Mechanism of Morphine-Induced Shifts in Blood Volume between Extracorporeal Reservoir and the Systemic Circulation of the Dog under Conditions of Constant Blood Flow and Vena Cava Pressures

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SUMMARY  The mechanism of a morphine-induced shift in blood volume between an extracorporeal reservoir and the systemic circulation of a dog under conditions of constant blood flow and vena cava pressures was investigated in 10 anesthetized dogs with the aid of right heart bypass. All blood returning to the heart was removed from the right atrial appendage and, after passing through a Starling resistor, drained by gravity into an extracorporeal reservoir. It then was pumped around a NaI(Tl) scintillation crystal, through a heat exchanger and a flowmeter, and returned directly into the pulmonary artery. The following data (mean ± SE) were obtained with the pump flow set at 80 ml min⁻¹ kg⁻¹. By the 5th minute following the administration of morphine, 4 mg/kg, arterial pressure fell from 99 ± 5 to 59 ± 6 mm Hg (P < 0.001). Reservoir volume fell by 578 ± 86 ml, and central hematocrit increased from 0.41 ± 0.02 to 0.47 ± 0.01 (P < 0.01) in 15 minutes. Plasma volume, determined by a ⁴⁷⁹Tc-tagged plasma tracer, decreased by 317 ± 32 ml in 15 minutes. Thus, following the administration of morphine, 58% of the decrease in reservoir volume was accounted for by fluid lost from the circulatory system. This filtration was preceded by an immediate 30% rise in the counts/min obtained from a scintillation probe positioned over the liver, suggesting a similar rise in liver volume. These counts remained elevated throughout the experiment. Ascites volume determined by an indicator dilution technique steadily increased from 78 ± 24 ml at the time of morphine administration to 276 ± 44 ml (P < 0.001) by the 60th minute following morphine. A mass balance calculated on the protein concentration of the ascites fluid suggested that the ascites produced by morphine resulted from the filtration of pure plasma. We conclude that morphine-induced decreases in extracorporeal reservoir volume at constant blood flow and vena cava pressure can be accounted for largely by blood trapped in the liver by constriction of the hepatic outflow vessels and plasma filtered at the liver sinusoids producing ascites.

IN THE prior paper,¹ we demonstrated that, at constant blood flows and vena cava pressures, morphine induces a translocation of a significant quantity of blood from an extracorporeal reservoir to the systemic circulation of the dog. When we analyzed every factor that we could in terms of our model, we were forced to conclude that the translocated volume was related to an increase in the unstressed vascular volume and/or an increase in volume trapped in the splanchnic system by an elevated effective splanchnic back pressure. Our method of assessing the effective splanchnic back pressure did not allow us to measure a sufficiently elevated back pressure to account for the translocated volume (our method only measured the lowest back pressure, but there could have been an increase in the effective back pressure in parallel channels). Another possible mechanism which could account for the translocated blood volume and which could not be assessed adequately by measuring only changes in mechanical parameters, was fluid filtered from the circulation, especially at the level of the liver, with the resulting formation of ascites. The elevated portal pressure and splanchnic venous resistance produced by morphine strongly suggested that both hepatic trapping of blood and hepatic fluid filtration could be parts of the overall mechanism resulting in the translocated blood volume. We therefore developed the necessary methods which allowed us to assess the actual mechanism of the morphine-induced shift in blood volume between the extracorporeal reservoir and the systemic circulation of the dog under conditions of constant blood flow and vena cava pressures.

