Mechanism of the Increased Vascular Capacity Produced by Mild Perfusion Hypothermia in the Dog

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SUMMARY The mechanism of the increased vascular capacity produced by perfusion hypothermia was investigated in 20 anesthetized dogs. A right heart bypass preparation separated cardiac output (CO) into splanchnic (Qs) and nonsplanchnic (termed peripheral, Qp) flows. Each channel drained by gravity into an external reservoir. Blood was then returned to the pulmonary artery at a constant flow of 80 ml/kg per min. Venous resistance and compliance of splanchnic (Rs and Cs) and peripheral (Rp and Cp) channels were calculated from transient and steady state volume shifts which occurred following rapid drops in venous pressure. Arterial pressure (Pa), hematocrit (H), plasma protein concentration, and changes in reservoir volume (ΔVres) were also measured. Filtered plasma (VF) volume was determined from changes in hematocrit; ascites (VA) volume was determined by an indicator dilution technique. Hypothermia to 33 °C decreased both Cs and Cp from 0.022 ± 0.002 (mean ± SE) to 0.014 ± 0.001 liter/mm Hg and 0.023 ± 0.002 to 0.017 ± 0.001 liter/mm Hg, respectively. Rp increased from 7.1 ± 1.0 to 9.0 ± 0.9 mm Hg/liter per min. Portal pressure increased from 7.5 ± 0.4 to 12.9 ± 1.3 mm Hg as H increased from 45.1 ± 1.1 to 49.4 ± 1.5%, and plasma protein concentration increased from 5.1 ± 0.2 to 6.7 ± 0.2 g/100 ml. Rp, Pa, and the steady state distribution of CO did not change. VA decreased 0.785 ± 0.063 liter during hypothermia, whereas VF increased 0.321 ± 0.031 liter. VM increased 0.082 liter during this period. We conclude that a large fraction of the decrease in plasma volume that occurs during mild perfusion hypothermia in the dog can be accounted for by the exudation at the liver of effectively pure plasma. The remaining percentage of filtered volume appears to be lost elsewhere in the circulatory system as an ultrafiltrate. Much of the hypothermia-induced increase in vascular capacity appears to be the result of an increase in the unstrained vascular volume and/or an increase in the volume sequestered in the splanchnic bed by a constriction of the hepatic outflow vessels.

PERFUSION hypothermia is the means by which body temperature can be lowered by selectively cooling the temperature of blood passing through an extracorporeal circuit. It has been long recognized that mild perfusion hypothermia (decreasing temperature to around 30°C) produces a decrease in extracorporeal reservoir blood volume at constant pump flow or a decrease in venous return at constant blood volume (i.e., an increase in vascular capacity) in both man and dog (Brown, 1962; Drew, 1966; Gollan et al., 1952; Yeh et al., 1961; Lindberg, 1959; Oz et al., 1960; Pierucci et al., 1960). Although there are several books (Cooper and Ross, 1960; Blair, 1964; Popovic and Popovic, 1974) and innumerable papers in the literature covering the subject of hypothermia, there are relatively few physiological reports of the effects of perfusion hypothermia. Furthermore, a review of the literature indicates that differences of opinion exist concerning the mechanism(s) of the increase in vascular capacity produced by perfusion hypothermia. This study was undertaken in an attempt to clarify the mechanism(s) responsible for this increase in vascular capacity.

Methods

Surgical Procedures

Twenty mongrel dogs with a mean weight of 23.0 ± 0.53 (SE) kg were anesthetized with sodium thiopental, 18 mg/kg, iv. The anesthesia was maintained with 70% nitrous oxide in oxygen and halothane. A tracheotomy was performed and the dog was ventilated at an appropriate tidal volume and frequency for its size. A ventral laparotomy was then performed, followed by a splenectomy. The abdominal incision was not closed. The extracorporeal perfusion system used in this study (Fig. 1) has been described previously (Green, 1977; Green et al., 1978a, 1978b). In brief, the hepatic venous outflow of the splanchnic circulation and the circulation from the rest of the body, the peripheral channel, were isolated and cannulated. The venous return from these beds drained by gravity through separate Starling resistors and a Carolina Medical square wave electromagnetic flowmeter into an ex-
tracorporeal reservoir. A Sarns roller pump then pumped blood around a NaI(Tl) scintillation crystal, through a Travenol mini prime heat exchange unit, and then directly into the pulmonary artery via a stainless steel cannula placed in the pulmonary artery through the right ventricular outflow tract. After the dog had been connected to the extracorporeal circuit, which had been primed with 1.58 to 2.78 liters of blood from donor animals, an umbilical tape previously placed around the pulmonary artery was drawn tightly around the cannula, thereby preventing blood from entering the pulmonary artery except through the cannula. The Starling resistors were placed at an appropriate level, relative to the right atrium, to maintain venous pressures at 2.5 mm Hg. The pump was set to maintain a flow of approximately 80 ml/min per kg (1.839 ± 0.065 liters). Splanchnic and peripheral venous flows; arterial, right atrial, inferior vena cava, hepatic venous, and portal venous pressures; as well as changes in reservoir volume were measured and recorded continuously on an Electronics for Medicine multichannel recorder.

