Influence of Arterial Occlusion on Hematocrit and Plasma Protein Concentration of Femoral Venous Blood in Rabbit

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Abstract The changes of venous outflow ($Q_v$), hematocrit (Hct), total plasma protein ($T_p$), electrolytes, crystalloid osmolality, $P_O_2$, $P_CO_2$, and pH were simultaneously measured in hindlimb preparation of anesthetized rabbits during 5 min occlusion of the femoral artery and the abdominal aorta and during the reactive hyperemia. During the occlusion of the femoral artery, $Q_v$ decreased to 66.4% at the end of the occlusion. Hct and $T_p$ decreased slightly and then tended to progressively increase. $P_O_2$ showed an increasing trend following an initial decrease. During the occlusion of the aorta, $Q_v$ showed a continuous drop to 38.0%, while Hct and $T_p$ declined to 95.4 and 93.8%, respectively. $Q_v$, Hct, and $T_p$ showed increase after the release of the occlusion of the femoral artery and the aorta. Crystalloid osmolality and $K^+$ were observed to rise slightly. From these results it was suggested (1) that the increase of Hct and $T_p$ which represents the hemoconcentration occurred during the reactive hyperemia and during the occlusion of the femoral artery, while the hemodilution occurred during the aortic occlusion, and (2) that the gradual increase of Hct and $T_p$ during the occlusion of the femoral artery would be caused by the collateral blood flow.

Key words: reactive hyperemia, hematocrit, arterial occlusion, plasma protein.

It has been reported that "hyperemia" plays an important physiological role during and after muscle exercise, and its influence on hematocrit (Hct) and total protein ($T_p$) in the venous effluent has been observed by several investigators (Kaltleider and Meneely, 1940; Astrand and Saltin, 1964; Jove and Poortmans, 1970; Takemiya, 1976). They have confirmed that hemoconcentration takes place in association with exercise hyperemia. Takemiya et al. (1981) revealed through prolonged exercise hyperemia in a local circulation of rabbits that $T_p$ showed a marked increase but Hct and blood flow did not change in parallel.

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with it. To explain the mechanism of this phenomenon, Nagashima (1972) and Takemiya et al. (1977) have proposed that there may be an alternative variation between the “nutritive” and “non-nutritive” microvascular flow.

In this study, the author first attempted to assess whether the marked increase of $T_p$ can also be observed during the process of “reactive hyperemia,” and then to analyze the mechanism of the change of $T_p$ and Hct. Especially, the relation of these values against the change of blood flow was investigated by occluding the femoral artery and the abdominal aorta. In addition, the changes in the values of plasma crystalloid osmolality, electrolyte concentration ($Na^+$, $K^+$, and $Cl^-$), $P_{O_2}$, $P_{CO_2}$, and pH were simultaneously measured during the process of the reactive hyperemia.

**MATERIALS AND METHODS**

Twenty seven rabbits of both sexes weighing 3.0–4.5 kg were used for the experiments. The animals were fixed in the supine position under urethane anesthesia (1.0 g/kg, i.p.). After tracheotomy was made, the lower abdomen and hindlimbs were operated on to expose the abdominal aorta, the femoral artery, and the femoral vein. The left femoral artery was cannulated for monitoring the systemic blood pressure after the intravenous administration of sodium heparin (400 unit/kg). The right femoral artery was left intact. A silicone cannula with a 1 mm i.d. was inserted into the peripheral end of the right femoral vein, and the outflow was led to a reservoir through a photocell drop counter. The orifice of the tip of the cannula was fixed at heart level, which was taken as the zero reference level for blood pressure measurements. The blood in the reservoir was returned to the animal through the left femoral vein by a roller pump (Corona Co., Japan). The electric pulse signal from the photocell circuit was fed into an interval-voltage converter so as to calculate the venous outflow ($Q_v$) in ml/min.

About 0.2 ml of the blood sample was collected at a 30 sec or 1 min interval with a small plastic spoon inserted underneath the orifice of the drop counter. The blood sample was taken into six microtubes (1.0 mm i.d., 75 mm long) for the measurement of Hct, $T_p$, and crystalloid osmolality. The residual blood in the spoon was returned to the reservoir. Hct was measured by the microcapillary method. $T_p$ was measured with a D-Z type refractometer (Kyoei Co., Japan) from a plasma sample obtained by cutting the capillary tube at the portion of plasma after determining Hct. The plasma sample was also used for the measurement of crystalloid osmolality with a cryoscopic osmometer (H. Knauer & Co., West Germany). $P_{O_2}$, $P_{CO_2}$, and pH were measured in the total blood in heparinized syringes with an IL meter (IL Co., U.S.A.). $Na^+$, $K^+$, and $Cl^-$ were measured in the plasma obtained by centrifugation of the capillary tubes with a flame photometer (Shimadzu Co., Japan).

The right femoral artery and the abdominal aorta were occluded by means of
clamps. For occluding the aorta, one of the clamps was placed at the level of the fourth lumbar artery, while for the femoral artery, the other clamp was placed at the level below the bifurcation of the circumflex artery. These occlusions were maintained for 5 min. Obtained data were expressed as mean±S.E. Statistical comparison of the data before, during, and after the occlusion was made by Student's t-test.

