was an 11.4% expansion. There were small transient increases in both the mean arterial blood pressure and saphenous vein pressure during the infusion. The plasma total protein concentration decreased from 60 to 43 mg/ml during the infusion and remained constant at the lower level. Lymph flow (L) increased while the lymph:plasma concentration ratio for total protein \( (R_T) \) decreased. The early increase in \( R_T \) is an artifact of the decreasing plasma protein concentration. Lymph flow reached a peak around the end of the infusion and then decreased to a level higher than baseline. This was a consistent finding in all the skin experiments.

Table 1 presents a summary of lymph flow and the lymph:plasma concentration ratio for total protein, albumin, and IgG. Control data were obtained from 10 animals. For the eight expanded animals (15 legs), both pre-infusion baseline and post-infusion data are presented. There were no significant differences between control and baseline values. There was a large variation in control lymph flows, which was probably due to the variation in the amount of skin drained by the cannulated lymphatic. With a net volume expansion \( \frac{8.9 ± 0.9}{8.9 ± 0.9} \) of body weight, the plasma total protein concentration decreased from 53.6 ± 2.6 to 39.4 ± 1.5 mg/ml \( (P < 0.01) \). Mean arterial blood pressure before infusion was 100 ± 3 mm Hg, whereas at the end of the experiment it was 91 ± 4 mm Hg \( (0.1 > P > 0.05) \). The pressure in the saphenous vein was 6.0 ± 0.7 and 6.7 ± 0.8 mm Hg \( (P > 0.5) \) during baseline and near the end of the experiment, respectively. At the end of the experiment lymph flow remained elevated above baseline, with decreased lymph:plasma concentration ratios for total protein, albumin, and IgG. The lymph flow and \( R_T \) for the second to last sample were 4.23 ± 0.84 µl/min and 0.230 ± 0.015. These values were not significantly different \( (P > 0.4) \) than those reported in Table 1. We have assumed that this indicates near steady state conditions for the protein concentration in lymph.

**Table 1** Effects of Saline Expansion on Skin Lymph Flow and Composition

<table>
<thead>
<tr>
<th></th>
<th>Control (n = 10)</th>
<th>Baseline (n = 15)</th>
<th>Expanded (n = 15)</th>
</tr>
</thead>
<tbody>
<tr>
<td>L(µl/min)</td>
<td>3.38 ± 1.02</td>
<td>1.56 ± 0.25</td>
<td>4.08 ± 0.63*</td>
</tr>
<tr>
<td>( R_T )</td>
<td>0.300 ± 0.026</td>
<td>0.346 ± 0.015</td>
<td>0.230 ± 0.016*</td>
</tr>
<tr>
<td>( R_A )</td>
<td>0.417 ± 0.031</td>
<td>0.491 ± 0.015</td>
<td>0.331 ± 0.019*</td>
</tr>
<tr>
<td>( R_G )</td>
<td>0.254 ± 0.022</td>
<td>0.312 ± 0.017</td>
<td>0.207 ± 0.020*</td>
</tr>
</tbody>
</table>

Lymph flow = L. Lymph:plasma concentration ratios for total protein \( (R_T) \), albumin \( (R_A) \), IgG \( (R_G) \). Values are expressed as mean ± SEM. Differences between control and baseline were not significant \( (P > 0.1) \) using a Mann-Whitney U test.

* Significantly different from baseline \( (P < 0.01) \) using Wilcoxon matched-pairs test. Nonparametric tests were used, since lymph flows did not appear to be normally distributed.
Lymph Response: Muscle

In Figure 2, an example of the effects of volume expansion on muscle lymph is shown. In this experiment, a net volume of saline equivalent to 10.8% body weight was given over a 3-hour period. There was a small increase in the pressure of the saphenous vein, but only a transient increase in mean arterial blood pressure. The total protein concentration in plasma decreased from 55.4 to 41.0 mg/ml and remained constant until the end of the experiment. Lymph flow increased while RT decreased. Unlike the response in skin, muscle lymph flow increased and remained at the elevated flow rate.

