Blood and Plasma Specific Gravity Changes During Acute Alterations in Hemodynamics in Splenectomized Dogs

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A variety of procedures were used to change hemodynamics acutely and thereby to produce changes in blood volume. Changes in plasma specific gravity showed no relation to those in aortic pressure or total peripheral resistance, but were related to central venous pressure. The simultaneous cell fraction tended to vary reciprocally with the plasma specific gravity, indicating a bulk transfer of either red cells or of plasma between the large blood vessels and peripheral beds.

Changes in blood volume in response to acute changes in hemodynamics have often been inferred solely from deviations in red cell fraction (hematocrit) seen in animals with intact spleens. Hence the textbooks still quote alleged changes in capillary permeability following the injection of such things as histamine or snake venom because of an increased hematocrit. It is true that petechial hemorrhages and a localized edema may be seen in such cases. But when the agents are given to splenectomized dogs, the cell fraction change is neither large nor consistent. Either the local edema is too small to produce a significant change in blood volume, or the fluid loss in damaged beds is being masked by a gain elsewhere, as a consequence of a hemodynamic effect of these toxins. Any agent which alters the intravascular pressure in the capillaries should produce a blood volume change. Unfortunately, the integrated action of tone changes in both arterioles and venules has not been well enough described to allow a firm speculation as to when, and in which direction, this hydrostatic pressure might vary. It has been shown that subjectation of an animal to cold is followed by an increase in blood specific gravity, which is presumably related to peripheral constriction. A blood pressure reduction after hemorrhage (also with vasoconstriction) is followed by hemodilution. It has not been shown that pressor or depressor agents would produce similar hematocrit changes.

In other studies, changes in plasma volume have been inferred from changes in plasma specific gravity. If a transcapillary shift of fluid is the only mechanism involved, then this gravity and the hematocrit should change in parallel and to proportionate degree. If there has been gain or loss of plasma protein, such a parallelism would be lost. Any significant change in the rate of lymphatic return, for example, could temporarily produce such a discrepancy. Further, much evidence has now accumulated that the body has plasma "depots." The most likely sites for such plasma stores would appear to be the tiny vessels of all peripheral beds, and reflect a red cell skimming and plasma accumulation as blood flows through small tubes. While one might anticipate that the volume of such a peripheral pool would vary with the rate of flow and with small vessel diameter, changes in the amount of pooled plasma have not been so demonstrated.

So far as we are aware, no systematic study of simultaneous changes in plasma specific gravity and in red cell fraction during acute changes in hemodynamics has been made. We have therefore followed these measures after the use of some common pressor and depressor agents. To assess possible changes in the size of the peripheral plasma pool, simultaneously drawn femoral arterial and venous samples have been compared.
METHODS

The dogs used were given 10 mg./Kg. morphine sulfate followed by an intravenous administration of 15 to 20 mg./Kg. of sodium pentobarbital. The spleens were removed immediately through a midventral incision, and the animal then allowed to rest for about 30 min., or until the circulation appeared stabilized. The surgery resulted in more rapid control heart rates than we usually expect in dogs anesthetized by this regime. Aortic pressure was recorded by a sound, connected to an optical manometer, passed down the left carotid artery into the ascending aorta. From the recorded pulse contours were calculated the cardiac indexes and total peripheral resistances (mean aortic pressure in mm. Hg/cardiac index in ml/sec). Central venous pressure was recorded from within the thoracic cage through a polyethylene tube passed down the right external jugular vein, connected to a saline manometer. The reference zero level was that of the apex beat. Intraocular pressure was not recorded, but in these experiments neither respiratory rate nor depth was appreciably altered. Readings were made between respirations.

The specific gravity of the blood and plasma were determined by the falling drop technique, changing the tower gravity when necessary so that all falling times were between 20 and 40 sec. Direct hematocrit determinations were made in Wintrobe tubes, centrifuged in an International centrifuge at 2800 r.p.m. for 30 min. Simultaneous blood samples were taken from the femoral artery and vein of the same leg, using syringes wetted with heparin solution, with short 26 G. needles. In some experiments, hepatic vein blood was sampled through a catheter, which had been placed under fluoroscopy. Any sample showing visible hemolysis was discarded.