Arterial Po$_2$, Pco$_2$, and pH were measured at 10-minute intervals from the time the dog was placed on the bypass circuit until the end of the normothermic control period. Acid-base balance was adjusted to achieve a pH of 7.4, a Pco$_2$ of 40 mm Hg, and a Po$_2$ greater than 100 mm Hg by appropriately fixing the frequency of ventilation and/or by infusing NaHCO$_3$ solution. To avoid osmotic transients, no further adjustments were made once normal acid-base balance was established. Although pH slowly dropped during the hypothermic period, it did not fall below 7.38 in any of the dogs. During the initial period of the right heart bypass procedure, blood in a secondary reservoir (Fig. 1) was continually mixed with blood in the rest of the perfusion system (by maintaining clamps "a" and "c" open). Thus, osmotic and oncotic equilibrium was achieved between the blood of the donor and experimental dogs.

During the normothermic control period, body temperature was maintained at 39°C. To produce hypothermia, cooled water was circulated through the water side of the mini prime heat exchange unit until a rectal temperature of between 32° and 34°C was achieved. The average time required to achieve this degree of hypothermia was 36.3 ± 3.6 minutes. Hypothermia was maintained by adjusting the flow rate of the chilled water.

Measurement of Systemic Mechanical Parameters

In all 20 dogs, systemic mechanical parameters were determined as follows (Green, 1977; Green et al., 1978a). All venous pressures were initially set at 2.5 mm Hg by adjusting the level of the Starling resistor relative to the right atrium. The venous pressure of one compartment was then raised to 10 mm Hg while the other venous pressure was maintained at 2.5 mm Hg. As venous pressures were held at these values, blood left the extracorporeal reservoir. When the transfer of blood from reservoir to dog had stopped, the Starling resistor was rapidly dropped, returning the venous pressure to 2.5 mm Hg. Immediately following the decrease in venous pressure, the blood volume of the extracorporeal reservoir increased as a single exponential. The time constant for venous drainage (R,C) is the time it takes, following the step decrease in venous pressure (ΔPv), for the blood volume in the external reservoir to rise by 0.63 (V_{new} - V_{old}). Where V_{old} = the initial reservoir volume, the volume when one venous pressure was elevated, V_{new} = the new steady state reservoir volume when both pressures equaled 2.5 mm Hg (Green, 1977). Since V_{new} - V_{old} is the inverse of the volume change to that
occurring in the dog, the steady state change in reservoir volume \(\Delta V_r\) could also be used to calculate the compartmental compliances as:

\[
\frac{\Delta V_r}{\Delta P_r} = C_r
\]

Time constants and compliances \(C\) of both splanchnic and peripheral compartments were measured in this manner. With the time constant and compliance, the venous resistance \(R_v\) of each compartment could be calculated by dividing the time constant by the compliance. The value obtained was corrected by subtracting the known hydrodynamic resistance of the extracorporeal tubing.

The effective splanchnic back pressure was measured by the method previously described (Green, 1975). The hepatic venous pressure was slowly elevated by raising the Starling resistor until portal pressure rose. The level of the hepatic venous pressure when portal pressure began to rise was taken as the effective splanchnic back pressure \(P_{sv}\).

The upstream venous pressures for both compartments \(P_a\) and \(P_p\) were calculated as:

\[
P_a = \frac{(Q_a - P_{sv})}{Q_a}, \quad P_p = \frac{(Q_p - P_{sv})}{Q_p}
\]

The effective downstream venous pressure of the peripheral compartment \(P_{pv}\) was measured with a catheter in the right atrium and was approximately 2.5 mm Hg. Arterial resistances for both compartments \(R_a\) and \(R_p\) were then calculated as:

\[
R_a = \frac{(P_a - P_{sv})}{Q_a}, \quad R_p = \frac{(P_p - P_{sv})}{Q_p}
\]

The total unstressed vascular volume \(V_u\) of the systemic circulation was calculated as:

\[
V_u = P_a C_a + P_p C_p
\]

The total unstressed vascular volume of the systemic circulation \(V_u\) was calculated by subtracting \(V_t\) from the dog's total blood volume (see below). A discussion of the model upon which these measurements are based and the physiological significance of the parameters termed stressed and unstressed vascular volume is presented in the Discussion.

