Estimation of Venous Blood Volume in the Dog by the Indicator-Dilution Method

By W. R. Milnor, M.D. and C. A. Bertrand, M.D.

The indicator-dilution method of determining cardiac output and mean transit times can be employed to estimate indirectly the volume of blood in the larger veins in vivo although it is difficult to define precisely the peripheral anatomic boundaries of the volume so measured. In 12 anesthetized dogs the "venous" blood volume obtained by multiplying cardiac output and mean transit time from femoral vein to right atrium averaged 16.6 ml./Kg., or 18 per cent of total blood volume. Rapid intravenous infusion of dextran solution caused no significant change in the blood volume of the large veins, heart or lungs.

The increased venous pressure and obvious distention of the superficial veins observed in clinical congestive heart failure in man have long suggested that the volume of blood in this part of the circulation must be larger than normal. There is, however, no direct evidence to show whether the volume of the systemic veins is actually increased in this condition, and whether such an increase is a passive concomitant of increased total blood volume, or is accomplished by active redistribution of blood.

The Stewart-Hamilton method of estimating the volume of a vascular compartment by the use of indicator-dilution curves offers one possible approach to these questions. It seems reasonable to assume that the mean transit time from peripheral vein to right heart is primarily a function of venous blood volume and cardiac output, and that streamlining of flow in the great veins does not invalidate the use of the Stewart-Hamilton formulas so long as mixing is accomplished by the heart and lungs before the dilution curve is measured. We have therefore undertaken to make such measurements in the dog, using T-1824 dilution curves to measure cardiac output and mean transit time from a peripheral vein to the right atrium. This report describes our findings in normal, closed-chest, anesthetized dogs, and the effects of acute hypervolemia brought about by intravenous infusion of dextran solution, as steps preliminary to an examination of the changes accompanying heart failure.

Methods

Mongrel dogs (6.8 to 18.6 Kg.) were premedicated with morphine (approximately 2.0 mg./Kg.) and subsequently anesthetized with intravenous doses of either pentobarbital sodium (50 to 40 mg./Kg.) or chloralose (30 to 100 mg./Kg.). T-1824 dye was used in a concentration of 1.5 to 4 mg./ml., and a total of 0.21 to 0.46 mg./Kg. was used for each injection. Dye was injected into the right atrium through a no. 6 cardiac catheter passed down the superior vena cava. The position of the catheter tip was checked by fluoroscopy, pressure recordings, and in several instances by autopsy at the end of experiment. Dye was rapidly injected into the femoral vein approximately 10 cm. distal to the caval bifurcation, through a No. 20 gage needle. The volume of the injection needles and catheters were calibrated, and the amount of dye injected was measured by weighing of syringes before and after injection.

Blood concentration of T-1824 was measured by a photodensitometer previously described, applied to a loop of polyethylene tubing (inside diameter 2.6 mm.) inserted in one carotid artery in such a way that the blood returned to the distal carotid artery after flowing through the densitometer cuvette. To discourage clotting, the lumen of the tubing was coated with silicone (General Electric silicone oil SF-96-100) and the animal was heparinized. The densitometer measurements were continuously recorded on a Sanborn Polyvigo recorder, and calibration factors for each dilution curve were obtained from blood samples.
drawn from a femoral artery. T-1824 concentration in the serum of the calibrating samples was measured in a Beckman model DU spectrophotometer.

Cardiac output, mean transit time, and dilution volumes were calculated by conventional procedures.1

To determine the mean transit time from femoral vein to right atrium one injection was made into the femoral vein, and a second injection 3 min. later into the right atrium. The mean transit times of the two resulting arterial concentration curves were calculated, and the mean transit time from vein to right atrium determined by subtracting the right atrium-to-carotid artery time from the vein-to-carotid artery time.

The volume obtained by multiplying cardiac output per second and mean transit time from right atrium to carotid artery loop, in seconds, was termed the “cardiopulmonary blood volume;” the product of cardiac output and femoral-vein-to-right-atrium mean transit time was termed “venous blood volume.” It is to be emphasized that the anatomic boundaries implied by these terms are far from exact.