Hemodynamics were acutely changed by: (a) 15 min. infusion of epinephrine (Adrenaline, Parke Davis) at a rate of 5 or 10 µg./Kg./min.; (b) the application of several ice bags to the whole ventral surface of the body (after close clipping) for 20 min.; (c) a 15 min. infusion of 5 or 10 µg./Kg./min. of acetylcholine; (d) a 9 min. infusion of isoproterenol (Isuprel, Winthrop) at 1 or 2 µg./Kg./min.; (e) a 15 min. infusion of histamine (15 µg./Kg./min. of histamine base). Infusions were made by a motor driven syringe, through polyethylene tubing into a femoral vein. It took 45 to 60 sec. from the start of the motor for the drugs to clear the tubing and exert their first noticeable effect.

Evaluation of Precision of Methods

Simultaneous femoral arterial and venous samples were taken at 10 min. intervals from a sedated dog which had suffered no surgery other than the exposure of the two vessels under local anesthesia, and who was resting quietly. For the 20 obtained samples, plasma (Gp) and blood (G) specific gravities were determined independently by two technicians, using different towers. At least three falling times were reported for each sample from each. Differences between the reported values were expressed in per cent of the mean of the two results (e.g., a difference of .0010 from a mean plasma gravity of 1.0250 would be called a 4 per cent error). The average errors were 0.4 per cent for Gp and 0.55 per cent for G.

On the same samples, Wintrobe hematocrit readings were done in triplicate. The average difference of any single reading from the mean of the triplicate was 1.3 per cent. The specific gravity determinations thus had an appreciable smaller error than the Wintrobe hematocrit determinations.

An indication of the spontaneous deviations in the animal over the 100 min. sampling time was had by comparing each sample to the mean of the whole series. The standard deviation for the 10 arterial samples was 0.7 per cent for Gp, 0.9 per cent for G and 2.0 per cent for Wintrobe hematocrit determinations.

To assess the difference between simultaneously drawn femoral arterial and venous blood, 58 pairs of samples were taken from 21 anesthetized dogs during steady state conditions and before any experimental procedures had been undertaken. No systematic A-V difference was found for any of the 3 measures. The standard deviation of this A-V difference was 2.3 per cent of the arterial value for Gp, 3.8 per cent for G and 8.4 per cent for Wintrobe hematocrit determinations.

Changes in Gp and G can be quantitatively compared only if the cell fraction and the cell specific gravity are known. Ashworth and Tigges presented the formula H = Gc + (1 - H) Gb, where H is the centrifuge hematocrit and Gc the red cell gravity. In several experiments done on 10 dogs in which hemoconcentration was produced by leg tourniquet release, intestinal trauma or intraperitoneal injections of hypertonic solutions, they found that the calculated Gc showed but minor variation from a mean of 1.0593 (standard deviation was .00205 or 2.1 per cent). Hence they used this value as a constant, calculating the cell fraction from G and Gp by CF = Gp - G / 1.0593 - G.

Assuming a constant Gc denies intraindividual differences in cell gravity and any appreciable cell volume change during an experiment. We tested the first of these by taking femoral venous samples from 130 anesthetized dogs, in stable state,
before any experimental procedures had been performed. H was determined by a single sample Wintrobe centrifugation. The average calculated Gc was lower (1.0935) and the standard deviation appreciably greater (.0071 or 7.6 per cent) than those cited above. It was also observed that in a number of animals studied in the summer months, and which showed low hematocrit values, the Gc values were significantly higher than those obtained in the cooler seasons. The whole series of animals was then divided into 13 groups of 10 each, arranged in ascending order of the Wintrobe readings. As shown in figure 1, the Gc values showed no significant differences between the groups. The Gc values showed the expected increase as the hematocrit increased, but a departure from a straight line relationship was clear. This deviation was due to a decrease in Gc as the cells became more concentrated. The reason for this trend cannot be given. When our mean Gc value of 1.0935 was used in the above formula, the average difference between measured hematocrit and calculated cell fraction was 4.2 per cent. If a correction was made for the changing Gc as shown in figure 1, this difference was reduced to 1.8 per cent.

