Redistribution of Cardiac Output during Hemorrhage in the Unanesthetized Monkey

By Ralph P. Forsyth, Ph.D., Barry I. Hoffbrand, B.M., M.R.C.P., and Kenneth L. Melmon, M.D.

ABSTRACT
Regional flow measurements were made, using the Rudolph and Heymann microsphere technique, before and after 10, 30, and 50% of the previously measured blood volume was withdrawn from five unanesthetized rhesus monkeys restrained in horizontally tilted primate chairs. Measurements at similar time intervals were also made in seven control monkeys. Systemic arterial and central venous pressures, cardiac output, stroke volume, and hematocrit decreased progressively at each level of bleeding. Heart rate (until severe hemorrhage), respiratory rate, and blood levels of α-glucosidase rose; bradykinin levels in arterial blood were unchanged. The fraction of cardiac output was found to be progressively increased to brain, heart, adrenal gland, and hepatic artery vascular bed at the expense of skin, spleen, and pancreas. The hepatic artery vascular bed was the only one that showed significant vasodilatation at either 30 or 50% hemorrhage.

During acute endotoxin shock in monkeys, we have previously found a similar fall in systemic arterial pressure due to a decrease in total peripheral resistance and a different pattern of regional blood flow changes. The lack of bradykinin production, the integrity of cardioregulatory mechanisms, and the fall of hematocrit levels found during hemorrhage may help account for some of the hemodynamic differences between these two types of shock.

ADDITIONAL KEY WORDS microspheres organ blood flow peripheral vascular resistance sympathetic nervous system blood pressure cardiac output α-glucosidase bradykinin endotoxin shock

Little information is available in primates on regional changes in blood flow during graded hemorrhage. Most work has been carried out on rabbits, rats or dogs, often with the complications of anesthesia or narcotics and acute surgical trauma. Despite these problems and the variety of experimental procedures used, it is generally agreed that the heart and the brain are favored in the redistribution of cardiac output during hemorrhage at the expense of skin, muscle, and splanchnic organs; there is less agreement regarding other vascular beds (1-3).

Sapirstein et al. (4), using the 85Rb and antipyrine techniques in unanesthetized rats, were the first to report circulatory changes due to hemorrhage in a variety of regional organs. The 85Rb method has the advantages that only one measurement can be made and tissue uptake of this isotope is not solely dependent on blood flow, the extraction ratio changing in varying conditions (5, 6). In contrast, radioactively labeled microspheres, 50μ in diameter, have an extraction ratio of 1.0 in major organs and can be used to measure the simultaneous distribution of cardiac output in many organs at several different times (7). This labeled-microsphere method has been used to evaluate regional.

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flow and resistance changes during hemorrhage in the rabbit (8) and the dog (9).

We have adapted the Rudolph and Heymann microsphere method to study the effects of three different levels of bleeding in the unanesthetized monkey free from the influences of recent surgery. Further, we compare these changes with those found with the same technique in monkeys during endotoxin shock (10, 11).

Materials and Methods

Twelve male rhesus monkeys (Macaca mulatta) weighing between 3.5 and 6.8 kg were studied. Using pentobarbital anesthesia (30 mg/kg), we placed sterile polyvinyl catheters into the inferior vena cava and abdominal aorta and the left external iliac vessels and into the left ventricle through the left common carotid artery. The animals were then transferred to primate restraining chairs which were modified to allow tilting inside isolation booths. Details of this preparation and the microsphere method for regional blood flow measurements have been previously reported (12, 13).

Seven to eight days following the operation, circulating blood volume was measured with the monkey tilted to a horizontal position in his chair, using T1834 dye and the large vessel hematocrit rather than the total body hematocrit (14). The experiments were performed 1 to 3 days later, the animal having been similarly tilted 1 to 2 hours previously.