Table 2 presents a summary of lymph flow and the lymph:plasma concentration ratios for total protein, albumin, and IgG. The control data were obtained from 10 animals, whereas the expanded data were taken from 8 animals (15 legs). In the control animals, we found that after ligation of the popliteal efferent lymphatic, lymph flow and protein concentration became constant with time after 2–3 hours. Therefore, we did not attempt to obtain pre-infusion baseline values due to prolonged anesthesia time. With a net volume gain of saline equivalent to 10.0 ± 0.4% of the body weight, the total protein concentration decreased from 53.0 ± 1.8 to 39.5 ± 1.3 mg/ml, which was similar to the experiments in which skin lymph was collected. Mean arterial blood pressure was 93 ± 3 mm Hg before the infusion and 89 ± 4 mm Hg (P > 0.5) at the end of the experiment. The pressure in the saphenous vein was 9.3 ± 0.4 and 10.1 ± 0.6 mm Hg (P > 0.4) during baseline and near the end of the experiment, respectively. Lymph flow after expansion was 3.6 times greater than control, while the lymph:plasma concentration ratios were decreased. In the expanded animals, lymph flow and RT for the second to last sample were 2.99 ± 0.38 µl/min and 0.366 ± 0.015, respectively. These values were not significantly different (P > 0.4) from the values for the last lymph collection reported in Table 2. As with the skin experiments, we have used this to support the assumption that the lymph protein concentration was not changing with time when we obtained the tissue samples.

Tissue Response

Table 3 presents a summary of the effects of saline expansion on the interstitial volume and extravascular protein masses for both skin and skeletal muscle. For skin, saline expansion resulted in a 20% increase in the interstitial volume, expressed as per gram dry weight. The wet-to-dry weight ratio corrected for hair was 3.96 ± 0.09 and 4.32 ± 0.06 for control and expanded conditions, respectively (P < 0.01). For muscle the interstitial space was more than double the control value. Muscle wet-to-dry weight ratio was 4.05 ± 0.05 for control and 4.70 ± 0.10 for saline expanded (P < 0.01). In terms of wet tissue weight, the interstitial volumes for skin were equivalent to 40.61 ± 0.88 and 44.26 ± 0.78 ml/100 g wet tissue weight for control and saline-expanded conditions (P < 0.01). For muscle, the interstitial volumes were equivalent to 7.65 ± 0.25 and 14.94 ± 0.68 ml/100 g wet tissue weight for control and saline expanded conditions (P < 0.01). For the skin samples, the values for the plasma volume ranged between 0.4 and 3.2 ml/100 g wet tissue. For muscle, the plasma volume ranged between 0.5 and 1.5 ml/100 g wet tissue.
weight. Activity of the vascular marker was not detectable in any of the final lymph samples.

Saline expansion did not significantly change the extravascular mass of albumin in skin but increased the albumin mass in muscle by 40%. The extravascular mass of IgG increased by 58% in skin and 50% in muscle. Correspondingly, plasma albumin concentration was around 20% less and the IgG concentration was around 30% less than control. The tissue samples were taken from 5 to 8.5 hours after the start of the saline infusion. This corresponds to 3.5 to 5.5 hours after the end of the saline infusion. There was no significant correlation (r < 0.6, F > 0.1) between the extravascular mass of albumin or IgG and the time of tissue sampling.

In Table 4, the lymph concentration for albumin or IgG is compared to the apparent interstitial concentration for these proteins. This latter quantity was calculated as the extravascular mass (M) for each protein divided by the interstitial volume (V). For all cases except IgG in skin following expansion, the apparent interstitial protein concentration was less than that in lymph. This can be attributed to exclusion of the proteins from portions of the interstitial volume. After saline expansion, the apparent interstitial concentration for IgG was 28% greater than that in lymph.

