Blood reservoir function of dog spleen, liver, and intestine

JOÃO J. CARNEIRO AND DAVID E. DONALD
Mayo Clinic and Mayo Foundation, Rochester, Minnesota 55901

CARNEIRO, João J., AND DAVID E. DONALD. Blood reservoir function of dog spleen, liver, and intestine. Am. J. Physiol. 232(1): H67-H72, 1977 or Am. J. Physiol.: Heart Circ. Physiol. 1(1): H67-H72, 1977.—The reflex decrease in blood volume of the spleen, the liver, and the intestine of vagotomized dogs was measured by plethysmographic techniques during bilateral carotid occlusion and moderate and severe hemorrhage. The volume of blood mobilized from each organ during carotid occlusion and moderate hemorrhage was from 6 to 30% of their respective blood volumes and from 55 to 81% during severe hemorrhage. In each experimental situation the spleen exhibited the greatest ability to release blood and the intestine, the least. During moderate hemorrhage (9 ml/kg) the spleen yielded a volume equal to 35% of the blood lost, the liver 14%, and the intestine 7%. Comparable figures for severe hemorrhage were 26, 13, and 5%, respectively. This order of ranking the component regions of the splanchnic circulation with regard to function as a blood reservoir may be specific for the dog.

splanchnic capacitance; hemorrhage; baroreceptors; splanchnic circulation

IT IS WELL RECOGNIZED that the splanchnic vascular bed constricts when the cardiovascular system is challenged by a variety of stresses. Since this region receives about 25% of the cardiac output at rest and contains approximately 30% of the total blood volume, the resultant redistribution of splanchnic blood flow and mobilization of splanchnic blood volume can contribute significantly to the support of arterial blood pressure and cardiac output (2, 6, 10, 15, 17, 19, 26). In a recent study in dogs it was shown that carotid sinus hypotension mobilized 14% of the splanchnic blood volume; during moderate hemorrhage the splanchnic region contributed 54 and 65% of the total volume of blood removed (3, 4). The present investigation extends these observations and describes the changes in blood volume in the spleen, liver, and intestine of anesthetized dogs during bilateral carotid occlusion and during hemorrhage. The spleen clearly was the principal blood reservoir. The findings differ from those recently described for the cat by Greenway and Lister (13). In this animal the gastrointestinal tract made the major contribution to the volume of blood removed during hemorrhage and the role of the spleen was negligible. Both studies are in general agreement regarding the overall capacity of the splanchnic circulation to mobilize blood.

METHODS

Dogs fasted for 24 h were anesthetized initially by an intravenous injection of sodium thiopental (30 mg/kg) followed by alpha-chloralose (80 mg/kg initially and 10 mg/kg hourly). Gallamine triethiodide (3 mg/kg iv) was given to increase relaxation of the abdominal muscles. The dogs were intubated and mechanically ventilated with oxygen using a Harvard respirator. The respiratory rate was between 10-14 cycles/min and the tidal volume was 20-22 ml/kg. Peak inspiratory pressures were between 10-12 cm H₂O. When the diaphragm was opened, the expiratory line was immersed to a depth of 3-5 cm in water to maintain a near-normal end-expiratory lung volume. Arterial Po₂, Pco₂, pH, and hematocrit were measured at regular intervals. If necessary, sodium bicarbonate (2-3 ml of an 8.4% solution) was given intravenously to maintain pH between 7.3 and 7.4 and the rate of ventilation was changed to maintain Pco₂ between 30 and 40 mmHg.

Aortic and central venous pressures were measured through catheters positioned in the abdominal aorta and in the inferior vena cava immediately above the diaphragm, and connected to Statham P23 strain gauges. Portal, mesenteric, and splenic venous pressures were recorded from catheters connected to PR23 Statham strain gauges.

A telethermometer (Yellow Springs Instrument Co.) placed in the esophagus monitored the body temperature, which was maintained between 38 and 39°C by a water-filled heating blanket.

Changes in organ blood volume. Changes in organ blood volume were measured using plethysmographic techniques.

A plethysmograph modified from that described by Greenway (11, 14) for the cat was used to measure changes in hepatic blood volume. The liver was exposed by a bilateral subcostal incision and the contents of the gallbladder were aspirated. The cystic duct was ligated and after the section of their diaphragmatic ligaments, the central and lateral lobes of the liver were placed within the plethysmograph. Their intact vessels passed through a 3.5-cm aperture which was sealed with a plasticized hydrocarbon gel (Plastibase, Squibb).