The five experimental monkeys, whose mean weight was 4.4 kg and mean circulating blood volume was 95 ml/kg (± 15 so), had similar protocols. After the cardiac output was measured, the first injection of microspheres was made; 10% of the previously measured circulating blood volume was then withdrawn from the arterial catheter by a Harvard pump in from 9 to 27 minutes (mean = 15 minutes). The first 10 ml of blood withdrawn in each case was used for blood assays. Following stabilization of the arterial pressure (from 5 to 10 minutes), the cardiac output was again measured and the second injection of microspheres was made. After this, a further 20% of the original blood volume was withdrawn in from 34 to 65 minutes (mean = 44 minutes) and the cardiac output and the third injection of microspheres was made. Another 20% of the original blood volume was withdrawn in a further 38 to 50 minutes (mean = 47 minutes) before the final cardiac output and the fourth injection of microspheres. A final 10-ml blood sample was then taken for measurements of the hematological and biochemical changes at 50% loss of the original blood volume.

The same hemodynamic and biochemical measurements were made in seven tilted controls (weighing on the average 4.4 kg, mean blood volume = 87 ml/kg (± 8) at similar time intervals with each of the bled monkeys.

Arterial (Pa), central venous (Pv), and left ventricular end-diastolic pressures (LVEDP) were measured during each experiment with Statham P533Cb strain gauges mounted at heart level. Heart rate was continuously recorded with a cardiotachometer coupled. Cardiac output (CO) was determined by the method of Stewart and Hamilton using left ventricular injection of indocyanine green dye and a Waters x302 densitometer. Arterial blood (usually 10 ml) was withdrawn from the aortic catheter at a rate of 10 ml/min through a 75 cm length of tubing with an internal volume of approximately 1 ml, and was returned after the dye curve was obtained. Each value was the mean of at least two dye-dilution curves. All these measurements were recorded on a Type R Beckman recorder. Total peripheral resistance was calculated as Pa-Pv/(liter . min⁻¹ . kg⁻¹). Respiratory rate was counted from the respiratory variations in the venous pressure waves.

Arterial blood samples were obtained at standard times throughout each experiment. Blood PaO₂, PaCO₂ and pH (corrected to 39°C) were measured with a Radiometer microelectrode unit using a glass/calomel electrode for pH, a Severinghaus type electrode for PaCO₂ and a Clark type of electrode (Radiometer) for PaO₂ measurement. Arterial blood was used for white blood cell counts and hematocrit determination. Plasma bradykininogen was determined by the method of Dinitz and Carvalho (15) and whole blood concentrations of bradykinin were measured by a major modification of Webster and Gilmore (16), as described by Niss et al. (10). Alpha-glucosidase, a lysosomal enzyme, was measured in 0.2 ml samples of heparinized plasma by the method of Williams (17).

For each measurement of regional blood flow, a batch of 5,000 to 10,000 50μm-diameter plastic microspheres (40 to 70μm with 95% at 50μm diameter, average specific gravity = 1.3) labeled with either 125I, 51Cr, 85Sr, or 90Sr (varying from 0.5 to 1.5 x 10⁶cpm) was injected over a period of 15 to 20 seconds into the left ventricle. These spheres mix well with blood in the left ventricle and travel with the blood until trapped by arterioles of the end organs. The number of spheres and, thus, the amount of radioactivity in each organ or tissue have been shown to be proportional to blood flow to it. Each injection of microspheres blocks only
about 0.1% of the total number of arterioles in the body, and has relatively little effect on the circulatory or biochemical measurements studied (13). The reliability of the measurements is good for those organs that receive more than 1% of the output (13).

At the end of each experiment the animals were killed with pentobarbital and dissected into 23 organs or tissues; the remaining tissue (average of 2 percent of the animal’s weight) was counted so that the total body radioactivity could be calculated. We measured the radioactivity present in all of the tissue in major organs, but only that in representative parts (20 to 30% total weight) of skeletal muscle, chest wall, skull, spine, and limb bone. The tissues to be analyzed were placed in glass vials which were automatically counted for 4 minutes apiece with a Nuclear Chicago Scintillation Counter. The amount of gamma emission from each nuclide for each vial and then each organ was calculated as previously described (11, 12).