Table 5 presents a summary of the excluded volume fractions for albumin and IgG based upon lymph concentration. In both skin and skeletal muscle, albumin and IgG were excluded from around 50% of the interstitial volume. After saline expansion, the excluded volume fraction for albumin decreased for both skin and skeletal muscle. The decrease in skin was greater than in muscle. In muscle, the IgG-excluded volume fraction decreased after expansion. However, the IgG-excluded volume fraction in skin following expansion could not be calculated. The large extravascular mass of IgG resulted in negative values for Fe.

The plasma volume in the animals was estimated based upon the dilution of the 125I-labeled albumin tracer after 10 minutes in the circulation. For control animals, the plasma volume was 2.89 ± 0.17% body weight. For the expanded animals, the plasma volume was 4.14 ± 0.27% body weight, which was significantly greater (P < 0.01) than the control animals.

**Discussion**

The results of these experiments support the proposal of Wasserman and Mayerson (1952) that acute saline expansion causes a redistribution of plasma proteins into the extravascular space. Four hours after saline expansion, there was an increase in the extravascular mass of albumin and IgG in muscle. In skin, there was an increase in the extravascular mass of IgG but no change in the mass of albumin. In the present study, we measured the endogenous content of albumin and IgG, using immunochemical techniques. The extravascular albumin masses, expressed in plasma volume equivalents, were 7.67 ± 0.33 and 3.03 ± 0.18 ml/100 g wet tissue weight for control skin and skeletal muscle, respectively. These values were similar to those previously reported for the dog (Bell et al., 1980) and rabbit (Owen and Triffitt, 1976; Bill, 1977). Also, our value for the extravascular IgG mass in muscle, which was equivalent to 1.75 ± 0.15 ml/100 g wet tissue weight, was similar to the value of 1.1 ml/100 g for tracer IgG in rabbit muscle reported by Bill (1977).

The change in extravascular protein mass does not appear to be due to an alteration in capillary permeability. Saline expansion resulted in an increased lymph flow accompanied by a decrease in the total protein lymph:plasma concentration ratio. This is not indicative of increased capillary permeability to plasma proteins (Taylor et al., 1981). However, the increased extravascular protein mass may have been due to a substantial increase in the available interstitial volume for protein distribution. This increased volume was due to edema and, possibly, a decrease in the excluded volume fraction.

The extracellular space was measured using 51Cr EDTA, which is a small molecule (mol wt 362) and uncharged at physiological pH (Poole-Wilson et al., 1975). The present values for the interstitial volume

**Table 3**

<table>
<thead>
<tr>
<th></th>
<th>Skin</th>
<th>Muscle</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control (n = 10)</td>
<td>Expanded (n = 15)</td>
</tr>
<tr>
<td>V (ml/g dry wt)</td>
<td>1.61 ± 0.06</td>
<td>1.90 ± 0.06*</td>
</tr>
<tr>
<td>Me (mg/g dry wt)</td>
<td>9.36 ± 0.61</td>
<td>10.69 ± 0.62</td>
</tr>
<tr>
<td>Mv (mg/g dry wt)</td>
<td>2.62 ± 0.47</td>
<td>4.13 ± 0.36*</td>
</tr>
<tr>
<td>Pα (mg/ml)</td>
<td>30.6 ± 1.0</td>
<td>23.3 ± 0.6*</td>
</tr>
<tr>
<td>P0 (mg/ml)</td>
<td>12.8 ± 2.3</td>
<td>8.5 ± 0.8*</td>
</tr>
</tbody>
</table>

V = extravascular distribution space for 51 Cr EDTA. Me and Mv are the extravascular masses of albumin and IgG, respectively. Pa and P0 are the plasma concentrations of albumin and IgG, respectively.

Results are expressed as means ± SE.