On this basis, a correction of the formula seems called for when an absolute agreement between hematocrit and cell fraction is desired. A survey of a fair number of comparisons taken from quite diversified experiments, which therefore do not lend themselves readily to tabulation, indicates that such a correction is probably not required when changes in cell fraction in a single animal, during a given procedure, are being followed. Despite a possible systematic difference, the Wintrobe hematocrit determination and the calculated cell fraction have almost always changed in nearly parallel fashion. Major exceptions have been seen in 3 types of experiments: 1. In a few dogs dying in shock after hemorrhage or muscle trauma, an increase in the Wintrobe hematocrit determination of about 5 per cent was seen when there was no corresponding change in blood gravity. In 2 cases where it was measured, hemoglobin determinations also failed to show the increase. 2. After the intravenous administration of snake venom, the calculated Gc was often reduced by as much as 10 per cent. Once again, the change in hemoglobin was similar to that in blood gravity, neither being as great as that in Wintrobe hematocrit determination. 3. When 5 per cent NaCl solution was given intraperitoneally to produce death by dehydration, the Gc value usually (but not always) increased by about 5 per cent. Such a change, which should be nearly maximal under physiologic conditions, would cause a decrease in the calculated cell fraction of 1.8 per cent.

We believe, therefore, that cell volume does remain essentially constant through most experiments, and that even when it does change, a cell fraction based on gravity measurements would be a more valid index to blood volume changes than the Wintrobe hematocrit determination.

Results

Epinephrine Infusion. A 15 min. infusion of epinephrine was given 12 times (10 dogs). Average values are shown in figure 2, with the standard errors depicted by vertical lines. The infusion maintained an increase in aortic pressure of about 24 mm. Hg, a slowed heart rate, and a rise in central venous pressure. The cardiac index was reduced by 12 per cent and calculated total peripheral resistance was elevated by 50 per cent.

The preinfusion arterial specific gravity value was taken as the reference, differences from it in both venous and arterial blood being tabulated separately. The average difference at a given time, and the standard error, were expressed in terms of per cent.
BLOOD AND PLASMA SPECIFIC GRAVITY

Fig. 2 Top Left. Average values of 12 responses to epinephrine infusions. Vertical lines, standard errors. For plasma gravity and cell fraction; solid line, femoral venous blood; broken line, femoral arterial blood; dotted line, hepatic venous blood. Values expressed in per cent differences from the preinfusion value for the arterial blood.

Fig. 3 Top Right. Average values of 7 responses to isoproterenol infusions. Legend as for figure 2.

Fig. 4 Bottom Left. Average values of 6 responses to acetylcholine infusions. Legend as for figure 2.

Fig. 5 Bottom Right. Average values of 7 responses to the application of ice bags to the ventral surface of the dogs. Legend as for figure 2.
deviation from the reference. Hence the preinfusion venous $G_p$ values averaged +0.4 per cent. During the infusion there was a small increase in $G_p$ in 9 out of 12 experiments. The average change was only 2 per cent and the probability of significance only 6 per cent as attained toward the end of the infusion. There was no significant A-V difference at any time. While the change in $G_p$ is of questionable significance here, it is in the same direction and of the same magnitude as that seen in 30 cases in which a single intravenous injection of 5 μg./Kg. epinephrine was given to splenectomized dogs. In this larger series, the $P$ value was 2 per cent.

The cell fraction did not rise as did the $G_p$, but fell about 4 per cent ($p = 3$ per cent at 9 min.) The reduced cell fraction was first evidenced in the venous sample, with a clearly significant A-V difference ($p = 0.4$ per cent) at 3 min.

Gravity determinations were also made on samples of hepatic venous blood in 6 cases. The $G_p$ values were close to those of the leg vein samples. The cell fraction, as shown by the dotted line in figure 2, did not show the abrupt decline seen in the leg, but fell only as the arterial blood value declined. Hence there was a significant difference between the two venous samples at 3 min. ($p = 1$ per cent).