**Measurement of Volume Shifts**

In 13 dogs, volume data were obtained as follows. Total circulating blood volume (intravascular blood volume minus splenic volume plus circulating blood volume of the extracorporeal tubing) was determined by the dilution of \(^{113}\text{mIn}\)-transferrin tracer. Details for this method have been presented previously (Green et al., 1978b). In brief, a New England Nuclear generator containing approximately 1 mCi of \(^{113}\text{mIn}\) on a zirconium oxide column was eluted with 0.05 N HCl and yielded about 700 mCi of \(^{113}\text{mIn}\) in the first 4 ml of eluate. Twelve to 15 ml of plasma (anticoagulated with heparin) were then mixed with sufficient eluate \((15 \text{mCi} \text{InCl}_3)\) to yield about 360 mCi of \(^{113}\text{mIn}\)-transferrin at the anticipated time of injection. Once the \(^{113}\text{mIn}\)-transferrin complex was injected, the radioactivity circulating in the preparation (dog plus extracorporeal system) was monitored by a 2- by 2-inch NaI(Tl) scintillation crystal surrounded by 110 ml of blood and adequately shielded with lead bricks. The detector was powered by an Ortec model 446 high voltage supply operated at 900 V. The output signal was fed to an Ortec model 276 preamplifier and then to an Ortec model 490A linear amplifier and analyzer. Pulse height analysis was achieved with a 60 keV window centered at the 393 keV gamma emission of \(^{113}\text{mIn}\). The analyzer output was scaled by a home-constructed four-channel buffered scaler which drove a teletype (ASR 33). Data were printed at 1-minute intervals, and the teletype-punched paper type output was fed into a computer for data analysis.

The \(^{113}\text{mIn}\)-transferrin complex was injected directly into the pulmonary artery at time \(t = 0\). Immediately prior to the injection, clamp “c” (Fig. 1) was closed, and a sufficient quantity of untagged blood was pumped from the primary to the secondary reservoir. Clamp “a” was then closed and clamp “b” opened. The mixing pump then mixed, for the rest of the experiment, the blood in the primary reservoir. After 15 minutes, adequate mixing had occurred, and initial base line counts were then obtained. Next, a known quantity of untagged blood \(\Delta V\), equilibrated with the circulating blood volume prior to \(^{113}\text{mIn}\) administration, was added to the extracorporeal system from the secondary reservoir (by opening clamp “c,” Fig. 1). The initial total circulating blood volume \(V_t\) before the addition of the untagged bolus was calculated as:

\[
V_t = \Delta V \cdot \frac{N_i}{N_f} - \frac{N_i}{N_f}
\]

where \(N_i\) and \(N_f\) are the initial and final \(^{113}\text{mIn}\) counts, respectively, after corrections for radioactive decay (Green et al., 1978b). The total circulating blood volume, following the volume addition, \(V_t\) was calculated as \(V = V_t + \Delta V\). \(V_t\) was considered the starting volume and was the volume to which all subsequent volume determinations were referenced.

Blood hematocrits were obtained at regular intervals throughout the experiment. Each hematocrit was determined in triplicate, read with a mm ruler, and then averaged. The value of the total circulating blood volume \(V_t\) at any time following the determination of \(V\) was then determined from a RBC volume balance as follows:

\[
V_i = V \left( \frac{H}{H_i} \right)
\]

where \(H\) and \(H_i\) are the starting hematocrit and the hematocrit (expressed as a fraction ranging from 0.0 to 1.0) at any subsequent time, respectively (Green et al., 1978b). The total blood volume of the dog was obtained by subtracting the known volume of the extracorporeal system (tubing plus reservoir) from the total circulating blood volume \(V_t\). The total volume filtered from the circulatory system at any subsequent time was then calculated as \(V_F = V - V_t\), or

\[
V_F = V \left( 1 - \frac{H}{H_i} \right)
\]
Since Equation 2 is based on a red cell volume balance, it can be correct only when there is no hemorrhage. Therefore, no attempt was made to measure blood volume until as near perfect hemostasis as possible had been achieved. This was determined by constancy of extracorporeal reservoir volume at constant cardiac output and vena caval pressures for a period of at least 10 minutes.

The plasma protein concentration, \( P \), was determined at regular intervals by withdrawing a sample of blood from the femoral artery, centrifuging the sample for 5 minutes at 5000 rpm, and analyzing the supernatant extract with an AO refractometer (model 10400). During the process of filtration, an inevitable mass of protein is lost from the circulatory system. If we assume that this protein is uniformly distributed in the entire filtrate, the concentration of proteins in the average filtered volume can be expressed as \( \alpha P \). The term \( \alpha \) (which is derived in the Appendix) is the ratio of average filtrate protein concentration to the plasma protein concentration and may be calculated from measured values of hematocrit and plasma protein concentration as follows:

\[
\alpha = \frac{(1 - H)P}{(1 - H_t)H_t} \tag{4}
\]

where the symbol without any subscript refers to the starting condition and the symbol with the subscript \( t \) represents any subsequent time. The term \( \alpha \) may assume values from 0 (ultrafiltration) to 1 (exudation of whole plasma). Consider, for example, the case where \( \alpha = 0.5 \). If \( P = 5.0 \text{ g/100 ml} \), the average filtrate protein concentration would be 0.5 \( \times \) 5.0 = 2.5 g/100 ml.