Methods

Surgical Procedures

Ten mongrel dogs with a mean weight of 23.6 ± 1.2(SE) kg were anesthetized with sodium thiopental, 18 mg/kg. Anesthesia was maintained with 70% nitrous oxide in oxygen and halothane at an end-expiratory concentration of 0.83%. A tracheotomy was performed and the dog ventilated at a tidal volume and frequency appropriate for its size. A ventral laparotomy and splenectomy were then performed. The abdominal incision was not closed. A ventral thoracotomy was performed and sodium heparin (3 mg/kg) administered. The right atrium was cannulated through the atrial appendage with a wide-bore Tygon tube. Blood returning to the heart, Q, was withdrawn from the right atrium through the Tygon cannula and, after passing through a Starling resistor (Penrose tube),...
was drained by gravity into an extracorporeal reservoir. A Sarns roller pump then pumped the blood around a NaI(Tl) scintillation crystal, through heat exchange unit, and a Carolina Medical square wave electromagnetic flowmeter. The blood returned to the circulation directly through the right ventricular outflow tract (Fig. 1). After the dog was placed on the extracorporeal circuit which had been primed with 1580-2780 ml of donor blood, 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 pulmonary arterial cannula. The Starling resistor was placed at an appropriate level, relative to the right atrium, to maintain right atrial pressure, P, at atmospheric pressure. The pump was set to maintain a flow of 80 ml·min⁻¹·kg⁻¹.

Right atrial pressure was measured with a catheter advanced into the right atrium through the jugular vein. Aortic pressure was measured through a catheter advanced into the aorta through the femoral artery. Volume shifts between the dog and the extracorporeal reservoir were determined by measuring the pressure exerted by the column of blood in the reservoir. Body temperature was measured with a rectal thermistor and maintained at 39°C by the heat exchange unit. Arterial Po2, Pco2, and arterial Po2 were measured at 10-minute intervals from the time the dog was placed on the bypass circuit until morphine was administered. The hematocrits were determined in triplicate immediately, read with a millimeter ruler, and averaged. After the hematocrit determinations, the blood was centrifuged at 5000 rpm for 5 minutes and the supernatant fluid was used for the determination of plasma protein concentration with an AO refractometer (model 10400) and osmolality measurements, using an Advanced Instruments osmometer. The osmolality measurements were made solely to establish the fact that no volume shifts were occurring due to osmotic pressure changes. The treatment of the abdominal fluid is described below.

Because one of the primary objectives of these experiments was to assess whether changes in plasma volume could account, in whole or in part, for the morphine-
induced translocation of blood volume between the extracorporeal reservoir and the systemic circulation under conditions of constant blood flow and vena caval pressures, we developed two independent methods to assess changes in plasma volume, one based on the dilution of a central hematocrit. Since preliminary experiments suggested that ascites was being produced by the morphine, we also developed a nonradioactive dye dilution technique to quantify the formation of ascites. We also took advantage of the radioactive plasma tracer to assess qualitatively changes in the liver volume with an external scintillation probe.

**$^{113m}$In as a Plasma Tracer**

A new England Nuclear generator containing 1 mCi of $^{113m}$Sn on a zirconium oxide column was eluted with 0.05 N HCl and yielded about 700 $\mu$Ci of $^{113m}$In in the first 4 ml of eluate. About 100 ml of venous blood were drawn from the experimental dog into 500 U of sodium heparin and centrifuged immediately at 5000 rpm for 10 minutes. Twelve to 15 ml of plasma were separated from the cells into a 20-ml plastic syringe to which sufficient $^{113m}$InCl$_3$ was added with constant mixing to give about 350 $\mu$Ci of $^{113m}$In-transferrin at the anticipated time of injection.

The chemical form of the injected tracer was tested in several ways. Electrophoresis on a 15-cm cellulose polyacrylate strip for 45 minutes, using 15 mA, 300 V, pH 8.6, NaBarbital (5,5'-diethylbarbituric acid) buffer showed that 100% of the activity migrated with a narrow band coincident with the $\beta$-lipoproteins, one of which is transferrin. No activity was found at the origin, where colloidal indium would remain indefinitely, or at the point where soluble indium complexes would migrate. Ultrafiltration through an Amicon B15 membrane filter confirmed that 100% of the $^{113m}$In-transferrin was bound to proteins of high molecular weight. A sample of $^{113m}$In-transferrin was also applied to a Sephadex G-100 column eluted with 0.15 M NaCl. The column void and bed volumes were 15 ml and 42 ml, respectively, whereas a single $^{113m}$In-labeled peak eluted at 22 ml, coincident with the elution of a standard of $^{59}$Fe-transferrin. We therefore concluded that, by our labeling method, all of the $^{113m}$In was chelated to transferrin and that none of it existed as a soluble inorganic complex or colloidal hydroxide at the time of intravenous injection.