A catheter advanced into the right branch of the portal vein through the pancreaticoduodenal vein was used to record portal venous pressure.
Changes in intestinal blood volume were measured using a plethysmograph similar to that described by Folkow et al. (9). The greater part of the jejunum and the ileum, together with the intact mesenteric pedicle, was placed within the plethysmograph. A catheter advanced into the mesenteric vein from a divided branch of the terminal ileal vein allowed measurement of mesenteric venous pressure from inside the plethysmograph.

A third plethysmograph was constructed to accommodate the spleen and its pedicle and was used to measure changes in splenic blood volume. The splenic pedicle was developed by dividing the short gastroepiploic veins. The greater omentum was removed. Splenic venous pressure was recorded by a catheter inserted in a divided gastroepiploic vein and advanced into a splenic vein inside the plethysmograph.

Plasticized hydrocarbon gel was used to seal the intestinal and splenic plethysmographs.

In order to avoid breaking the Plastibase seal, the diaphragm was opened in all experiments to reduce the movement due to inflation of the lungs.

The plethysmograph was connected to the system for recording changes in volume and both were filled with Krebs-Ringer-bicarbonate solution at 37°C. The system consisted of a vertical glass cylinder in which the liquid surface was set at the level of each organ pedicle. A photoelectric cell sensed the position of the liquid surface and its output activated a small motor to raise or lower the glass cylinder, and thus maintain a constant pressure within the plethysmograph. The electrical signal from the motor was used to register changes in organ blood volume. A sensitivity of 1 cm of recorded deflection for 1-ml change in volume was obtained without loss of stability. Eighty percent of the response to a near square-wave change in volume was achieved in 1 s. In all experiments hepatic, intestinal, and splenic venous pressures either did not change or increased by less than 0.5 cm H₂O on placing the organ within the plethysmograph. Systemic arterial and venous pressures and changes in volume of blood of the spleen, intestine, and liver were recorded continuously on an ultraviolet oscillograph (Honeywell Visicorder).

**Experimental procedures and data analysis.** Three different groups of dogs were used. Each animal was exposed to all the three experimental procedures but only one organ (liver, intestine, or spleen) was studied in each animal. Changes in organ blood volume and blood pressures were measured during 1) bilateral carotid occlusion, 2) brief moderate hemorrhage, and 3) severe hemorrhage. In each experimental procedure a steady state was obtained before the intervention was terminated. Procedures 1 and 2 were done alternately and a sufficient period of time was allowed between tests for blood pressures and organ blood volumes to return to or near (+5%) control values. The experiment was terminated following procedure 3.

**Bilateral carotid occlusion.** The common carotid arteries were stripped from the level of the manubrium to the carotid sinus. The anterior thyroid arteries and both vagosympathetic nerve trunks were divided. A snare was placed around each common carotid artery for later carotid occlusion. Occlusion was maintained for 2 min and an interval of 15 min was observed between successive tests. Two or more occlusions were carried out in each dog.

**Hemorrhage.** A large catheter was introduced into the right femoral artery and advanced into the abdominal aorta for the withdrawal and reinfusion of blood.

Hemorrhage equal to 9 ml/kg body wt was achieved by withdrawal of blood into heparinized warmed syringes at a rate of 50 ml/min. After 2 min the blood was reinfused at the same rate. An interval of at least 30 min was observed following reinfusion before further experimental intervention. Two such hemorrhages were carried out in each dog.

To produce severe hemorrhage the dog was heparinized (Liquemin 2.5 mg/kg), and the arterial catheter connected to an open reservoir set at such a level that the mean systemic arterial pressure was reduced to 40 mmHg. Changes in organ blood volume and blood pressures and in the amount of blood in the open reservoir were recorded during 20 min of sustained hypotension.

**Total organ blood volume.** To allow comparison between the organs, the change in blood volume was presented as a percentage of the total volume of blood within the organ as well as in absolute values.

Different methods were used to estimate the total volume of blood within the organ or that section of it placed within the plethysmograph. The splenic pedicle was clamped following 20 min of severe hypotension and the spleen removed from the plethysmograph. The splenic nerves then were stimulated electrically until the spleen was maximally contracted. The contracted spleen was weighed and the volume of expressed blood was measured. This volume was added to that lost by the spleen during the severe hemorrhage to give the total splenic volume. Changes in splenic volume during the different experimental procedures were expressed as a percentage of this total volume.

Similarly, the mesenteric pedicle was clamped at the end of the period of severe hemorrhage, the intestine removed from the plethysmograph, and weighed. The volume of blood within the intestinal segment was estimated by the red cell washout technique (9). This volume was added to that expressed during the severe hemorrhage to give the total volume of blood (ml/100 g) within the section of intestine.