RESULTS

Venous blood flow

Figure 1 shows the percent changes of Q, Hct, T, P02, Pco2, and pH of the venous blood sample during and after the femoral (solid circle) and aortic occlusion (open circles). The values of Q before the occlusion of the femoral artery and the aorta were 8.5±0.7 (n=10) and 8.3±0.5 ml/min (n=13), respectively. Immediately after the initiation of the occlusion of the femoral artery, the relative Q value started to decrease, reaching 56.4±7.2% (p<0.001) of the control level within 2 min, and then progressively recovered to 6.64±4.1% (p<0.001) at the end of the 5 min occlusion. Immediately after the release of the occlusion, Q increased markedly up to the maximal level, 158.6±6.4% (p<0.001) and then decreased rapidly.

During the occlusion of the aorta, Q decreased rapidly to 51.1±2.6% (p<0.001) of the control within 1 min and then decreased to 38.0±3.2% (p<0.001) at the end of the occlusion. In a few cases Q decreased to around zero. After the release of the occlusion of the aorta, Q increased to 170.2±7.9% (p<0.001) and then returned to the control level within 5 min.

Hct and Tp

The values of Hct before the occlusion of the femoral artery and the aorta were 43.1±1.3 (n=14) and 43.0±1.2% (n=13), respectively. The relative values of Hct decreased to 98.9±0.3% (p<0.05) at 1 min after the initiation of the occlusion of the femoral artery, and then gradually increased to 102.0±0.4% (p<0.01) at the end of the occlusion. These values increased to 105.4±0.4% (p<0.01) immediately after the release of the occlusion and returned to the control level within a few minutes (Fig. 1).

During the occlusion of the aorta, the Hct values continued to decrease, reaching a minimum value of 95.4±0.7% (p<0.01). After the release of the occlusion these values rapidly increased to 104.4±0.5% (p<0.01) and then decreased gradually in parallel with the decrease of Q.

The values of Tp before the occlusion of the femoral artery and the aorta were 5.9±0.2 (n=11) and 6.0±0.1 g/dl (n=10), respectively. During the occlusion of the femoral artery, Tp showed almost the same tendency as that of Hct; its relative value increased to 102.0±0.7% (p<0.05) at the end of the occlusion, and then
further increased to $103.9 \pm 0.7\%$ ($p<0.01$) after the release. During the occlusion of the aorta, the $T_p$ value declined to $93.8 \pm 1.0\%$ ($p<0.01$) and increased to $101.9 \pm 0.8\%$ ($p<0.05$) after its release.

$P_{O_2}$, $P_{CO_2}$, and pH

The values of $P_{O_2}$ in venous effluent before the occlusion of the femoral artery and the aorta were $41.6 \pm 2.5$ ($n=7$) and $38.3 \pm 2.1$ mmHg ($n=7$), respectively. During the occlusion of the femoral artery, the relative value of $P_{O_2}$ in venous effluent decreased to $59.5 \pm 4.9\%$ ($p<0.001$) of the control within 3 min and then gradually rose to $65.5 \pm 4.9\%$ ($p<0.001$) at the end of it (Fig. 1). A short period of overshooting of $P_{O_2}$ was observed within 2 min after the release of
the occlusion. On the contrary, during the occlusion of the aorta, $P_{O_2}$ gradually declined to $54.7 \pm 4.3\%$ ($p<0.001$) at the end of it, returning to the control level at about 10 min after its release.

The values of $P_{CO_2}$, before the occlusion of the femoral artery and the aorta were $35.8 \pm 2.2$ (n=8) and $39.6 \pm 2.5$ mmHg (n=6), respectively. The relative values of $P_{CO_2}$ rose during the occlusion of the femoral artery and of the abdominal aorta, showing maximum values of $123.7 \pm 3.8$ ($p<0.01$) and $132.6 \pm 3.3 \%$ ($p<0.001$), respectively. A relatively rapid return of the value was observed after the release of the occlusion of the femoral artery as compared with that of the aorta.

The values of pH in both cases were $7.353 \pm 0.003$ (n=7) and $7.321 \pm 0.003$ (n=7), respectively. The relative values of pH dropped slightly to $99.3 \pm 0.1 \%$ ($p<0.01$) at the end of the occlusion of the femoral artery. During the occlusion of the aorta, these values also decreased to $98.6 \pm 0.2 \%$ ($p<0.01$) and returned to the control level within about 8 min after its release.

Crystalloid osmolality, $Na^+$, $K^+$, and $Cl^-$

The values of osmolality before the occlusion of the femoral artery and the aorta were $336.5 \pm 4.6$ (n=10) and $335.7 \pm 1.9$ mOsm/kg H$_2$O (n=13), respec-
tively. During both of these occlusions, their relative values did not show a significant change. But, after the end of the occlusions, slight increases of these values were observed, indicating 102.5±0.3 and 102.7±0.5%, respectively (p<0.001, Fig. 2).