A citrate-phosphate-dextrose (CPD) anticoagulant which is the standard anticoagulant used for the preparation of radiosotopic plasma tracers was also tested for preparing $^{113m}$In-transferrin. The CPD was mixed 15% vol/vol with whole blood but, otherwise, was treated exactly as described above for indium labeling of plasma collected in heparin. Under these conditions, we found that up to 40% of the indium formed a citrate coordination compound and was not available as a plasma protein tracer. The greater thermodynamic stability of $^{113m}$In-transferrin relative to $^{113m}$In-citrate means that, ultimately, all of the $^{113m}$In would be bound to transferrin; however, the process is slow kinetically. Any injected $^{113m}$In-citrate is rapidly excreted via the kidneys and hence lost to the experiment; therefore, CPD was not used for the plasma anticoagulant in the preparation of $^{113m}$In-transferrin.

The radioactivity circulating in the dog was monitored by two 2- x 2-inch NaI(Tl) scintillation crystals. One was surrounded by 110 ml of blood in the extracorporeal circuit (Fig. 1) and the other was positioned over the liver in a right anterior oblique position normal to the midline and centered 3-5 cm below the costal margin. The blood probe was shielded with 4 inches of lead. It was impractical to shield the liver probe with more than a standard 2-inch parallel hole steel collimator with 1-cm walls; however, this probe was pointed away from any major volumes of activity, e.g., the heart or any extracorporeal reservoirs, to minimize its radiation background. The external probe was used to give a qualitative estimate of change in hepatic blood volume.

Both detectors were powered by an Ortec model 446 high-voltage supply operated at 900 V. Their output signals were collected by preamplifiers (Ortec model 276) and fed to linear amplifiers (Ortec model 490A) prior to pulse height analysis. A 60-keV window centered at the 393-keV emission of $^{113m}$In was selected. The analyzer output was scaled by a home-constructed four-channel buffered scalar which drove a teletype (ASR 33). Data were printed at 1-minute intervals, and the teletype-punched paper tape output was analyzed by computer.

The $^{113m}$In-transferrin complex was injected directly into the pulmonary artery at time $t = 0$. Immediately prior to the injection, clamp "a" (Fig. 1) was closed, and a sufficient quantity of untagged blood was pumped from the primary to the secondary reservoir. Clamp "a" then was opened and clamp "b" opened. The mixing pump then mixed, the blood in the primary reservoir for the rest of the experiment. A period of 15-30 minutes was allowed to elapse while control data were taken. Figure 2, which illustrates the $^{113m}$In method of determining total blood volume and changes in plasma volume, is a plot of the natural logarithm of the decay-corrected counts per min-

![Figure 2](http://circres.ahajournals.org/)

**Figure 2** Illustration of $^{113m}$In method of determining total blood volume and changes in plasma volume. $M =$ time of morphine injection; $V_f =$ filtered volume. For details see text.
ute, \(N\), obtained from the extracorporeal scintillation crystal vs. time. These counts have been corrected for radioactive decay of \(^{113}\text{In}\) as follows: \(\ln N = \ln N_0 + 0.006931t\), where \(N_0\) is the observed counts/min, and \(t\) is the minutes after injection of the \(^{113}\text{In}\)-transferrin complex. The constant 0.006931 is \(\ln 2/100\text{ min}^{-1}\), reflecting the 100-minute nuclear decay half-life of \(^{113}\text{In}\).

The total blood volume in the dog plus that in the extracorporeal circuit was determined prior to morphine injection by tracer dilution. A known volume, \(\Delta V\), of untagged blood, equilibrated with the circulating blood volume prior to \(^{113}\text{In}\) administration, was added to the extracorporeal system from the secondary reservoir (by opening clamp “c,” Fig. 1). This diluted the concentration of radioactivity in the blood and thus caused a sharp drop in the concentration measured by the blood probe. The count rate prior to volume addition, \(N_i\), and the count rate after volume addition, \(N_f\), were determined by regression of \(\ln N\) vs. \(t\) before and after the volume addition and extrapolated to the time of volume addition. The total circulating blood volume, \(V\), before the addition of the untagged bolus was calculated as

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

Equation 1 is derived in the Appendix.