Isoproterenol Infusion. The average of 7 responses (6 dogs) to a 9 min. infusion of this drug is given in figure 3. The aortic pressure fell by 30 mm. Hg, the heart rate was markedly increased, and the venous pressure showed an initial rise and then a remission to control levels. Cardiac index was greatly increased in all cases (+80 per cent) and the total peripheral resistance reduced (−62 per cent). The $G_p$ showed an initial significant increase in the venous blood, with a later return to preinfusion levels. The arterial blood did not show the initial rise, but a slight fall below the preinfusion value at 6 min. ($p = 6$ per cent). The negative A-V difference seen initially had a $p$ value of 1 per cent. The change in $G_p$ in the venous blood was directionally the same as with epinephrine (although shorter lasting) even though the effects of the two drugs on aortic pressure, cardiac index and total peripheral resistance were opposite; in common was the rise in central venous pressure.

The cell fraction showed a slight reduction of doubtful significance. There was no significant A-V difference at any time. As after epinephrine, there was no clear parallel between the changes in cell fraction and those in $G_p$.

Acetylcholine Infusion. In figure 4 are shown the average values obtained in 6 experiments (6 dogs) with a 15 min. infusion of acetylcholine. The mean aortic pressure was reduced by 25 mm. Hg, the heart rate accelerated, and the central venous pressure either remained the same or rose slightly. Cardiac index increases were much less than after isoproterenol, and were less steady during the infusion. Total peripheral resistance showed a moderate fall (−25 per cent), a partial reversal, and a secondary fall.

$G_p$ changes were small and variable, so that a significant change for the whole series was not present. The cell fraction showed a significant initial fall in the venous blood ($p = 1$ per cent), but the difference was later lost.

Exposure to Cold. The responses to ice bag application in 7 dogs are summarized in figure 5. There was no appreciable change in aortic pressure, cardiac index or total peripheral resistance for the first 10 min. In the next 10 min., the cardiac index was gradually reduced by 20 per cent, and resistance increased. Heart rate changes were not significant. The central venous pressure showed an initial rise and then remained constant, although variation in the amount of change between animals was large. There was no change in rectal temperature in 4 dogs, and in the others it fell only in the last 10 min. of exposure, and by about 1 F.

Despite the minor hemodynamic effects, $G_p$ showed the greatest change found in the present series of experiments ($p = 0.5$ per cent). The concentration of protein was noted first in the venous blood, later in the arterial. The A-V difference was significant at a 2 per cent.
level at 5 min., but only at a 5 per cent level for the later part of the experiment, when the arterial values were elevated as well. In 4 dogs, hepatic venous samples (fig. 5 dotted line) showed Gpn changes similar to those seen in the leg venous blood. By 10 min., the hepatic values had fallen to become equal to those of the arterial blood.

The cell fractions of the arterial blood showed no change for the first 10 min., and then rose by an amount which could be explained simply by the same water shift that produced the rise in Gpn. The leg venous blood cell fractions were more erratic, showing an initial increase which was not significant, a fall at 10 min. (p = 2 per cent), and then a second rise. The A-V difference was not significant. The decline in cell fraction was seen earlier in the hepatic samples (p = 4 per cent).

Histamine Infusions. In figure 6 are shown the results obtained with 15 min. histamine infusions in 10 experiments (10 dogs). Of these, hepatic vein samples were taken in 4. The general response differed from that seen with isoproterenol or acetylcholine by virtue of a large fall in venous pressure and in cardiac index (−50 per cent). The total peripheral resistance showed an immediate fall (−50 per cent), then a partial return to a level which was sustained. This resistance change is therefore more like that reported by Johnson and Blalock17 than that found by Deyrup.1 However, the former found high cardiac outputs after histamine, which were not observed here. Near mid infusion, the heart rate slowed and the venous pressure often showed a slight elevation. A cardiac impairment is strongly suggested, which might have been related to an inadequate coronary circulation, or perhaps to a direct action of the drug on the heart.18 Three animals died of what appeared to be acute heart failure toward the end of the infusion.