The fraction of cardiac output for each organ was calculated as the amount of nuclide radioactivity in that organ divided by the amount of counted radioactivity of that nuclide found in the total body. Blood flow to each organ was the fraction of cardiac output to that organ times the cardiac output measured by dye-dilution. Organ resistance was calculated as (P_a-P_v)/flow (in liters/min).

Changes in the fraction of cardiac output, regional blood flow and resistance in the 23 organs or tissues for each monkey at each of the three bleeding periods were expressed as a percent of the baseline measurement. Mean and median percent changes were calculated for the experimental and control groups. Because of the skewed distribution of many of the regional variables, the regional statistical differences were evaluated with the nonparametric Mann-Whitney U-test (18).

Results

Cardiovascular and Chemical Changes.—The hemodynamic and blood chemistry measurements for the seven control and five bled monkeys are shown in Table I. The control group had a significantly higher initial heart rate compared to the experimental group. After 10% bleeding only the systolic arterial pressure was significantly different from the controls; this was partly due to the slightly lower baseline pressure in the experimental group and the increase in pressure in the control group.

After 30% bleeding, arterial and central venous pressures, cardiac output, stroke volume, and pH were significantly lower in the experimental group, while heart rate and respiratory rate were higher. Hematocrit and plasma bradykininogen levels also fell significantly, indicating the occurrence of hemodilution at this time. Levels of the lysosomal enzyme α-glucosidase rose, probably indicating poor tissue perfusion.

After 50% bleeding, two of the five monkeys were moribund; their heart rate, arterial pressure, cardiac output and total peripheral resistance were all lower than in the other three monkeys. The three nonmoribund monkeys had a significantly higher total peripheral resistance and heart rate than the controls, but the entire group did not show significant changes. There were no significant differences in white blood cell counts at any of the bleeding periods and blood bradykinin levels never increased from near zero values.

Regional Circulatory Changes.—There were no significant differences between the experimental and control groups in the baseline distribution of cardiac output in any of the organs measured. These measurements were similar to the average values in supine monkeys already reported (13) and are therefore not presented.

The mean values of the distribution of cardiac output in the control group during the course of the experiment were remarkably constant, except in those organs receiving less than 1% of the cardiac output. The largest mean percent change of the fraction of cardiac output in the control group in the 15 organs receiving more than 1% of the cardiac output was ±14% (to bone at the third microsphere injection). For those eight organs receiving less than 1% of the cardiac output, the largest change in the control group was a 27% rise (at the fourth microsphere injection to the adrenals); most of the values were in the range of ±15%. These changes have been previously reported for five of the control monkeys (13) and are not repeated in this paper. Changes in the flow and resistance values showed somewhat more variability due
### TABLE 1
Measurements in the Five Experimental and Seven Control Monkeys