In the example illustrated in Figure 2, an untagged bolus of 286 ml was added to the system at minutes 35; this caused the count rate to drop from 474,492 to 438,011 counts/min and yielded a calculated total volume of 4259 ml. This example compares very favorably with the average value of 4247 ± 112 (SEM) ml, obtained from all 10 dogs. When the known volume of the extracorporeal system was subtracted, we obtained a blood volume of 1811 ± 125 ml or 77.8 ± 5.9 ml/kg for the average dog.

To assess the accuracy of this procedure for determining total blood volumes, several successive boluses of untagged volumes were added in a number of dogs. The volume was calculated from the known total blood volume, prior to the addition, \(V_i\), from the corrected count rates before and after the addition of \(N_i\) and \(N_f\) as follows:

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

The calculated volume addition (\(\Delta V\)) was then compared with the known addition. Figure 3 is a plot of calculated volumes vs. known volumes. The points scatter tightly about the line of identity. Even with very small volume additions, the technique yielded excellent agreement because of the high count rates obtained from the extracorporeal scintillation crystal surrounded by 110 ml of blood. Typical count rates were about 500,000 counts/min, yielding a standard deviation for Poisson distribution of 707 counts/min or 0.14%. Since \(N_i\) and \(N_f\) were determined by linear regression of \(\ln N\) vs. \(t\), using a time interval of at least 10 minutes, the error was reduced even further. Thus the major source of error in this system stems not from our ability to observe changes in the concentration of the \(^{113}\text{In}\) tracer but, rather, from our ability to resolve changes in reservoir volume (from which we determined the exact amount of untagged volume addition). This resolution, using Statham Db transducers, is about ±0.75% (error due to nonlinearity and hysteresis, combined). Thus, the resolution of our system is easily of the order of ±1% and could be improved further if \(\Delta V\) were determined gravimetrically.

In every experiment, the slope of the plot of \(\ln N\) vs. \(t\) decreased after morphine administration (Fig. 2). This indicates a decrease in the total volumetric concentration of the \(^{113}\text{In}\)-transferrin. If it is assumed that this decrease results from a loss of whole plasma from the intravascular space and that the total red cell volume remains constant, the total volume of plasma lost at any point in time, \(\Delta V_{\text{in}}\), may be calculated as

\[
\Delta V_{\text{in}} = \frac{\left(1 - \frac{N}{N_0}\right) V_o}{1 - H_o - \frac{N}{N_0}},
\]

where \(N_0\) is the corrected counting rate, \(H_o\) is the hematocrit/100, \(V_o\) the total volume immediately after the volume determination described above, and \(N\) the corrected counting rate at the time for which \(\Delta V_{\text{in}}\) is to be calculated. Justification for the major assumptions of this model will be deferred until the Discussion section. Equation 3 also is derived in the Appendix.

**Volume of Filtered Plasma, Calculated from Hematocrit Data**

If it is assumed that the total volume of red blood cells within the vascular space is constant during the course of any plasma change, it is possible to calculate any decrease in plasma volume, \(\Delta V_{\text{hi}}\), from hematocrit values, as shown in the Appendix.

\[
\Delta V_{\text{hi}} = V_o \left(1 - \frac{H_o}{H}\right).
\]

where \(H\) is the observed hematocrit at the time of interest and \(H_o\) is the hematocrit observed immediately before the administration of morphine. It is important to note that
Equations 3 and 4 present entirely independent observations of the filtered volume. Equation 3 is based on a mass balance for a tagged plasma tracer whereas Equation 4 is based on a red cell volume balance.