We have found it useful in these experiments to carry this conceptual approach to whole body filtration one step further. The total amount of volume filtered (\( V_F \)) may be thought of as being composed of two fractions, one which is essentially exudation of whole plasma (\( \alpha = 1 \)) and the other, filtration of pure water (\( \alpha = 0 \)). If we assume that the mass of exudated proteins could be concentrated together, \( \alpha \) allows us to estimate the percentage of the actual filtered volume (\( V_F \)) which would be necessary to establish a filtrate with a protein concentration equal to plasma. This “plasma” fraction is calculated as \( \alpha V_F \), whereas the water fraction is calculated as \( (1 - \alpha) V_F \).

With the aid of the \( ^{113m}\text{In}-\text{transferrin} \) tracer, we can gain additional information concerning the filtration process. With \( \beta \) representing the ratio of the concentration of \( ^{113m}\text{In}-\text{transferrin} \) in the “plasma” fraction of the filtrate to the concentration of \( ^{113m}\text{In}-\text{transferrin} \) in the actual intravascular plasma, this ratio may be calculated (see Appendix) as:

\[
\beta = \frac{N \frac{H}{H_t}}{(1 - H_t) \frac{P_t}{H_t} - (1 - H) \frac{P}{H_t}} \tag{5}
\]

where \( N \) is the decay corrected counts per minute for \( ^{113m}\text{In} \). If \( \beta = 1 \), the concentration of \( ^{113m}\text{In}-\text{transferrin} \) in the “plasma” fraction of the filtrate is equal to that in the plasma itself. This indicates that the \( ^{113m}\text{In}-\text{transferrin} \) molecule leaves the circulation with the same ease as does the average plasma protein molecule. If \( \beta \) is less than unity, the \( ^{113m}\text{In}-\text{transferrin} \) molecule has more difficulty leaving the circulation than the average plasma protein molecule. Hence the concentration of \( ^{113m}\text{In}-\text{transferrin} \) is less in the “plasma” fraction of the filtrate than in the plasma itself. Conversely, a value of \( \beta \) greater than unity indicates that the \( ^{113m}\text{In}-\text{transferrin} \) molecule leaves the circulation with greater ease than the average plasma protein molecule. As will be seen in the Results section, for the dogs studied here, the average value of \( \beta \) was indistinguishable from unity, indicating that transferrin was typical of the average plasma protein molecule. This is reasonable in light of the fact that the bulk of plasma protein is albumin, which has a molecular weight of 70,000, compared with 90,000 for transferrin. Therefore, it appears that the indium-tagged transferrin molecule is a suitable tracer to study the filtration of plasma proteins when the filtrate contains a significant amount of albumin.

Finally, the formation of ascites fluid was determined quantitatively by a dye dilution technique using a tracer made up of 0.5 g of a fluorescent-tagged dextran of molecular weight 150,000 (Pharmacia FITC 150) dissolved in 0.5 liter of Ringer’s solution. After the dog had become stable on bypass, a measured volume of this dye (0.205 ± 0.019 liter) was poured directly into the open abdominal cavity. The solution was then mixed by gentle external manipulation of the abdomen, and a 5-ml sample was collected via a catheter placed at the base of the cavity. Additional 5-ml samples were withdrawn at regular intervals during the experiment. Before each sample was collected, the abdomen was manipulated to mix the abdominal fluids. Immediately after collection, all samples were centrifuged at 5000 rpm for 10 minutes to remove any solids and then were sealed in glass vials for later analysis of protein and dye content. To detect bleeding, hematocrits on the abdominal fluids were measured. In none of the animals was an abdominal hematocrit greater than 5% observed and, in most dogs, values were considerably smaller.

At the completion of the experiment, the abdominal samples were analyzed for protein concentration with a refractometer (AO model 10400), and a
portion was diluted 100-fold with saline solution and analyzed for dye concentration with a Farrand Optical Mark I spectrophotometer with the stimulation monochromator set at 494 nm and the emission monochromator at 520 nm. A sample of the original dye solution served as a reference.

Total ascites production was calculated from the time the dye solution was poured into the abdomen. If the added dye solution had volume, \( V_d \), and concentration, \( C_s \), then the volume of ascites formed at any point in time \( \Delta V_m \) is given by simple mass balance of the dye as

\[
\Delta V_m = \left( \frac{V_d C_s}{C} \right) - V_d,
\]

where \( C \) is the dye concentration at the time ascites volume is to be determined. Substituting the relationships \( C_s = \frac{M_d}{V_d} \), where \( M_d \) is the mass of dye yields:

\[
(6)
\]

Because of sample withdrawal, \( M_d \) decreases with each sample. This loss is calculated by multiplying the sample concentration by the sample volume.

Statistical significance was determined by Student's t-test for paired variants.

**Results**

**Arterial Parameters**

The mean steady state values of the arterial parameters are presented in Table 1. Lowering body temperature to 33°C did not significantly change the arterial resistance of either the splanchnic or the peripheral compartments from their control values of 149.6 and 61.9 mm Hg/liter per min, respectively. Therefore, mean arterial pressure, at a constant cardiac output of 1.8 liters/min, did not change from its control value of 84 mm Hg. As a consequence, the steady state distribution of cardiac output remained constant at approximately 31% to the splanchnic compartment \( (F_s) \) and 69% to the peripheral compartment \( (F_p) \). The distribution of cardiac output was, however, highly variable during the transient cooling period, favoring the splanchnic compartment in some animals and the peripheral compartment in others.