<table>
<thead>
<tr>
<th>Variable</th>
<th>1st microsphere injection (baseline)</th>
<th>2nd microsphere injection (10% bleeding)</th>
<th>3rd microsphere injection (40% bleeding)</th>
<th>4th microsphere injection (50% bleeding)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean ± SD</td>
<td>Mean ± SD</td>
<td>Mean ± SD</td>
<td>Mean ± SD</td>
</tr>
<tr>
<td>Systolic arterial pressure (mm Hg)</td>
<td>C 143 ± 18</td>
<td>153 ± 21</td>
<td>158 ± 27</td>
<td>150 ± 29</td>
</tr>
<tr>
<td>Diastolic arterial pressure (mm Hg)</td>
<td>E 133 ± 14</td>
<td>126* ± 15</td>
<td>96* ± 24</td>
<td>46* ± 6</td>
</tr>
<tr>
<td>Mean arterial pressure (mm Hg)</td>
<td>C 84 ± 14</td>
<td>99 ± 15</td>
<td>94 ± 19</td>
<td>90 ± 18</td>
</tr>
<tr>
<td>Heart rate (beats/min)</td>
<td>C 114 ± 10</td>
<td>121 ± 17</td>
<td>124 ± 21</td>
<td>118 ± 21</td>
</tr>
<tr>
<td>Cardiac output (ml·min⁻¹·kg⁻¹ body wt)</td>
<td>C 316 ± 70</td>
<td>301 ± 64</td>
<td>312 ± 65</td>
<td>283 ± 73</td>
</tr>
<tr>
<td>Stroke volume (ml/kg⁻¹ body wt)</td>
<td>C 1.7 ± 0.1</td>
<td>1.6 ± 0.4</td>
<td>1.6 ± 0.3</td>
<td>1.6 ± 0.4</td>
</tr>
<tr>
<td>Total peripheral resistance (mm Hg/cardiac output (in liters·min⁻¹·kg⁻¹))</td>
<td>C 377 ± 101</td>
<td>417 ± 98</td>
<td>409 ± 83</td>
<td>436 ± 105</td>
</tr>
<tr>
<td>Mean central venous pressure (mm Hg)</td>
<td>C 2.4 ± 0.9</td>
<td>2.1 ± 0.9</td>
<td>2.8 ± 1.3</td>
<td>2.4 ± 1.4</td>
</tr>
<tr>
<td>Left ventricular end-diastolic pressure (mm Hg)</td>
<td>E 8.7 ± 1.7</td>
<td>8.9 ± 2.3</td>
<td>10.1 ± 2.4</td>
<td>8.6 ± 2.8</td>
</tr>
<tr>
<td>Respiratory rate (per minute)</td>
<td>E 31 ± 11</td>
<td>39 ± 19</td>
<td>45* ± 11</td>
<td>73* ± 15</td>
</tr>
<tr>
<td>Hematocrit</td>
<td>C 34 ± 4</td>
<td>33 ± 6</td>
<td>32 ± 5</td>
<td>31 ± 4</td>
</tr>
<tr>
<td>Arterial pH (units)</td>
<td>C 7.41 ± 0.07</td>
<td>7.41 ± 0.07</td>
<td>7.43 ± 0.06</td>
<td>7.43 ± 0.03</td>
</tr>
<tr>
<td>Arterial blood (ng/ml)</td>
<td>E 95 ± 11</td>
<td>91 ± 12</td>
<td>90 ± 6</td>
<td>93 ± 8</td>
</tr>
<tr>
<td>FCO₂ (mm Hg)</td>
<td>E 96 ± 7</td>
<td>93 ± 9</td>
<td>100 ± 17</td>
<td>117* ± 9</td>
</tr>
<tr>
<td>Arterial blood bradykinin (ng/ml)</td>
<td>C 42 ± 7</td>
<td>40 ± 8</td>
<td>36 ± 7</td>
<td>27 ± 6</td>
</tr>
<tr>
<td>Arterial blood bradykininogen (% control)</td>
<td>C 100 ± 0</td>
<td>98 ± 16</td>
<td>101 ± 7</td>
<td>99 ± 7</td>
</tr>
<tr>
<td>α-glucosidase (cpm/g protein) (% control)</td>
<td>C 100 ± 0</td>
<td>100 ± 8</td>
<td>100 ± 7</td>
<td>116 ± 16</td>
</tr>
</tbody>
</table>

* = mean, † = SE, ‡ = SEM (SD), ± = standard error
REGIONAL FLOW CHANGES DURING HEMORRHAGE

There were only two significant ($P < 0.05$) changes in the measured organ values of the experimental animals, compared to the controls, after 10% bleeding. The heart received a higher fraction of cardiac output (mean 146% of baseline), while the skin had a lower blood flow (67% of baseline).