**Determination of Ascites Fluid**

To quantify the formation of ascites fluid, a dye dilution technique was employed. A solution of 500 mg of a fluorescent-tagged dextran of molecular weight 150,000 (Pharmacia FITC 150) was dissolved in 500 ml of Ringer's solution. After the dog had become stabilized on bypass, a measured volume of this dye (225 ± 8 ml) was poured directly into the open abdominal cavity. The solution then was 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 the then sealed in glass vials for later analysis of protein dye content. To detect bleeding, hematocrits were also determined on the abdominal fluids. In none of the dogs reported in this paper was an abdominal hematocrit value of greater than 3% observed.

On completion of the experiment, the abdominal samples were analyzed for protein concentration, using a refractometer (AO model 10400), and a portion was diluted 100-fold with saline solution and analyzed for dye concentration, using a Farrand Optical Mark I spectrofluorometer 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. The response of the spectrofluorometer was found to be extremely linear at the dilutions employed here. We estimate total concentration errors to be less than 2%, mostly due to a slight electronic drift in the spectrofluorometer. Although we have no direct measure of the thoroughness of mixing in the abdominal cavity, the smooth trend of the data strongly indicates that mixing was nearly complete.

Total ascites production was calculated from the time the dye solution was poured into the abdomen. If the added dye solution had volume, $V_o$, and concentration, $C_o$, then the volume of ascites formed at any point in time, $\Delta V_{as}$, is given by $\Delta V_{as} = [(V_oC_o)/C] - V_o$, where $C$ is the dye concentration at the time ascites volume is to be determined. Substituting the relationship $C_o = M_o/V_o$, where $M_o$ is the mass of dye yields,

$$\Delta V_{as} = \frac{M_o}{C} - V_o. \tag{5}$$

Because of sample withdrawal, $M_o$ decreases with each sample. This loss is calculated by multiplying the sample concentration by the sample volume.

**Results**

The means and standard errors of the means for the variables measured in these experiments are presented in Figure 4 as functions of time after administration of morphine. Statistical significance was determined by the one-tail $t$-test for unpaired variants unless otherwise specified (9 df).

**Blood Measurements**

Morphine produced a drop in arterial pressure from 99 ± 5 to 59 ± 6 mm Hg ($P < 0.001$) by the 5th minute; pressure then rose slowly to 80% of control in 60 minutes. Reservoir volume fell by 578 ± 86 ml by the 15th minute and remained essentially unchanged until 45 minutes, at which time there was a gradual decrease that was associated with bleeding (see Discussion). Central hematocrit increased from 0.41 ± 0.02 to 0.47 ± 0.01 ($P < 0.01$) by the 15th minute, then remained unchanged throughout the rest of the experiment. Filtered volume (decrease in plasma volume) calculated from the $^{113}$In tracer ($\Delta V_{in}$) was 317 ± 32 ml by the 15th minute and increased slightly.

![Figure 4](http://circres.ahajournals.org/)

**Figure 4** Means and standard errors of the means for the variables measured as a function of time following the administration of morphine. $H$ and $In$ refer to filtered volumes calculated by the hematocrit and indium methods, respectively.
to 378 ± 59 ml (P < 0.3) in 60 minutes. Filtered volume calculated by the hematocrit method (ΔV_H) was 349 ± 58 ml by the 15th minute and rose to 415 ± 61 ml (P < 0.4) in 60 minutes. Both methods of calculating filtered volume gave values that were not statistically different from each other (t-test of paired variants). This conclusion was reinforced by a linear regression of ΔV_H against ΔV_m, pairing values at the appropriate time interval after morphine, and yielding a regression equation of ΔV_H = 12.5 + 0.905 ΔV_m, R = 0.893. Thus by the 15th minute after morphine was administered, approximately 58% of the decreased in reservoir volume could be accounted for by fluid lost from the circulatory system. The protein concentration of the blood remained essentially unchanged from its control value of 5.2 ± 0.2 g/100 ml until 30 minutes (P < 0.7). In 60 minutes, the protein concentration of the blood had increased slightly to 5.6 ± 0.1 g/100 ml (P < 0.1).