**Venous Parameters**

The mean steady state values of the venous parameters are presented in Table 1. On the average, lowering body temperature to 33°C decreased both splanchnic and peripheral compartment compliances from 0.022 ± 0.002 to 0.014 ± 0.001 liter/mm Hg \( (P < 0.001) \) and 0.023 ± 0.002 to 0.017 ± 0.001 liter/mm Hg \( (P < 0.001) \), respectively. Splanchnic venous resistance increased from 7.1 ± 1.0 to 9.0 ± 0.9 mm Hg/liter per min \( (P < 0.05) \), whereas peripheral venous resistance showed a statistically insignificant increase from 3.2 ± 0.4 to 4.7 ± 0.4 mm Hg/liter per min \( (P < 0.1) \). The effective splanchnic back pressure increased from 3.2 ± 0.2 to 4.6 ± 0.3 mm Hg \( (P < 0.001) \), whereas portal pressure increased from 7.5 ± 0.4 to 12.9 ± 1.3 mm Hg \( (P < 0.001) \).

**Volume Data**

The mean steady state values of the volume data are presented in Table 2. The extracorporeal reservoir blood volume decreased following hypothermia by an average 0.785 ± 0.063 liter. In 13 dogs, 0.072 ± 0.007 liter of this volume was attributed to drawn blood samples, for a net decrease in reservoir volume of approximately 0.713 liter. Simultaneously with the decrease in extracorporeal reservoir blood volume, the dog's total blood volume increased from 1.823 ± 0.129 to 2.001 ± 0.070 liters \( (P < 0.2) \). This increase in the dog's blood volume was the result of the combined decrease in stressed vascular volume from 0.314 ± 0.025 to 0.273 ± 0.024 liter \( (P < 0.2) \).

**Table 1 Mechanical Parameters**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Normothermia</th>
<th>Hypothermia</th>
<th>( P ) value*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Weight (kg)</td>
<td>23.9 ± 0.5</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cardiac output (liters/min)</td>
<td>1.839 ± 0.065</td>
<td>1.814 ± 0.066</td>
<td>NS</td>
</tr>
<tr>
<td>Distribution of cardiac output to splanchnic region (%)</td>
<td>30.5 ± 2.0</td>
<td>31.7 ± 2.0</td>
<td>NS</td>
</tr>
<tr>
<td>Splanchnic arterial resistance (mm Hg/liters per min)</td>
<td>149.6 ± 18.7</td>
<td>143.0 ± 16.0</td>
<td>NS</td>
</tr>
<tr>
<td>Splanchnic compliance (liters/mm Hg)</td>
<td>0.022 ± 0.002</td>
<td>0.014 ± 0.001</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Splanchnic venous resistance (mm Hg/liters per min)</td>
<td>7.1 ± 1.0</td>
<td>9.0 ± 0.9</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Effective splanchnic backpressure (mm Hg)</td>
<td>3.2 ± 0.2</td>
<td>4.6 ± 0.3</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Portal venous pressure (mm Hg)</td>
<td>7.5 ± 0.4</td>
<td>12.9 ± 1.3</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Peripheral arterial resistance (mm Hg/liters per min)</td>
<td>61.9 ± 5.7</td>
<td>64.0 ± 5.0</td>
<td>NS</td>
</tr>
<tr>
<td>Peripheral compliance (liters/mm Hg)</td>
<td>0.023 ± 0.002</td>
<td>0.017 ± 0.001</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Peripheral venous resistance (mm Hg/liters per min)</td>
<td>3.2 ± 0.4</td>
<td>4.7 ± 0.4</td>
<td>&lt;0.1</td>
</tr>
<tr>
<td>Mean arterial pressure (mm Hg)</td>
<td>84.0 ± 4.0</td>
<td>83.0 ± 5.0</td>
<td>NS</td>
</tr>
</tbody>
</table>

* Determined by the t-test for paired variants.
Table 2 Volume Data

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Normothermia</th>
<th>Hypothermia</th>
<th>P value*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blood volume of dog (liters)</td>
<td>1.834 ± 0.129</td>
<td>2.001 ± 0.079</td>
<td>&lt;0.1</td>
</tr>
<tr>
<td>Stressed vascular volume (liters)</td>
<td>0.314 ± 0.025</td>
<td>0.273 ± 0.024</td>
<td>&lt;0.2</td>
</tr>
<tr>
<td>Unstressed vascular volume (liters)</td>
<td>1.542 ± 0.129</td>
<td>1.767 ± 0.082</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Ascites volume (liters)</td>
<td>0.965 ± 0.014</td>
<td>0.147 ± 0.037</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Filtered volume (liters)</td>
<td>0.221 ± 0.031</td>
<td>0.221 ± 0.031</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Change in reservoir volume (liters)</td>
<td>0.785 ± 0.063</td>
<td>0.785 ± 0.063</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Hematocrit (%)</td>
<td>45.1 ± 1.1</td>
<td>49.4 ± 1.5</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Plasma protein concentration (g/100 ml)</td>
<td>5.1 ± 0.2</td>
<td>5.7 ± 0.2</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>( \alpha ) ratio</td>
<td>0.566 ± 0.046</td>
<td>0.566 ± 0.046</td>
<td></td>
</tr>
<tr>
<td>( \beta ) ratio</td>
<td>1.026 ± 0.096</td>
<td>1.026 ± 0.096</td>
<td></td>
</tr>
</tbody>
</table>