**Hepatic blood volume was estimated in two dogs by the washout technique described for the intestine.**

The weight of liver or of intestine within and without the plethysmograph was obtained in each experiment, and the changes in blood volume were corrected for the total mass of the organ. This was not necessary with the spleen which was wholly contained within the plethysmograph. These total changes in organ blood volume then could be compared in absolute values or as milliliters per kilogram body weight.

**Data analysis.** In each test situation, change was measured as the difference between observations in the control and in the steady-state experimental condition. The results from each group of dogs were summed to obtain the mean and standard error. The difference in mean values was evaluated statistically by the t test, the level of significance was taken as 0.05%.
RESULTS

The changes in blood volume, expressed as a percentage of total organ blood volume in the spleen, intestine, and liver during carotid occlusion and hemorrhage in the individual dogs of each group, are shown in Fig. 1. The grouped data (means ± SE) are presented in Fig. 2.

Changes in splenic blood volume. Six dogs of mean weight equal to 17 kg (SE ± 1.0) were studied. The mean weight of the maximally contracted spleen was 40 ± 1.9 g and the mean splenic blood volume was 180 ± 17 ml. The mean control arterial and splenic venous pressures were 138 ± 5.3 mmHg and 13 ± 0.5 cmH₂O, respectively.

During bilateral carotid occlusion, the splenic blood volume decreased by a mean of 42 ml (SE ± 10), corresponding to 23% of the mean splenic blood volume. The mean increases in arterial blood pressure and splenic venous pressure were 75 ± 8 mmHg and 2.2 ± 0.1 cmH₂O, respectively.

A mean decrease of 54 ml (SE ± 7.5) equal to 30% of the mean splenic blood volume was observed during a brief moderate hemorrhage. The arterial blood pressure decreased by 7 mmHg (SE ± 0.9) and the splenic venous pressure decreased by 0.6 cmH₂O (SE ± 0.3).

Severe hemorrhage caused a mean decrease in splenic blood volume of 145 ml (SE ± 15) corresponding to 81% of the mean splenic blood volume. Splenic venous pressure decreased by 2.6 ± 0.4 cmH₂O. The mean volume of blood held within the open reservoir during the 20 min of sustained hypotension was equal to 33 ± 3.0 ml/kg.

Changes in intestinal blood volume. The mean weight of the six dogs in this group was 17 ± 0.7 kg and that of the total intestine was 385 ± 47 g. A mean of 73% of the total intestinal mass was contained within the plethysmograph. The mean blood volume of the intestinal segment within the plethysmograph was 34.5 ± 2.5 ml, corresponding to 12.2 ml/100 g.

Bilateral carotid occlusion decreased the intestinal blood volume by a mean of 2.6 ml (SE ± 0.4) or 0.7 ml/
100 g, corresponding to a decrease of 5.5% in total intestinal blood volume. The mean increases in arterial blood pressure and mesenteric venous pressure were 60 ± 7.5 mmHg and 2.7 ± 0.5 cmH₂O, from mean controls of 117 ± 2.8 mmHg and 11.6 ± 1 cmH₂O, respectively.

Bilateral carotid occlusion was repeated in all dogs of this group following complete obstruction of inflow to and outflow from the spleen by inflation of a pneumatic cuff placed around the splenic pedicle. One such experiment is illustrated in Fig. 3. The mean decrease in the intestinal blood volume during carotid occlusion was 6 ± 0.9 ml and the mean increase in mesenteric venous pressure was 0.5 ± 0.1 cmH₂O. These values are significantly different from those of 2.6 ml and 2.7 cmH₂O observed when the splenic pedicle was not occluded. The arterial blood pressure increased by a mean of 54 ± 6 mmHg, a value not significantly different from that of 60 ± 8 mmHg obtained with the nonoccluded spleen.

The mean decrease in intestinal blood volume caused by a brief moderate hemorrhage was 11 ± 1.2 ml or 2.9 ml/100 g. This is equivalent to a decrease of 23% in total intestinal blood volume. Arterial blood pressure and mesenteric venous pressure decreased by 8 ± 1 mmHg and 0.6 ± 0.1 cmH₂O, respectively.

During severe hemorrhage, intestinal blood volume decreased by a mean of 28.5 ± 1.2 ml, or 7.4 ml/100 g, corresponding to a decrease of 61.5% in total intestinal blood volume. The decrease in volume was sustained throughout the period of severe hypotension. Mesenteric venous pressure decreased by 2.8 ± 0.3 cmH₂O. The mean amount of blood contained within the open reservoir during the 20 min of sustained hypotension was equal to 34 ml/kg (SE ± 3.2).