* Significantly different from control (P < 0.05).
† Significantly different from control skin (P < 0.01).
in control skin and skeletal muscle were in the range of previously reported values (see review: Aukland and Nicolaysen, 1981). Saline expansion, equivalent to 10% of the body weight, resulted in an increase in the interstitial volume of 20% for skin and 2.3 times for muscle. Thus, there was a much greater increase in the interstitial volume in muscle than in skin. This occurred even though lymph flow increased 3-fold in both tissues. Assuming that lymph flow was a function of interstitial pressure, the data were consistent with the finding that the muscle interstitial space has a higher compliance than in skin (Aukland and Nicolaysen, 1981). Thus, the prevention of edema by changes in lymph flow appears less significant in muscle than in skin.

As reviewed by Comper and Laurant (1978) and, more recently, by Aukland and Nicolaysen (1981), the interstitium is composed of an interlinked matrix of collagen and glycosaminoglycans. Many of the intersticies between the matrix fibers are too small to permit entry of plasma proteins, although large enough to permit entry of water and small solutes. Molecules the size of albumin can distribute in only a small portion of this fiber matrix and are sterically or electrostatically excluded from other portions of the matrix. Based upon in vitro studies with collagen or hyaluronic acid, Granger and Shepherd (1979) have proposed that edema would dilute the interstitial matrix and lead to a small decrease in the excluded volume fraction for plasma proteins. This would occur as the intersticies between the matrix fibers become greater due to the dilution. A greater fraction of the interstitial water would become available for the distribution of extravascular plasma proteins.

The albumin excluded volume fraction has been calculated for lung (Parker et al., 1979), intestine (Granger et al., 1980) and dog skin and skeletal muscle (Bell et al., 1980). Furthermore, the albumin-excluded volume fraction decreased with increased tissue hydration in lung and intestine, which is consistent with the in vitro studies.

In both skin and skeletal muscle in the rabbit, albumin (Stokes-Einstein radius of 36Å) and IgG (Stokes–Einstein radius of 56Å) were calculated to be excluded from approximately 50% of the interstitial space during control. The excluded volume fraction for albumin decreased in both tissues after saline expansion. In muscle, there was a 20% reduction in the excluded volume fraction after a doubling in the interstitial volume. This change was smaller than that found in the lung, where a doubling of the interstitial volume resulted in a 74% decrease in the excluded volume fraction (Parker et al., 1979). However, in skin, a 20% increase in the interstitial volume resulted in a 50% decrease in the calculated excluded volume fraction. The in vitro studies indicate that 20% dilution of a collagen or hyaluronate gel will result in a much smaller change in the excluded volume fraction (Aukland and Nicolaysen, 1981; Granger and Shepherd, 1979). This discrepancy between the in vitro studies and our change in the albumin-excluded volume fraction calculated for skin may indicate that there are

### Table 4

<table>
<thead>
<tr>
<th></th>
<th>Albumin</th>
<th>IgG</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>C_L (mg/ml)</td>
<td>M/V (mg/ml)</td>
</tr>
<tr>
<td>Skin</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control (n = 10)</td>
<td>12.9 ± 1.2</td>
<td>5.83 ± 0.39*</td>
</tr>
<tr>
<td>Expanded (n = 15)</td>
<td>7.73 ± 0.47</td>
<td>5.70 ± 0.35*</td>
</tr>
<tr>
<td>Muscle</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control (n = 10)</td>
<td>24.3 ± 1.6</td>
<td>12.8 ± 1.2*</td>
</tr>
<tr>
<td>Expanded (n = 15)</td>
<td>13.4 ± 0.6</td>
<td>8.11 ± 0.81*</td>
</tr>
</tbody>
</table>

Results are expressed as mean ± se.
* Indicates values of M/V significantly different from C_L (P < 0.05) using a paired t-test.
Mullins and Bell/Skin and Muscle Edema after Saline Infusion

Factors other than exclusion which affect the distribution of albumin in skin. As will be discussed with IgG, there may be sieving of plasma proteins through the interstitial matrix in skin. This will affect the assumptions used for calculation of the excluded volume fraction.