The organs with significant changes in the fraction of cardiac output or resistance after 30% bleeding are shown in Table 2. In addition, blood flow fell significantly (from a mean of 43 to 61% of baseline) in kidneys, skeletal muscle, small intestine, large intestine, spine, mesentery, and diaphragm. Blood flow to the remaining six organs (cecum, bronchial artery, limb bone, skull, chest wall, and eyes), as well as the heart, brain, liver, and adrenals were unchanged.

After 50% bleeding (Table 3) 16 of the 23 organs showed significant changes in the fraction of the cardiac output they received. In the seven unlisted organs (stomach, large intestine, cecum, lung (bronchial artery), skull, spine, and chest wall) the blood flow decreased significantly in proportion to the decrease in cardiac output. The changes in distribution of blood flow in the two moribund monkeys were qualitatively similar to those in the other three monkeys and, thus, the data on all five monkeys were grouped together in Table 3.

The absolute mean percents of cardiac output received in eight major organs or tissues of the experimental group at baseline, 30% and 50% bleeding are shown in Figure 1. The absolute changes in blood flow/100 g tissue in some of the organs showing significant differences during bleeding are given in Table 4.

Pathological Changes.—At autopsy, we found no remarkable gross pathological findings. The gastrointestinal tract and other visceral organs were not congested or necrotic.

Behavioral Changes.—Observations of the monkeys during the hemorrhagic period indicated they felt little distress. Although they did not become comatose until about 40 to 50% bleeding, they did not struggle or show other signs of discomfort.

Discussion

One of the advantages of the methods used in this report is the use of primates who have not been exposed to recent anesthesia, surgery, or other traumatic experience. These influences, as well as those hormonal and cardioregulatory alterations caused by anesthesia or trauma, are known to obscure many of the hemodynamic changes caused by hemorrhage (1). However, the labeled-microsphere technique, in contrast to electromag-

C = seven control monkeys; E = five experimental monkeys.

* = differences from controls at $P < 0.05$, t-test.
† = differences from controls at $P < 0.01$, t-test.
$N = 5$ for the control group.

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### TABLE 2

<table>
<thead>
<tr>
<th>Body</th>
<th>Percent cardiac output Mean</th>
<th>Flow Mean</th>
<th>Resistance Mean</th>
</tr>
</thead>
<tbody>
<tr>
<td>(total)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Heart</td>
<td>196*</td>
<td>103</td>
<td>71</td>
</tr>
<tr>
<td>Brain</td>
<td>146†</td>
<td>84</td>
<td>85</td>
</tr>
<tr>
<td>Skin</td>
<td>56*</td>
<td>31*</td>
<td>247*</td>
</tr>
<tr>
<td>Stomach</td>
<td>66</td>
<td>36*</td>
<td>20†</td>
</tr>
<tr>
<td>Spleen</td>
<td>61†</td>
<td>36*</td>
<td>400 (211)</td>
</tr>
<tr>
<td>Pancreas</td>
<td>61†</td>
<td>30*</td>
<td>249*</td>
</tr>
<tr>
<td>Liver (hepatic artery)</td>
<td>302† (198)</td>
<td>135</td>
<td>57†</td>
</tr>
<tr>
<td>Adrenals</td>
<td>223†</td>
<td>112</td>
<td>58</td>
</tr>
<tr>
<td>Lymph nodes</td>
<td>53</td>
<td>30*</td>
<td>292† (219)</td>
</tr>
<tr>
<td>Fat</td>
<td>69†</td>
<td>37*</td>
<td>187</td>
</tr>
</tbody>
</table>

All values are expressed as percent of baseline values before bleeding. When very different from mean, median is given in parentheses.

* = difference from controls at \( P < 0.01 \); Mann Whitney U-test.
† = difference from controls at \( P < 0.05 \); Mann Whitney U-test.

The netic flowmeters that measure flow continuously, is only able to measure flow in regional vascular beds at a particular moment and, thus, misses the initial changes and resulting compensations that occur with a decrease in blood volume and blood pressure.