Immediately after the release of these occlusions, Na+ and Cl− showed no significant change, but K+ showed a slight increase to 103.3±0.8 (p<0.01) and 104.0±0.5% (p<0.01), respectively. The values of K+ before both occlusions were 3.3±0.1 (n=7) and 3.2±0.1 mEq/liter (n=7).

**DISCUSSION**

*Hct and Tp changes during the arterial occlusions.* It is generally accepted that the values of Hct and Tp are useful indexes to evaluate a transvascular fluid movement in the capillary channel (ALBERT, 1971). The decrease of Hct and Tp during the occlusion of the aorta may indicate that hemodilution took place due to the decrease of intravascular hydrostatic pressure as suggested by the hypothesis proposed by PAPPENHEIMER (1953). During the occlusion of the femoral artery, on the other hand, Hct and Tp showed a gradual increase following a slight decrease. This may also suggest that hemoconcentration occurred in the capillary channel.

Observing the change in the venous outflow, Qv, its maximum percent decrease during the occlusion of the femoral artery reached 66.4% of the control level, while that of the aorta 38.0%. This may indicate that there are collateral pathways functioning during both types of these occlusions. Thus, this evidence would be due to the difference of the blood flow coming through the collateral pathway. The collateral flow during the femoral occlusion should be larger than that during the aortic occlusion.

Even if the aorta was completely occluded, the collateral flow may come through the skin and the surrounding tissues. The collateral flow during the femoral occlusion seems to come through the deep femoral, the medial femoral circumflex, and the descending branch of the lateral circumflex artery, and the skin vasculature. The slight and gradual increase of P0i following the initial decrease during the occlusion of the femoral artery also suggests that there was some amount of arterial blood of the collateral origin flowing into the concerned muscle.

Using plethysmography MORIMOTO (1959) observed a gradual increase following the initial decrease of the hindlimb volume in dogs when the pressure in the femoral artery was reduced to about 50 mmHg by the compression of the aorta. This was not observed during the complete occlusion of the aorta. It is suggested by this evidence that the plasma fluid filtration may occur if intravascular pressure is maintained above some level, as stated by PAPPENHEIMER (1953).

It has been reported that the dilation of the arterioles due to the relaxation of the vascular muscle occurred during the occlusion of the artery (BAYLISS, 1902;
JOHNSON and INTAGLIETTA, 1976). From the rheological point of view, the increases of diameter causes the decrease of the blood flow and its velocity, and then the decrease of the ratio of the thickness of the plasma layer to the diameter (MERRIL, 1969; HOCHMUTH et al., 1970). In other words, the degree of the axial accumulation decreases in the blood flow with lowered flow velocity. The increase of plasma filtration may also occur during this state (KLITZMAN and DULING, 1979). The relation between the vessel diameter and Hct was investigated by TAKEMIYA et al. (1978). They reported that the Hct decreases during the vasoconstriction caused by the application of norepinephrine. By these factors Hct also increases, and such dilation will be accelerated by local hypoxia (DETER and BOHR, 1968) and by the accumulation of such chemical substances as K+, lactate, adenosine etc. (CRAWFORD et al., 1959; SKINNER and GOSTIN, 1970; CHEN et al., 1972; STOCK and ISSELHARD, 1972).

Hct and $T_p$ changes during reactive hyperemia. After the release of these two types of arterial occlusions, a similar pattern of venous outflow increase, "reactive hyperemia," was observed. During the time Hct also increased in parallel with $T_p$. Such a parallel change in Hct and $T_p$ has never been observed in the exercise hyperemia. As shown by POORTMANS (1969) and TAKEMIYA et al. (1981), the rate of increase of $T_p$ is larger than that of Hct in exercise hyperemia. Although the mechanism is still obscure, the high increase rate of $T_p$ in exercise hyperemia seems to be closely related to muscle contraction.

The increase of Hct and $T_p$ may also indicate that the hemoconcentration occurred in the capillary during the reactive hyperemia. Possible factors causing the hemoconcentration would be as follows: (1) The increase of intravascular pressure. (2) Re-opening of the closing capillaries. This would occur by the increase of blood flow, and then the effective filtration area would subsequently increase. BURTON and JOHNSON (1972) estimated that about 30% of the increased flow may be due to this factor. (3) Accumulation of chemical substances in the interstitial fluid. Effect of chemical substances on the vasodilation has been stated above. SKINNER and GOSTIN (1970) reported the increase of filtration due to the rise of osmolality which is caused by the accumulation of metabolites in the interstitial fluid.

Although plasma crystalloid osmolality and K+ increased slightly, after the release of the 5 min occlusion, the amount of the change was below 10 mOsm/kg H2O and 0.3 mEq/liter, respectively. Thus, their vasoactive effects in reactive hyperemia would be mild as compared with those in exercise hyperemia (KJELLMER, 1964; MELLANDER and JOHANSSON, 1968; LUNDVALL, 1972).

In conclusion, it is considered that the gradual increase of Hct and $T_p$ during the occlusion of the femoral artery are caused by the collateral blood flow to maintain the intravascular pressure sufficient for keeping the filtration pressure. Several microrheological effects play a complementary but inevitable role in this phenomenon.
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