Data are presented as mean ± standard error of the mean; \( n = 13 \).
* Determined by the \( t \)-test for paired variants.

and the increase in unstressed vascular volume from 1.542 ± 0.129 to 1.767 ± 0.082 liters (\( P < 0.05 \)). Hypothermia also increased the protein concentration of the ascites from 0.273 ± 0.024 g/100 ml to 0.221 ± 0.031 g/100 ml (\( P < 0.001 \)). The ratio of the total volume filtered to the plasma protein concentration, \( \alpha \), was 0.566 ± 0.046 following hypothermia. This suggests that 56.6% of the total volume filtered had a protein concentration equal to plasma itself and amounts to approximately 182 ml. The remainder of the filtered volume was lost as an ultrafiltrate. The ratio \( \beta \) was 1.026 ± 0.096 following hypothermia suggesting that the "plasma" fraction of the filtrate had the same concentration of transferrin as did plasma.

**Ascites Protein Mass Balance**

To determine the nature of the fluid producing the ascites, a protein mass balance was calculated as follows. At the end of the normothermic control period (immediately before the onset of cooling) there was, on the average, 0.027 liter of abdominal fluid consisting of 0.065 ± 0.014 liter of ascites and 0.205 ± 0.019 liter of instilled fluorescent dye. This abdominal fluid had an average protein concentration of 1.0 g/100 ml. When the body temperature had been reduced to 33°C, an additional 0.082 liter of ascites had been produced, and the average protein concentration of the abdominal fluid was 2.2 g/100 ml. Thus, 0.270(1.0) + 0.082(X) = 0.352(2.2), where X is the protein concentration of the ascites produced. Solution of this equation yielded a value of 6.1 g/100 ml for X. Since the 99% confidence interval for the blood protein concentration during hypothermia was 5.1 to 6.2 g/100 ml, this value of X (6.1 g/100 ml) strongly suggests that the ascites fluid was produced by the exudation of essentially pure plasma. When viewed in context with the above-reported \( \alpha \) ratio, these data suggest that a large part of the volume lost as pure plasma (volume with a protein concentration equal to plasma) was filtered from the vascular system at the liver.

**Discussion**

This study represents, to the best of our knowledge, the first attempt to investigate the effects of perfusion hypothermia on the mechanical properties of the systemic circulation. Although various studies have reported the apparent loss of vascular tone induced by perfusion hypothermia (Peirce and Law, 1962; Neville et al., 1961; Ankeney and Murphy, 1962; Sealy et al., 1963; Lesage et al., 1962), and still others have measured the effects of both perfusion and surface cooling on arterial resistance (Oz et al., 1960; Sealy et al., 1963; Lesage et al., 1962; Sealy et al., 1958; Lopez-Belio et al., 1960; Salisbury et al., 1963; Svanes et al., 1970; Chen and Chien, 1977), changes in venous compliance, unstressed vascular volume, and venous resistance have not been reported. In contradistinction, there is abundant literature, most recently summarized by Chen and Chien (1977), covering the effects of all types of hypothermia on fluid balance. The evidence presented in the literature clearly demonstrates that a decrease in body temperature is accompanied by a decrease in plasma volume, although there is much disagreement as to the exact mechanism responsible for this decrease. Our contribution begins to add the missing knowledge concerning the effects of perfusion hypothermia on systemic circulatory mechanics and presents evidence suggesting the exact mechanism for the decrease in plasma volume observed in the dog.

The interpretation of the mechanical parameters measured in this study depends in large part upon the two-compartment lumped parameter model of the systemic circulation which served as the conceptual basis for this study. This model divides the systemic circulation into parallel splanchnic and extrasplanchnic (termed peripheral) channels (Green, 1977; Green et al., 1978b). Each channel is
considered to be composed of a compliant locus, fed by a high arterial resistance and drained by a small venous resistance. The distinguishing characteristic of each channel is its venous time constant, which is the product of the compartmental venous resistance and compliance.

There are two implicit assumptions underlying this model. First, it is assumed that each channel may be described by a single time constant; second, it is assumed that the time constant of the two channels are measurably different. Evidence supporting both of these assumptions has been presented previously (Green, 1977; Green et al., 1978b). There is, in addition, another reason for using the two-compartment model to describe the systemic circulation in the canine species. There is a pronounced hepatic outflow sphincter in the dog which has a profound influence on venous return by virtue of its direct effect on the splanchnic channel (Green, 1975). It is, therefore, not only reasonable to conceptually separate the splanchnic channel from extraspinalchic vascular beds, but it is also important to do so experimentally.