The known error in estimation of dye-dilution cardiac output due to undetected recirculation during hemorrhage with right atrial dye injections led us to inject the dye into the left ventricle. Oriol et al. (19) have shown that during moderate or severe hemorrhage measurements based on the left ventricle injection site did not differ systematically from those using the right atrial site and Dow's corrections (20). Previously, we have found good agreement in a series of comparisons in both normal and hypotensive conditions between cardiac output measured by dye injected into the left ventricle and the Fick method (13).

The total body and regional cardiovascular responses of monkeys to acute hemorrhage are different, in many respects, from those we and others have found during endotoxin shock, although similar levels of hypotensive systemic arterial pressures were reached (10, 11, 21). During acute endotoxemia, we found that cardiac output fell only slightly while total peripheral resistance fell markedly and the blood vessels in all organs studied, except the spleen, vasodilated to some extent; these findings were associated with elevated blood levels of bradykinin. The fraction of cardiac output, like that at severe bleeding (50%), was elevated to the myocardium, small intestine, liver (hepatic artery), and adrenals and reduced to the spleen and pancreas. The brain and skin, however, were quite differently affected; during acute endotoxemia, there was a significantly smaller fraction of cardiac output going to brain but the fraction going to skin did not change significantly (11).

In the primate, blood levels of bradykinin and histamine have been reported to be elevated in the acute phase of endotoxemia (10, 22); in our experiments bradykinin levels were not elevated after hemorrhage. In contrast, blood catecholamine levels do not rise in the monkey during early endotoxemia.
(23, 24) but are elevated during hemorrhage (1, 3). Although such evidence is as yet circumstantial, the pattern of hormonal release could help account for the observed hemodynamic differences in the two types of shock.

Another possibility is that endotoxemia, but not hemorrhagic shock, causes alterations in nervous system function which prevent reflex vasoconstriction in response to hypotension. For example, Trank and Visscher (25) have reported that after the onset of endotoxin shock in cats, the carotid nerve baroreceptor discharge frequencies exceeded control values for most intrasinusal stimulus pressures. They concluded that this baroreceptor "resetting" might explain the poor compensatory response to the endotoxemic hypotension.

Part of the regional flow changes during hemorrhage might have been due to the fall of hematocrit and subsequent alterations of blood viscosity which are found after bleeding in monkeys (26) and man (27). We have found that a mild fall of hematocrit secondary to blood loss during surgery is associated with a significantly increased fraction of the cardiac output going to heart, brain, liver (hepatic artery), and small intestine. The fraction going to kidney and muscle was significantly reduced. Although the fraction to skin falls sharply with hemorrhage, we found it to be elevated in anemic monkeys. Anemia was also associated with a rise in cardiac output and a fall of total peripheral resistance which would...
oppose the changes seen during hemorrhage (28).

The regional changes in our monkeys during severe hemorrhage agree in most respects with those in the rat (4), rabbit (8), and dog (9) in which simultaneous measurements of the distribution of cardiac output were made.

Studies which record only portal vein or total liver flow may miss the considerable redistribution of blood to the organs contributing to these flows. After moderate hemorrhage, we found no significant changes in the fraction of output to total liver flow because the increase to the hepatic artery offset the decreases to the spleen and pancreas. With severe hemorrhage, however, the fraction of output received by the total liver (through hepatic artery and portal vein) increased significantly.

The variability of figures for bronchial flow precluded any significant changes except for the decrease of blood flow at severe bleeding. However, there was a progressive decline in the fraction of output received (to a median of 55% of baseline in severe hemorrhage) associated with a continued increase in resistance (median of 407%). This fall in the percent of radioactivity counted in the lungs during hemorrhage suggests that portal-systemic or systemic-arteriovenous shunting was not occurring.

The increase in the fraction of output to the adrenals after both moderate and severe hemorrhage was similar to data obtained in the dog (29) and rat (4) but not the rabbit (8). The increase in the fraction of output we found was enough to maintain normal blood flow at moderate, but not severe, hemorrhage.
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27. ADAMSON, J., AND HILLMAN, R. S.: Blood volume


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