In muscle, the decrease in excluded volume fraction for IgG was similar to that for albumin. However, in skin, the IgG-excluded volume fraction could not be calculated since the apparent interstitial concentration of IgG was greater than that in lymph (Table 4). The apparent interstitial concentration was calculated as the extravascular protein mass divided by the interstitial volume. If IgG distributed in the total interstitial volume at lymph concentration (no exclusion from portions of the matrix), the apparent interstitial concentration would be equal to lymph. If IgG were excluded from portions of the interstitial volume, the apparent interstitial concentration would be less than lymph, as was the case for albumin. The IgG data in Table 4 may indicate adsorption of IgG to some tissue component following saline expansion or sieving through the matrix. Macrophages are reported to bind small amounts of IgG (Phillips-Quagliata et al., 1971). In lichen planus, IgG may bind to the epithelial cells of human skin (Shousha and Svirbely, 1977). However, this would be unexpected in the presumably normal rabbit skin tissue in our studies. A more probable explanation may be that the lymph IgG concentration does not entirely reflect the interstitial concentration in skin following saline expansion due to concentration gradients within the matrix.

Calculation of the excluded volume fraction assumes that the interstitial protein concentration was equal to that in lymph, and that the extravascular protein mass and lymph concentrations were in steady state. A comparison of lymph flow and protein concentration between the last half-hour collection and the second to last showed less than a 3% difference, which was not significant. Tissue samples were collected from 3.5 to 5.5 hours after the end of the infusion, and there was no indication that the extravascular protein masses were changing with time. Thus, we have assumed that the present data represent near steady state conditions following acute saline expansion.

The other assumption was that the protein concentrations in the available interstitial volume and lymph were equal. Support for this hypothesis has come from a number of studies that have shown no consistent differences in the protein composition between prenodal lymph and interstitial fluid collected from micropipettes, wicks, or implanted capsules (see reviews: Renkin, 1979; Aukland and Nicolaysen, 1981). However, these techniques may be too coarse to detect small regional protein concentration gradients within the matrix. Fox and Wayland (1979) studied the diffusion rates of fluorescein-labeled albumin and large size dextrans in the rat mesentery. They found that the restricted diffusion coefficient for albumin was about 7% of the free diffusion coefficient and that the interstitial matrix behaved as if it had between 50 and 70 Å pores. If this is the case, there could be steady state concentration gradients for plasma proteins within the interstitial matrix in addition to across the capillary wall. Consequently, the apparent interstitial concentration for large proteins would be greater than lymph. Whereas the apparent interstitial concentration of IgG in skin following saline expansion is 28% greater than lymph, it is still 72% less than plasma, indicating that most of the sieving still would occur across the capillary wall.

Renkin (1979) has discussed the possible circumstances for protein concentration gradients within the interstitial matrix. If there are free-fluid channels within the interstitial matrix, the protein concentration in channels of higher flow will be less than in channels of lower flow. He suggests that this would lead to a protein concentration in the interstitial fluid greater than in lymph. The interstitial concentration will be a weighted average of the channel flows. Since free fluid channels are not considered to restrict macromolecules sterically, this case should apply to both albumin and IgG. Since, in these two proteins, there was a difference in behavior, this explanation for concentration gradients appears unlikely. Another possible source for protein gradients would be sieving through the restrictive gel matrix (Renkin, 1979). This would occur if the size of the intersticies between the matrix fibers were of the order of the pore sizes in the capillary, as has been suggested for the rat mesentery (Fox and Wayland, 1979). Since albumin is smaller in size than IgG, the interstitial sieving of albumin would be less than for IgG. Since macromolecular sieving across the capillary or interstitium depends upon the lymph flow rate (Landis and Pappenheimer, 1963), the degree of sieving across the gel matrix may be small at normal lymph flows. With increased flow through the matrix, sieving may become substantial for molecules the size of albumin or IgG and may create concentration gradients within the interstitium. This would occur more in skin—where there was an increase of only 20% in interstitial volume, with a 3-fold increase in lymph flow—than in muscle—where there was a 2.3-fold increase in interstitial volume, with a similar change in lymph flow.