Variability in Gpn was large. A significant fall in the arterial level was seen in 8 cases shortly after the infusion was started (p = 2 per cent). In 4 cases the arterial value remained low, in the others it returned to the preinfusion levels. Two animals showed an elevated Gpn. There was a gross trend for a decline in venous Gpn in mid infusion, but the change from the control was not significant. Nor was there a significant reduction in the Gpn of the hepatic venous blood. The only significant change in cell fraction was a decline seen in the hepatic samples at 9 min. This preceded a trend for the arterial values to decline somewhat.

DISCUSSION

Variation in the amount, and even in direction, of change in Gpn and in cell fraction between animals was such that group differences from control levels of clear statistical significance were infrequent. This was perhaps inevitable when no account was taken of differences in sensitivity of the animals to the various procedures, and the results simply grouped on the basis of time elapsed. There was a significant increase in the Gpn of both venous and arterial blood after exposure to cold, and an increase in the venous blood after the infusion of isoproterenol. While not significant here, there was a definite trend for an increase in both bloods after epinephrine. Since these various procedures had quite different actions on aortic pressure and cardiac
index, it would seem that total peripheral resistance offers no reliable criterion to \( G_p \) change, and hence, by inference, to changes in capillary hydrostatic pressure. A better case could be made for a parallel between \( G_p \) change and that in central venous pressure. The relation is qualitative only (e.g., compare the responses to cold and to epinephrine). The central venous pressure is, of course, no necessary index to the venous pressures in the tissues, but in the anesthetized supine dog it would seem that both would vary together.

In keeping, when the venous pressure was elevated after cold exposure or after isoproterenol, the \( G_p \) of the plasma returning from the leg was greater than that of the arterial plasma entering it. Our only experiment which involved a decline in central venous pressure was the histamine infusion. The results here are difficult to interpret. The \( G_p \) of the arterial blood fell as expected. But the values in the venous blood returning from the leg did not show this fall. The plasma seemingly was being diluted in some bed other than the leg. Our hepatic vein determinations offer no support for such a dilution taking place in the visceral circulation. The classic explanation for the higher venous \( G_p \) values would be that an altered capillary permeability allowed protein leakage from the plasma. We need only postulate that such a loss was predominantly of the smaller molecular proteins. But we must also conclude that the loss was not a generalized one.

We have no direct measure of the rate of leg blood flow. Past observations indicate that changes in this flow are directionally the same as those found here in cardiac index. If the leg blood flow had become very sluggish after histamine, then a part of the apparent A-V difference in \( G_p \) could have reflected simply a slow circulation time of the diluted arterial plasma through the leg bed.

Parallel changes in \( G_p \) and in cell fraction were the exception rather than the rule in these experiments. Hence the effect of plasma volume changes due to transcapillary fluid shifts on the cell fraction would appear negated by the movement of either red cells or plasma to or from "depots." No suggestion has been made as to the location of a red cell depot in splenectomized dogs, unless it be in the liver.10 If the hemodilution seen in 4 of these experiments were to reflect an increase in stored cells in this organ, one would expect to observe a change in cell fraction in hepatic venous and in arterial blood before one in leg venous blood. This was not true. The only possible contradiction to this was after cold exposure, where a small hemodilution of hepatic blood developed before the late concentration of red cells seen in blood taken from all sites. The alternative is that cells were being packed into peripheral beds such as the leg.

A more rational explanation would be that the changes in cell fraction, observed first in the femoral venous blood, reflected capacity changes of a peripheral plasma pool. This could mean either a change in the differential rate of passage of red cells and of plasma through peripheral vessels, or could constitute a bulk transfer of previously accumulated plasma back into the larger vessels. The first does not appear likely. An increased flow rate, as after acetylcholine, would, if vessel size was not greatly altered, produce an increased cell fraction in the venous blood, rather than the observed decrease. If we were to say that the dilator effect of the drug was paramount, and the decreased cell fraction a reflection of increased tube size, then it is not clear why a similar dilution was seen after the use of epinephrine, which certainly must reduce peripheral tube diameter.