Total plasma volume was calculated from the amount of dye injected and the serum dye concentration 10 minutes after injection, minus the concentration immediately before injection. Values for whole blood were calculated from plasma values and the arterial hematocrit readings, and are therefore subject to the error introduced by differences between arterial and “total body” hematocrit.

Hematocrit readings were measured on samples taken at intervals throughout the experiment, in Wintrobe tubes spun for 30 min. at 3000 r.p.m. in a centrifuge with a radius of 12.5 cm. No correction was applied for trapped plasma.

In 5 animals 6 per cent dextran solution (Plavolex) was administered intravenously after a control period of 50 to 90 min. to determine the effects of increasing total plasma volume. From 430 to 880 ml. of dextran solution, equivalent to 105 per cent of the control plasma volume, were given at a rate of 30 to 69 ml./min. The experiments were continued from 40 to 120 min. after the infusion of dextran, and data for calculation of cardiac output, "venous," "cardiopulmonary" and total blood volume were obtained at least twice during this period.

**RESULTS**

The data obtained are listed in table 1. Control values given in this table are averages of 2 or more measurements over a period of at least 30 min. in each animal. Individual transit time measurements during the control period varied less than ±13 per cent from this average value. Determinations after dextran were made 15 to 80 min. after the end

**Table 1.—Summary of Experimental Data**

<table>
<thead>
<tr>
<th>Dog no.</th>
<th>Weight (Kg.)</th>
<th>Cardiac output (ml./sec.)</th>
<th>Mean transit times (sec.)</th>
<th>Blood volumes (ml./Kg.)</th>
<th>Vol. dextran infused (ml.)</th>
<th>Cardiac output (ml./sec.)</th>
<th>Mean transit times (sec.)</th>
<th>Blood volumes (ml./Kg.)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>FV-RA RA-CA</td>
<td>FV-RA RA-CA Total</td>
<td></td>
<td>FV-RA RA-CA</td>
<td>FV-RA RA-CA Total</td>
<td></td>
<td></td>
</tr>
<tr>
<td>B15</td>
<td>9.1</td>
<td>26.2</td>
<td>3.37 7.29</td>
<td>9.7 21.0 81</td>
<td>470</td>
<td>52.0 1.35 3.91</td>
<td>7.7 22.3 132</td>
<td></td>
</tr>
<tr>
<td>B16</td>
<td>11.4</td>
<td>34.3</td>
<td>3.16 8.97</td>
<td>9.5 27.0 86</td>
<td>500</td>
<td>65.6 1.70 5.61</td>
<td>9.8 32.3 147</td>
<td></td>
</tr>
<tr>
<td>B19</td>
<td>6.8</td>
<td>29.0</td>
<td>3.99 7.97</td>
<td>17.0 34.0 104</td>
<td>430</td>
<td>49.1 2.46 4.29</td>
<td>17.8 31.0 171</td>
<td></td>
</tr>
<tr>
<td>B21</td>
<td>12.7</td>
<td>42.2</td>
<td>4.61 9.34</td>
<td>15.3 31.0 88</td>
<td>880</td>
<td>76.2 1.90 4.16</td>
<td>11.4 25.0 116</td>
<td></td>
</tr>
<tr>
<td>B26</td>
<td>18.6</td>
<td>62.0</td>
<td>4.76 7.64</td>
<td>15.9 25.4 89</td>
<td>820</td>
<td>112.0 2.66 4.05</td>
<td>16.0 28.0 151</td>
<td></td>
</tr>
<tr>
<td>B2</td>
<td>13.2</td>
<td>35.0</td>
<td>7.20 16.50</td>
<td>19.1 43.7 103</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>B20</td>
<td>16.8</td>
<td>25.8</td>
<td>8.82 14.31</td>
<td>33.5 54.3 103</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>B22</td>
<td>9.1</td>
<td>32.5</td>
<td>3.36 7.95</td>
<td>12.0 28.4 96</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>B31</td>
<td>13.6</td>
<td>15.2</td>
<td>12.30 16.91</td>
<td>13.7 18.9 82</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>B32*</td>
<td>14.1</td>
<td>28.7</td>
<td>7.56 13.08</td>
<td>15.4 20.6 80</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>B33*</td>
<td>15.9</td>
<td>60.5</td>
<td>5.78 8.35</td>
<td>22.0 31.8 103</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>B35*</td>
<td>17.2</td>
<td>73.5</td>
<td>3.88 9.83</td>
<td>16.6 42.0 103</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean</td>
<td>12.4</td>
<td>38.7</td>
<td>5.73 10.68</td>
<td>16.6 32.0 93</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*Anesthetized with chloralose. Remaining 9 animals anesthetized with pentobarbital.