Before proceeding, some discussion of the concept of the unstressed vascular volume is needed. The total vascular capacity or the volume held within the systemic circulation, at any point in time, is the sum of two separate volume entities: the stressed and unstressed vascular volumes. The unstressed vascular volume, \( V_o \), is the volume which the vasculature would contain if the right atrium was at atmospheric pressure and the remainder of the vasculature were in hydrostatic equilibrium with atmospheric right atrial pressure. The stressed vascular volume, \( V_s \), is the difference between the total vascular volume and the unstressed vascular volume. In terms of the two-compartment model, the stressed volume in each compartment is the product of the pressure in each compliant bed (above atmospheric plus hydrostatic) and the compliance. \( V_s \) is often defined as the intercept of the static pressure-volume curve of the systemic circulation. This intercept will be the unstressed vascular volume only if the volume plotted is the total vascular volume and there is no volume sequestered in the vasculature at pressures above hydrostatic equilibrium under conditions of zero flow and atmospheric right atrial pressure. \( V_o \) is important physiologically because it is the volume that must be applied to the systemic circulation to produce elastic recoil of the veins. Since there can be no pressure gradients until the veins begin to recoil, \( V_o \) is also important physiologically because there can be no venous return (and therefore no cardiac output) until the vascular volume exceeds \( V_o \).

There are two ways of assessing changes in the unstressed vascular volume. First, one can determine the systemic volume-pressure (Shoukas and Sagawa, 1971; Drees and Rothe, 1974) or volumeflow (Green, 1975) curves and then extrapolate these curves to zero pressure and flow, respectively. Second, as was done in these experiments, one can determine the total blood volume of the animal and then subtract the stressed vascular volume as calculated from the mechanical parameters.

The unstressed vascular volume becomes important within the context of the experiments reported herein because of its apparent ability to change. An increase in \( V_o \) at any given total blood volume would result in a reciprocal decrease in the stressed vascular volume, reducing venous pressures and venous return. This could occur even if there were no change in venous compliance.

We refer to changes in \( V_o \) as apparent because there are several problems in assessing changes in this parameter. First, there is the uncertainty that stems from assessing changes in any extrapolated parameter. Next, volume may be trapped in the splanchnic bed due to constriction of the hepatic outflow vessels. This sequestered splanchnic volume cannot be distinguished from an increase in \( V_o \) (Green et al., 1978b; Green, 1975). Finally, a volume which has been injected into the intravascular space but eventually lost from this space (e.g., by filtration) may be interpreted as a change in \( V_o \) unless the lost volume can be accounted for (Green et al., 1978b).

The principal effect of hypothermia on the systemic circulation was a generalized vasoconstriction of the venous system. This was manifest both as a decrease in venous compliance and as an increase in venous resistance. The constriction of the hepatic outflow vessels was particularly marked. The increase in vascular capacity (decrease in reservoir volume at constant pump flow) was consistent with other reports (Brown, 1962; Drew, 1966; Gallan et al., 1952; Yeh et al., 1961; Lindberg, 1959; Oz et al., 1960; Pierucci et al., 1960) but was inconsistent with a decrease in stressed volume (product of compliance and static venous pressure). What then was the mechanism for this increase in vascular capacity? It was the increase in unstressed vascular volume. But was the increase in \( V_o \) real or apparent? To assess this last point it was necessary to assess carefully fluid shifts, a phenomenon known to occur during hypothermia (Chen and Chien, 1977; Bass and Henschel, 1956; Rodbard et al., 1951; Hervey, 1973; Barbour, 1943), and to assess the possible role of the hepatic sphincter as a means of sequestering blood volume in the splanchnic bed. In the current study, the proportion of the decrease in extracorporeal reservoir blood volume that was due to the decrease in plasma volume was measured and accounted for before the change in \( V_o \) was calculated. Thus, the change in this parameter cannot be attributed to fluid filtered from the circulatory system. The role played by the constriction of the hepatic outflow vessels (increase in the effective splanchnic back pressure, \( P_w \), and the splanchnic venous resistance, \( R_w \)) is not as easy to assess. The increase in \( P_w \), \( R_w \), and portal pressure suggests that blood was sequestered in the splanchnic bed.
Unfortunately, our method of measuring $P_{hv}$ does not allow us to make an accurate enough determination of this parameter to measure reliably the sequestered volume (our method measures only the lowest back pressure, but there could have been greater increases in the effective back pressure in parallel channels) (Green et al., 1976). Thus we cannot separate increases in volume sequestered in the splanchnic bed from increases in $V_v$. The effect on venous return is, however, the same. As the hepatic outflow vessels constrict, sequestering volume in the splanchnic bed, an intravascular redistribution of blood volume occurs, reducing the stressed vascular volume in the extrasplanchnic vascular beds. In other words, both an increase in sequestered volume and an increase in $V_v$, produce a decrease in extrasplanchnic stressed volume. The end result is a reduction in venous return.