Change in Liver Volume

The change in the count rate (corrected for radioactive decay) recorded from the external collimated scintillation crystal placed over the liver was 22 ± 7% 5 minutes after the administration of morphine, increased to 28 ± 9% in 15 minutes, and remained essentially unchanged for the rest of the experiment (Fig. 4). This suggests that the filtered volume was preceded by an immediate and rapid increase in liver volume. This is illustrated further in Figure 5, which presents the time course of the change in liver counts and filtered volume as a percent of their 30-minute values.

Ascites Measurement

Ascites volume steadily increased from 78 ± 24 ml at the time of morphine administration to 276 ± 44 ml (P < 0.001) in 60 minutes for a total accumulation of 198 ± 50 ml. The protein concentration of the abdominal fluid (instilled dye plus ascites) increased rapidly during the first 5 minutes following morphine administration from 1.1 ± 0.2 to 2.0 ± 0.2 g/100 ml (P < 0.01), then slowly and steadily increased to 2.7 ± 0.2 g/100 ml in 60 minutes. Thus, during the course of the experiment, morphine increased the protein concentration of the abdominal fluid by 1.6 ± 0.3 g/100 ml.

Ascites Protein Mass Balance

To determine the nature of the fluid producing the ascites, a protein mass balance was calculated as follows. At the time of morphine injection, there was, on the average, 303 ml of abdominal fluid consisting of 78 ml of ascites and 225 ml of instilled fluorescent dye. This abdominal fluid had a protein concentration of 1.1 g/100 ml. In 60 minutes, an additional 198 ml of ascites had been produced, and the protein concentration of the abdominal fluid was 2.7 g/100 ml. Thus, 303 (1.1) + 198 X = 501 (2.7) where X is the protein concentration of the produced ascites. Solution of this equation yielded a value of 5.15 g/100 ml for the value of X. Since this is nearly identical to the observed control plasma protein concentration and since the 99% confidence interval for the blood protein concentration was 4.55 to 5.85 g/100 ml, we conclude that the 198 ml of ascites produced by the morphine resulted from the filtration of essentially pure plasma.

Discussion

The plasma tracer selected for these experiments was 113mIn-transferrin prepared in vitro. It was selected over the more traditional plasma marker, 125I-albumin, because of the lower radiation exposure to the investigator per detectable gamma emission, the increased radiation safety associated with shorter-lived nuclides, the availability of a 113mSn/113mIn generator for our use, and the simplicity with which 113mIn-transferrin can be prepared. The safe radiation practice associated with the 100-minute half-life of this nuclide was particularly advantageous to us because of the complexity of our preparation, with its potential for serious contamination.

Indium binds to transferrin as well as iron; however, 113mIn-transferrin remains in the circulation and is not removed rapidly for erythrocyte production as is tracer iron from 58Fe-transferrin.2 The clearance of 113mIn-transferrin has been compared with 125I-albumin in dogs and rabbits.3 The plasma clearance curves of the two tracers were parallel; however, the zero-time intercept or initial volume of distribution was 32-35% greater for 113mIn-transferrin than for 125I-albumin. This probably reflected the presence of colloidal hydroxide in 113mIn-transferrin labeled by direct intravenous injection of acidic 113mInCl3. Evidence presented earlier demonstrated that all of our injected 113mIn was bound to protein and is, therefore, a valid marker for plasma volume.

The major consumptions underlying both methods of measuring changes in plasma volume were (1) that all filtered volume was pure, unfractionated plasma and (2) that the total circulating volume of red blood cells remained constant. Justification for these assumptions rests mainly with the results of the experiments themselves. The decrease in indium concentration (counts/min) which occurred after morphine administration could have occurred by either plasma loss or water absorption. If water absorption were the cause, then the hematocrit value

![Figure 5](#)
would fall rather than rise as it did. This alone is strong evidence to support the assumption that the filtered volume was plasma. This assumption is strengthened considerably by the protein mass balance calculated on the protein concentration of the abdominal fluid. This leaves no doubt that the ascites was produced from a fluid of protein concentration equal to that of plasma, i.e., plasma itself. In addition, the constancy of the plasma proteins suggests that it was plasma that was lost.