FV, femoral vein; RA, right atrium; CA, carotid artery. All volumes given are for whole blood, calculated from plasma volumes and arterial hematocrit.
of the infusion. The values given in table 1 represent the first postinfusion measurement.

In the control experiments, the average total blood volume was 93.1 ml./Kg.; average venous blood volume was 16.6 ml./Kg., or 18 per cent of the total blood volume (S.D. = ±4.23 ml./Kg.); average cardiopulmonary blood volume was 32.0 ml./Kg., or 34 per cent of total blood volume (S.D. = ±5.06 ml./Kg.). Cardiac output in the control runs varied considerably in different animals depending on the size of the animal, depth of anesthesia, surgical manipulation during insertion of the carotid loop and other factors. The observed blood volumes were not significantly different in animals anesthetized with pentobarbital and those in which chloralose was used, although the latter had slower heart rates. Heart rate was relatively constant in each animal during the control periods, but varied from 82 to 198/min. in different animals. The relative constancy of the measurements during control periods under pentobarbital anesthesia was somewhat surprising, in view of reports by Etsten and Li, and by Johnson that the intrathoracic blood volume decreases progressively under barbiturate anesthesia. The reported changes always accompanied decreases in cardiac output, however, which did not occur in our animals. Comparison of anesthetized and unanesthetized animals was not carried out in these experiments.

Infusion of dextran solution increased cardiac output (+69 to +98 per cent) and total blood volume (+31 to +71 per cent) but had no significant effect on the venous or cardiopulmonary blood volume (p > 0.7) (fig. 1). Changes in mean right atrial pressure after the infusion varied from —2 to +8 mm. Hg as compared with control levels. Heart rate was essentially unchanged. No auscultatory or other signs of pulmonary edema appeared during or after the infusion. Plasma volume increased continuously during the postinfusion period in each experiment. The total increase in plasma volume at the end of the experiments was from 40 to 141 per cent of the volume of dextran solution given, suggesting a hypertonic osmotic effect of infused solution in some cases.

**DISCUSSION**

Our finding that the average cardiopulmonary blood volume in the dog is equivalent to 34 per cent of the total blood volume is in agreement with the measurements of Rabino-witz and Rapaport, and of Nahas, Visscher, Mather, Haddy, and Warner.

The limited information available on the volume of the larger veins is compatible with our finding of 18 per cent of total blood volume. On the basis of anatomic measurements Green gives the proportion of blood in veins 6 mm. and more in diameter in a hypothetical 13 Kg. dog as 19.6 per cent of total blood volume.

**Validity of Method.** Physiologic interpretation of volumes determined by the Stewart-Hamilton formula is limited by the difficulty in defining their precise anatomic boundaries. The volume measured by this technic includes all the blood which contributes to the dilution of dye from the point of injection to the point of sampling, including all points in the vascular tree which are equidistant in time with the injection and collection sites. In other
words, the observed mean transit time of 5.7 sec. from femoral vein to right atrium implies that the calculated venous blood volume extends out the venous tree to include all points 5.7 sec. "travelling time" from the right atrium.

Injections at multiple venous sites suggest that this region probably includes most of the primary and secondary branches of the venae cavae. In 3 dogs the mean transit time from a foreleg vein of approximately 2.0 mm. diameter to the carotid artery was from 104 to 114 per cent of the mean transit time from a site in the femoral vein of the same diameter to the carotid. In 2 experiments the mean transit time from splenic vein to carotid artery averaged 185 per cent of the femoral vein-to-carotid time, indicating that little if any of the portal circulation is included in the "venous blood volume" measured by the method described. In a relatively short circulatory pathway such as the coronary circulation, on the other hand, the calculated "venous blood volume" probably includes coronary arteries and capillaries as well as veins.