If a bulk transfer is involved, 2 mechanisms must be invoked to explain these changes. First, a reduction in peripheral bed size could displace plasma. This would explain the venous hemodilution after epinephrine, and also the smaller change in the same direction seen in the first part of the response to cold. In the latter case, similar patterns were seen for both the visceral and the leg beds. The absence of venous dilution of the hepatic samples after epinephrine might be related to the observation that the constriction is less acute.
and of shorter duration in visceral beds than in the leg. Second, the vasodilators could produce a more transient fall in venous cell fraction because of the increased peripheral flow rate.

Why the hemodynamic response to histamine should have been so unlike those to other dilator agents is problematic. At smaller dosage levels, this drug can act to lower total peripheral resistance and increase the cardiac index. In the present experiments, a severe reduction in venous return was evidenced by the marked fall in venous pressure and in cardiac index. One explanation could be that a constriction of the hepatic sphincter restricted flow through the visceral beds. If so, the plasma and blood gravity values of the hepatic venous blood gave no indication of such a change. Or there could have been a large generalized peripheral bed expansion. The action of such an expansion to produce reductions in the amount of “actively circulating” blood has been given quantitative documentation in the case of isoproterenol. It is possible that the action of histamine is considerably greater than that of isoproterenol because the cardiac action seems to be handicapped in the former and definitely augmented in the latter case. The small amount of change in \( G_p \) and the absence of a clear increase in cell fraction speak against any massive loss of plasma from the vascular tree.

**SUMMARY**

Changes in plasma specific gravity, and in cell fraction calculated from plasma and blood gravity, were followed in samples simultaneously drawn from the femoral artery and vein of dogs during exposure to cold and infusions of epinephrine, acetylcholine, isoproterenol and histamine. In some cases, changes in these measures in hepatic venous blood were also determined. Changes in gravity were not large, and changes of clear statistical significance infrequent. An increase in plasma gravity, of varying degrees, was seen after the first 4 of these procedures, and a decrease followed histamine. These changes are not related to those in aortic pressure or total peripheral resistance, but do appear related to the direction of change in central venous pressure. In almost all cases, the cell fraction changed reciprocally to the plasma gravity. The arteriovenous difference in this cell fraction was consistent with the notion that extra plasma was being returned from peripheral beds to the central vessels after either a reduction in bed size (epinephrine, cold) or an increased peripheral flow (acetylcholine, isoproterenol). The hemodynamic response to histamine presents problems in interpretation. There was no clear evidence of a gross plasma leakage after this drug.

**SUMMARIO IN INTERLINGUA**

Le alterationes del gravitate specific del plasma e del fraction cellular calculate super le base del gravitate de plasma e de sanguine esseva observate in specimen simultaneamente obtenite ab le arteria e le vena femoral de canes in le curso de lor exposition a frigido e durante infusiones de epinephrina, acetylcholina, isoproterenol, e histamina. Le alterazione de gravitate non esseva grande. Alterationes de definite significacion statistic esseva rar. Un augmento de varie grados in le gravitate del plasma esseva notate post le prime quatro del supra-listate interventiones; un reduction post histamina. Iste alterationes non es relacionate a alterationes del pression aortie o del total resistentia peripheric, sed illos pare esser relacionate al direction del alteration in le pression venose central. In quasi omne le casos, le fraction cellular se alterava reciprocamente al gravitate del plasma. Le differentia arterio-venose in le fraction cellular esseva compatible con le notion que le excesso de plasma retornna ab le vasculatura peripheric verso le vasos central si ben post un reduction del magnitude del vasculatura (epinephrina, frigido) como etiam post un augmento del fluxo peripheric (acetylcholina, isoproterenol). Le responsa hemodynamic a

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histamina presenta problemas de interpretación. Post le uso de iste droga, nulle signos definite de un grossier escappamento de plasma esseva notate.

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