It was reported that increased venous pressure decreased the extravascular albumin mass and did not alter the calculated albumin-excluded volume fraction in rabbit skin and skeletal muscle (Bell and Mullins, 1982). Thus, venous hypertension affects the interstitial matrix differently from saline expansion. One explanation for the different responses may be derived from consideration of an interaction between the capillary wall and a heterogenous interstitial gel. The interstitium may be composed of two phases: a non-restricting free fluid phase and a restrictive gel phase (Wiederhielm, 1971; Watson et al., 1980). An increase in capillary pressure may preferentially increase capillary filtration across the venular capillary, which is more permeable to water and solutes than...
the arterial capillary (Landis and Pappenheimer, 1963). This fluid may flow directly to the lymphatics through expanded free fluid channels and not disrupt the gel matrix. On the other hand, saline expansion decreases plasma oncotic pressure; this may result in increased filtration into the interstitial gel matrix along the entire capillary, and may cause either a disruption of the gel matrix, decreasing the excluded volume fraction for albumin, or sieving of plasma proteins within the gel matrix.

Recently, Kramer et al. (1981) reported that reduced plasma protein concentrations in sheepe by plasma- pheresis resulted in a sustained elevation in pulmonary lymph flow after the calculated hydrostatic and oncotic pressure gradients across the capillaries had returned to baseline. They propose that hypopro- teinemia increased the hydraulic conductivity of the interstitial gel. In the present experiments, muscle lymph flow was 3-fold greater than control 3 or more hours after the end of the infusion. At that time, there was no change from the baseline mean arterial pressure, the saphenous vein pressures, and the lymph- plasma oncotic pressure gradient, calculated using the albumin and globulins equation of Landis and Pap- penheimer (1963). Thus, the increase in lymph flow cannot be explained by these forces. It could be attributed to an increase in hydraulic conductivity as proposed by Kramer et al. (1981). Alternatively, a decrease in pre- to post-capillary resistance could account for the greater flow. Because the increase in lymph flow was associated with a fall in Rp, surface area changes are not the explanation (Taylor et al., 1981). The results of the present experiment suggest another explanation. If protein concentration gra- dients occur in the interstitium, a higher oncotic pressure could exist in the pericapillary fluid than in the lymph. It remains to be shown how the persistent elevation in lymph flow after saline infusion can be explained.

In summary, saline expansion resulted in a decrease in the protein concentration in lymph from both skin and skeletal muscle. Accompanying the increased interstitial volume, there was either no change or an increase in the extravascular mass of albumin and IgG. Assuming that the interstitial protein concentration was equal to that in lymph, albumin and IgG were excluded from 50% of the interstitial volume in both tissues under normal conditions. Saline expansion resulted in a decrease in the calculated excluded volume fraction for albumin in both tissues and IgG in muscle. In skin, saline expansion resulted in an apparent interstitial concentration for IgG greater than that in lymph. This may indicate that there was sieving of this molecule through the interstitial matrix, causing steady state concentration gradients within the interstitium.

We are grateful to Nancy J. Carr and Karen Krook Ward for their technical assistance, and to Maureen Davis and Susan Churan for typing the manuscript. Dr. Frank Blumenstock provided assistance with the purification of albumin and IgG standards.
Mullins and Bell/Skin and Muscle Edema after Saline Infusion


INDEX TERMS: Peripheral lymph • Interstitial exclusion • Protein sieving • Gel matrix • Free fluids

Downloaded from http://circres.ahajournals.org/ by guest on October 18, 2017
Changes in interstitial volume and masses of albumin and IgG in rabbit skin and skeletal muscle after saline volume loading.
R J Mullins and D R Bell

Circ Res. 1982;51:305-313
doi: 10.1161/01.RES.51.3.305
Circulation Research is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 1982 American Heart Association, Inc. All rights reserved.
Print ISSN: 0009-7330. Online ISSN: 1524-4571

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://circres.ahajournals.org/content/51/3/305.citation