Since the volume of the entire venous side of the systemic circuit including all venous channels beyond the capillaries is approximately 65 per cent of the total blood volume, it is clear that the volume determined by the present method includes only a part of the vascular bed which is subject to veno-motor activity. Moreover, the boundaries of the measured volume could be changed by veno-motor activity in one part of the bed if such activity altered the local flow-volume relationship.

Another theoretic objection to this method arises from the presence of laminar flow in the veins. Fortunately, as Dow points out, local streamlining does not invalidate flow and volume measurements by this method provided mixing is accomplished at some point upstream of the sampling site, and presumably all of the venous inflow is eventually mixed completely by passage through the heart and lungs.

Effects of Dextran Infusion. Since the increase in cardiac output was accompanied by a proportionate decrease in "venous" and "cardio-pulmonary" mean transit time (table 1) it follows that the volume of these beds did not change significantly. The increment in total plasma volume appears to be accommodated in other parts of the vascular system, presumably the smaller vessels.

These results differ from those of Doyle, Wilson, Estes, and Warren who reported that the pulmonary blood volume increased proportionately with the total blood volume after saline infusion in normal humans. Also, Witham, Fleming, and Bloom, using pulmonary artery injections, found that the pulmonary blood volume increased proportionately more than the total blood volume after dextran infusion in 3 of 5 patients studied. These discrepancies may reflect an inherent difference between dog and man in their response to acute hypervolemia.

SUMMARY
The dye dilution method was used to measure cardiac output and mean transit time between femoral vein, right atrium, and carotid artery in 12 intact anesthetized dogs. The "venous blood volume," defined as the volume calculated by the Stewart-Hamilton formula using the mean transit time from femoral vein to right atrium, averaged 16.6 ml. of blood/Kg. body weight, or 18 per cent of total blood volume, and presumably approximates the volume of the large veins. "Cardio-pulmonary blood volume," calculated from cardiac output and the right atrium-to-carotid artery mean transit time, averaged 32.0 ml./Kg., or 34 per cent of total blood volume.

Rapid intravenous infusion of 6 per cent dextran solution in amounts sufficient to increase the total blood volume 31 per cent to 71 per cent produced no significant change in venous or cardio-pulmonary blood volumes, suggesting that the increment in total blood volume was accommodated in the smaller systemic vessels.

ACKNOWLEDGMENT
The technical assistance of Mrs. Joanne Chester in these experiments is gratefully acknowledged.
We are indebted to the Warner-Chilcott Laboratories, New York, for supplies of T-1824, and to Eli Lilly Co., Indianapolis, for the heparin sodium used.

**Summario in Interlingua**

Le metodo a dilution de colorante esseva usate pro mesurar le rendimento cardiac e le tempore medie de transito inter vena femoral, atrio dextere, e arteria carotic in 12 intacte canes anesthesiate. Le "volumine de sanguine venose," definite como le volumine calculate per le formula de Stewart-Hamilton super le base del tempore medie de transito inter vena femoral e atrio dextere, amontava a un valor medie de 16,6 ml de sanguine per kg de peso corporeo o de 18 pro cento del volumine total de sanguine. Il es probable que iste valor representa approximativemente le volumine del grande venas. Le "volumine cardio-pulmonar de sanguine," calculate super le base del rendimento cardiac e le tempore medie de transito inter le atrio dextere e le arteria carotic, amontava a un valor medie de 32,0 ml per kg de peso corporeo o de 34 pro cento del volumine total de sanguine.

Le rapide infusion intravenose de un solution de 6 pro cento de dextrano in quantitates sufficiente pro augmentar le volumine total de sanguine per 31 a 71 pro cento produceva nullle significative alteration in le volumines venose e cardio-pulmonar de sanguine. Isto pare indicar que le augmento del volumine total de sanguine esseva acceptate per le minor vasos systemic.

**REFERENCES**


Estimation of Venous Blood Volume in the Dog by the Indicator-Dilution Method

W. R. MILNOR and C. A. BÉRTRAND

doi: 10.1161/01.RES.6.1.55

*Circulation Research* is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 1958 American Heart Association, Inc. All rights reserved.
Print ISSN: 0009-7330. Online ISSN: 1524-4571

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://circres.ahajournals.org/content/6/1/55

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in *Circulation Research* can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to *Circulation Research* is online at:
http://circres.ahajournals.org/subscriptions/