Although hypothermia produced changes in two principal circulatory functions, mechanics and fluid dynamics, the former appears to have in large part produced the latter. The decrease in total circulating plasma volume, increase in ascites volume, and the increase in portal pressure are all consistent with the mechanical obstruction of the hepatic outflow vessels, producing a rise in hepatic sinusoid pressure which resulted in an exudation of pure plasma. This hypothesis is consistent not only with the protein mass balance of the ascites fluid but also with the conclusion of other investigators. Greenway and Lautt (1976) as well as Brauer et al. (1959) presented evidence that the hepatic sinusoidal wall is permeable to substances of high molecular weights (i.e., plasma proteins) and suggested that the reflection coefficient of the sinusoidal wall to plasma proteins is nearly zero. Thus a rise in hepatic sinusoid pressure is all that is necessary to lose plasma from the intravascular space. The $\alpha$ ratio (ratio of the protein concentration in the filtrate to plasma protein concentration) which we obtained (0.566) further suggests that a significant amount of the fluid "filtered" during hypothermia was lost as a plasma exudate.

Recently Chen and Chien (1977) reported that, in the dog during surface cooling to 26°C, a decrease in plasma volume and thoracic duct lymph flow occurred with an increase in hematocrit but no increase in plasma protein concentration. Since the lymph flow decreased at the same time as the plasma volume, they suggested that the reduction in plasma volume in hypothermia (in animals with no increase in plasma protein concentration) represents intravascular sequestration. They also suggested that another possible explanation was the trapping of extravasated plasma in the interstitial space, but they rejected this as being unlikely. If the results we obtained with mild perfusion hypothermia can be applied to the more profound cooling produced by Chen and Chien, our experiments offer an explanation consistent with both the observations of Chen and Chien and others; i.e., pure plasma volume can leave the vascular compartment at the liver due to elevated sinusoid pressures, secondary to constriction of the hepatic outflow vessels. Our additional observation that water can also be lost during hypothermia as an ultrafiltrate is consistent with some (Svanes et al., 1970; Rodbard et al., 1961; Barbour, 1953; D'Amato and Hennan, 1953), but not all, of the observations of a decrease in plasma volume during hypothermia. Finally, it should be emphasized that the conceptual partitioning of the lost plasma (through the use of the $\alpha$ ratio) into a pure plasma compartment and an ultrafiltrate compartment is somewhat artificial and results in a purely operational concept. It nevertheless has helped us to appreciate the complicated mechanisms responsible for the fluid loss resulting from hypothermia. This is in large part attributed to the fact that we could explain a large part of the loss of pure plasma by the formation of ascites.

In summary, the results of these experiments suggest that approximately 57% of the decrease in plasma volume which occurs during perfusion hypothermia can be accounted for by the exudation at the liver of pure plasma. The remaining percentage of filtered volume appears to be lost elsewhere in the circulatory system as an ultrafiltrate. The increase in vascular capacity which occurs during perfusion hypothermia is believed to be the result of an increase in unstressed vascular volume and/or an increase in the volume sequestered in the splanchnic bed by a constriction of the hepatic outflow vessels.

**Appendix**

The ratios $\alpha$ and $\beta$ yield information on the nature of the volume filtered from the intravascular space. If we assume that the mass of protein lost from the circulatory system during filtration is uniformly distributed in the entire filtrate, an expression for $\alpha$ may be derived from a total protein mass balance: total intravascular protein initially present $= \text{total intravascular protein remaining} + \text{protein in the filtered volume}$, or

$$V_pP = V_{pt}P_t + V_{pt}P_t,$$

where $V_{pt}$ is the intravascular plasma volume and $\alpha$ the ratio of filtrate protein concentration to plasma protein concentration. Replacing $V_{pt}$ with $V,(1-H_t)$, $V_t$ with Equation 2, and $V_r$ with Equation 3, yields upon rearrangement:

$$\alpha = \frac{(1-H)P}{P_t} - \frac{(1-H_t)H}{H_t}.$$

An implicit assumption in the above relationship is that all of the protein lost from the plasma is associated with filtration. Since estimated diffusive protein flux is approximately 2% of the total avail-
In activity in the plasma and $\frac{1}{\alpha V_F N}$ is proportional to the activity in the "filtered plasma." The activity balance may be expressed as follows: starting activity = remaining activity + activity in the filtrate or

$$V_N = V_N + \left(\frac{\beta N}{1 - H}\right)$$  \hspace{1cm} (A3)

Solving Equation A3 for $\beta$ gives:

$$\beta = \frac{V_N - V_N}{\alpha V_F N} \left(1 - H\right).$$  \hspace{1cm} (A4)

Inserting Equations 1, 2, 3, and A2 into A4 yields upon rearrangement:

$$\frac{N_H}{N_i} = \frac{1 - H}{P_i} \left(\frac{P - H}{1 - H}\right).$$  \hspace{1cm} (A5)

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