The assumption of a constant red cell volume is harder to document, at least for the first 45 minutes of the experiment. Measurements of osmotic pressure and plasma protein concentration were constant throughout this experimental period in all dogs. Thus, red cell volume changes due to osmotic or oncotic pressure changes seem unlikely. Further, there was no evidence of bleeding during this period which would result in a loss of red cell volume. During the last 15 minutes of the experiment, there was slight bleeding. Thus, during this period, the total circulating red cell mass inevitably decreased and this resulted in a slight overestimation of the calculated filtered volumes. However, the major conclusions drawn from this study rest on data obtained during the first 30 minutes. Although it may not be of any consequence as far as this study is concerned, it is nevertheless of academic interest why bleeding commenced 45 minutes after morphine administration (or approximately 1 hour and 45 minutes from the start of bypass). The bleeding that we observed is not unlike the altered hemostasis observed in patients following cardiopulmonary bypass. Although the pathophysiology of the altered hemostasis created by cardiopulmonary bypass is not fully understood, there appear to be at least two primary causes: (1) a functional platelet defect of unclear etiology and (2) a hyperfibrinolytic defect.

We suspect that the bleeding that we observed was produced by the same or similar mechanisms induced by the extracorporeal bypass circuit.

The results reported herein are consistent with those reported in the preceding paper and more accurately define the mechanism responsible for the morphine-induced translocation of blood volume which occurs between the extracorporeal reservoir and the systemic circulation of a dog under conditions of constant blood flow and vena caval pressures. When analyzed purely on the basis of the two-compartment model of the systemic circulation, which considers only mechanical parameters, the mechanism for the translocated blood volume produced by morphine appeared to be an increase in volume trapped by the effective splanchic back pressure and/or an increase in the unstressed vascular volume. The current study demonstrates that at least 58% of the translocated volume could be accounted for by plasma being filtered from the circulatory system at the liver and eventually finding its way into the abdominal cavity as ascites. The rapid change in the liver volume, which increased 30% by the 15th minute following morphine administration, suggests that the remaining volume may be accounted for by volume trapped in the liver by a constriction of the hepatic outflow vessels.

Our conclusion that the ascites resulted from the hepatic filtration of essentially pure plasma is in keeping with similar conclusions of others. Greenway and Lautt presented evidence that the hepatic sinusoidal wall is permeable to substances of high molecular weight (i.e., plasma proteins) and suggested that the reflection coefficient of the sinusoidal wall to plasma proteins is nearly zero and, thus, plays no role in hepatic trans sinusoidal fluid exchange. Brauer et al. also presented similar evidence.

Although we can account for the morphine-induced translocation of blood volume from the extracorporeal reservoir to the systemic circulation in the dog largely by hepatic trapping of blood volume and fluid filtration, we do not imply that there was no change in the true unstressed vascular volume. Blood volume at zero transmural pressure can be properly called the unstressed vascular volume. However, there are several problems in assessing changes in the unstressed vascular volume. First, there is the uncertainty that stems from assessing changes in any extrapolated parameter. Second, and probably most important, unless one is absolutely certain that the effective splanchnic back pressure is zero for all parallel splanchnic channels (a situation most certainly impossible) there is no way to separate changes in the volume trapped in the splanchnic system from changes in the true unstressed vascular volume. Indeed it is entirely conceivable to us that changes in the true unstressed vascular volume in the dog do not occur and that hepatic trapping of blood volume, therefore, represents an apparent change in unstressed vascular volume. Furthermore, as this study demonstrates, a volume which is injected into the intravascular space but eventually is filtered from the circulatory system also can appear as an apparent change in unstressed vascular volume.

In summary, we have studied the mechanism of the morphine-induced shift in the blood volume-flow curve of the systemic circulation of the dog and the shift in blood volume which occurs between its extracorporeal reservoir and systemic circulation under conditions of constant blood flow and vena caval pressure. Our results suggest that these effects are largely caused by (1) volume trapped in the liver by constriction of the hepatic outflow vessels and (2) fluid lost from the circulation at the liver by the filtration of plasma and the production of ascites.