Changes in hepatic blood volume. Nine animals with mean weight of 15 ± 1.5 kg were studied in this group. The mean liver weight was 399 ± 39 g. A mean of 60% of the total hepatic mass was contained within the plethysmograph. In the two dogs in which hepatic blood volume was measured, this was 28 and 25 ml/100 g of liver. To estimate volume changes in terms of total hepatic blood volume, the previously reported mean value of 25 ml/100 g was used (10).

Bilateral carotid occlusion caused a decrease in hepatic blood volume of 17 ml (SE ± 4.0) or 4.3 ml/100 g. This is equivalent to a decrease of 16% in total hepatic blood volume. Arterial blood pressure increased by 68 ± 4.0 mmHg from a mean control of 130 ± 3 mmHg and portal venous pressure increased by 3.8 ± 1.2 cmH₂O from a mean control of 12 ± 0.5 cmH₂O.

During moderate hemorrhage, hepatic blood volume decreased by 19 ± 2.9 ml, or 4.8 ml/100 g. This is equivalent to a decrease of 19% in total hepatic blood volume. Arterial blood pressure and portal venous pressure decreased by 11 ± 2 mmHg and 0.8 ± 0.2 cmH₂O, respectively. When the animals were submitted to severe hemorrhage the hepatic blood volume decreased by 57 ± 9.5 ml, or 14.3 ml/100 g, corresponding to a decrease of 55% in total hepatic blood volume. The decrease in liver blood volume was sustained through the period of severe hypotension. Portal venous pressure decreased by 3.1 ± 0.6 cmH₂O. The mean amount of blood contained within the open reservoir during the 20 min of sustained hypotension was equal to 30 ml/kg (SE ± 2.0).

When comparing the three different groups of animals, there is no significant difference between the groups in the mean increases in arterial blood pressure caused by bilateral carotid occlusion (75 ± 8, 60 ± 7.5, and 68 ± 4.0 mmHg) or the mean decreases due to moderate hemorrhage (7 ± 0.9, 8 ± 1.0, and 11 ± 2 mmHg). Also, the mean volumes of blood withheld from the dogs during severe hemorrhage (33 ± 3.0, 34 ± 3.2, and 30 ± 2.0 ml/kg) do not differ significantly.

**DISCUSSION**

The advantages and disadvantages of the plethysmographic method of measuring changes in the blood volume of an organ have been discussed (11, 14). It should be noted, however, that only a portion of the total liver (mean 60%) and of the intestine (mean 73%) was enclosed within the plethysmograph so that volume changes were extrapolated on a weight basis to the whole organ. Also, the recorded volume changes represent the total contribution of intravascular and extravascular compartments to the mobilization of fluid from each organ. In the spleen the volume changes represent the release of blood with a high hematocrit (7, 20, 24). In the liver the volume mobilized is probably entirely blood (11, 12). In the intestine a shift of fluid from the extravascular to the intravascular compartment might be anticipated. However, it has been shown in the cat that during hemorrhage (7 ml/kg), vasocstructor fiber stimulation, and lowering of carotid sinus pressure, once the intestinal blood flow was stable at the reduced level and initial changes in volume were com-

![FIG. 3. Reflex decrease in intestinal blood volume during carotid occlusion in a dog with spleen included (upper panel) and excluded (lower panel) from circulation. Zero position of scale for change in intestinal blood volume is set arbitrarily at beginning of experiment and not changed thereafter.](http://alphalnary.physiology.org/Downloadedfrom http://ajpheart.physiology.org/)
ple, an isovolumetric state again was attained (8, 22). A transfer of fluid across the capillaries of the intestine evidently does not take place when vasoconstrictor fiber activity is reflexly augmented.

It would thus seem reasonable to regard the changes recorded by the plethysmographic technique in the present experiments as changes in the blood volume of the organ.

The installation of each plethysmograph required laparotomy and considerable further surgery, which could have influenced the results. However, there is reasonable agreement with a previous study of changes in splanchnic blood volume, wherein a different method of measuring volume was used and the abdomen remained intact (3, 4). Summation of the decreases in organ blood volume during bilateral carotid occlusion (spleen 2.5, liver 1.1, and intestine 0.2 ml/kg, respectively) gave an estimate of 3.8 ml/kg for the changes in splanchnic blood volume. In the previous study, reducing the carotid sinus pressure from 140 to 40 mmHg gave a decrease in splanchnic blood volume of 3.1 ml/kg (mean body wt 14.6 kg, mean volume change 45 ml). Similar summation of the changes in organ blood volume during moderate hemorrhage (spleen 3.2, liver 1.3, and intestine 0.6 ml/kg, respectively) gave an estimate of a 5.1 ml/kg decrease in splanchnic blood volume. The earlier studies using a lesser degree of blood loss (7.2 ml/kg) gave values of 3.8 and 4.7 ml/kg.

Thus the surgical interference necessary to the plethysmographic technique had not materially affected the capacity of the splanchnic circulation to mobilize its blood volume.

The present study demonstrated that the spleen, the liver, and the intestine can contribute a considerable portion of their respective blood volumes to the splanchnic capacitance system. Moderate hemorrhage and carotid occlusion mobilized from 6 to 30% of organ blood volume and severe hemorrhage mobilized from 55 to 81%. However, the several components differed considerably in their ability to function as blood reservoirs. As shown in Fig. 4, the spleen provided the largest amount of blood in the three experimental situations and the intestine the smallest. This is due in part to the fact that the spleen contained a greater volume of blood (mean 10.5 ml/kg body wt) than did the liver (6.6 ml/kg) or the intestine (2.8 ml/kg) and in part to the fact that the spleen yielded a greater percentage of its blood volume than did the liver or intestine. This ranking of reservoir function is illustrated by calculation of the percentage contribution of each organ to the total blood loss in moderate and severe hemorrhage. In the former situation the splenic contribution was 35%, that of the liver 14%, and that of the intestine 7%; comparable values for severe hemorrhage are 26, 13, and 5%.

In the cat the situation is rather different. In this animal a hemorrhage of 8 ml/kg decreased splenic, hepatic, and intestinal blood volume by 1.5, 1.7, and 1.7 ml/kg body wt, respectively (13). In the present study, in the dog a hemorrhage of 9 ml/kg decreased the respective organ blood volumes by 3.2, 1.3, and 0.6 ml/kg. If, however, the changes in blood volume of the separate organs are summed to give an estimate of the change in total splanchnic volume, the results indicate that in each species the total splanchnic circulation makes a similar contribution to the volume lost during hemorrhage. Values for the cat are 4.9 ml/kg or 62% of the blood withdrawn and for the dog, 5.1 ml/kg or 56%. The major difference is that the gastrointestinal tract makes the greater contribution in the cat and the spleen in the dog.

Greenway and Lister (13) suggested as a working hypothesis that the mobilization of blood from the splanchnic region after a moderate hemorrhage involved active constriction of the capacitance vessels mediated by a sympathetic reflex from atrial pressure receptors. However, in the present study both cervical vagosympathetic nerve trunks were divided prior to any experimental intervention. Also, it has been shown that in the presence of normally functioning arterial baroreceptor reflexes, interruption of afferent vagal traffic from the cardiopulmonary area has minor effects on the cardiovascular system, and the contribution of the vagal cardiac nerves to the compensatory cardiovascular adjustments to blood loss is small (21, 24, 25). Further experiments seem indicated to determine if the systemic arterial, or the cardiopulmonary mechanoreceptors, or both, form the afferent arm of the reflex response to moderate hemorrhage.

That units of the splanchnic circulation may interact during a general reduction in splanchnic vascular capacity is indicated by the observation that the decrease in intestinal blood volume during carotid occlusion was greater when the spleen was excluded from the circulation. Presumably the expulsion of blood of high hematocrit from the spleen into the common mesenteric vein increased the resistance to outflow from the intestinal vascular bed and helped to maintain intestinal blood volume relatively constant. There is evidence both in the cat and the dog that the movement of blood into and out of the intestinal vasculature is strongly influenced by venous outflow pressure (7, 18, 21). Thus, in the normal animal the expulsion of blood by the spleen may further reduce the limited ability of the intestinal vascular bed to act as a blood reservoir.

The preeminence of the spleen as a blood reservoir in
the dog is in agreement with previous studies (1, 7, 20). The present study indicates that in this animal the liver also has a definite, though limited, capacity to mobilize blood during hemorrhage and activation of the sympathethic adrenergic system (16). Though the volumes of blood translocated are moderate, the mobilization is rapid and, thus, may make a significant contribution to the maintenance of cardiac filling pressure.

The authors acknowledge the courtesy and assistance of Dr. C. V. Greenway, Dept. of Pharmacology and Therapeutics, University of Manitoba Medical School, Winnipeg, Canada, in demonstrating the non-hypotensive hemorrhage and blood volume expansion in anesthetized cats. J. Physiol., London 237: 279–294, 1974.

REFERENCES


Received for publication 28 May 1976.