Appendix

In the following, all count rates are assumed to be corrected for radioactive decay.

I. Determination of total vascular volume by dilution of a radioactive tracer.

Since the total number of radioactive particles per unit of blood volume at any instant of time is directly proportional to the count rate per unit volume, a radioactive particle mass balance may be determined with the substitution of count rates per unit volume for particle concentration. Thus, the total count rate for blood can be calculated as the product of the total blood volume, \( V \), and the count rate per unit of volume seen by the scintillation crystal, \( \gamma \). The total count rate is then \( V(N_t - N_t) \), where \( N_t \) is the initial count rate. After the addition of a given volume of untagged blood, \( \Delta V \), the count rate will decrease from \( N_t \) to \( N_t \), the final count rate. Since the total count rate (reflecting the number of radioactive
particles) remains unchanged, we can equate the initial and final rates as follows: \( V(N_i/V_s) = (V + \Delta V)(N_f/V_s) \). Solving for \( V \) yields,
\[
V = \Delta V \cdot \frac{N_f}{N_i - N_f}.
\] (1)

II. Determination of plasma filtration from decrease in \(^{113m}\text{In} \) count rates.

The assumptions involved in this calculation are (1) that all filtered volume is pure, unfractionated plasma and (2) that the total circulating volume of red blood cells remains constant at the value obtained immediately prior to morphine administration. Justification for these assumptions may be found in the Discussion. The count rate prior to morphine, \( N_0 \), is proportional to the plasma fraction, \( V_{P,o}/V_o \), where \( V_{P,o} \) and \( V_o \) represent the plasma and total blood volumes prior to morphine. Thus,
\[
N_0 = V_{P,o}/V_o = 1 - H_o,
\]
where \( H_o \) is the hematocrit prior to morphine. Similarly, the count rate at some point during the morphine-induced filtration, \( N \), is proportional to the then existing plasma fraction \( V_{P,\Delta}/V \). Therefore,
\[
\frac{N}{N_0} = \frac{V_{P,\Delta}/V}{V_{P,o}/V_o}.
\]

Since both the plasma and total volumes are diminished by the amount of plasma filtered, \( \Delta V_{in} \), this may be written as
\[
\frac{N}{N_0} = \frac{(V_{P,o} - \Delta V_{in})/(V_o - \Delta V_{in})}{V_{P,o}/V_o}.
\]

Solving for \( \Delta V_{in} \) yields
\[
\Delta V_{in} = \left(1 - \frac{N}{N_0}\right)\frac{V_o}{V_{P,o}} - \frac{N}{V_{P,o} - N_0}.
\]

Since \( V_{P,o}/V_o = 1 - H_o \), the above equation becomes
\[
\Delta V_{in} = \frac{V_o}{1 - H_o} \frac{1 - N}{N_0}.
\] (3)

III. Determination of plasma filtration from increase in hematocrit.

Using the same assumptions described above, the volume of red cells present before morphine is given by \( V_o H_o \), and that after morphine, by \( V_H \). Equating these two volumes since red cell volume is conserved, yields \( V_o H_o = V_H \). \( V \) may be replaced by \( V_{P,\Delta} - \Delta V_{in} \), where \( \Delta V_{in} \) is the volume filtered (as determined from hematocrit data). Therefore, \( V_o H_o = (V_o - \Delta V_{in})H \), and solving for \( \Delta V_{in} \) yields
\[
\Delta V_{in} = V_o \left(1 - \frac{H}{H_o}\right).
\] (4)

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References

2. McIntyre PM: Comparison of the metabolism of iron-labeled transferrin (Fe-TF) and indium-labeled transferrin (In-TF) by the erythropoietic marrow. J Nucl Med 15: 856-862, 1974
Mechanism of morphine-induced shifts in blood volume between extracorporeal reservoir and the systemic circulation of the dog under conditions of constant blood flow and vena caval pressures.
J F Green, A P Jackman and K A Krohn

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