Influence of Short, Prolonged and Ischemic Exercise on Local Blood Flow, Hematocrit, Plasma Protein Concentration and Osmolality of Rabbit Hindlimbs

by
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Introduction

Several investigators have reported an increase in hematocrit and plasma protein concentration during and after muscular exercise in man (1, 8, 15, 16). It is generally accepted that hematocrit and protein increase are mainly the result of a loss of plasma fluid. However, hematocrit changes cause by whole body exercise include a concomitant effect of the movement of plasma fluid and change of circulating red cell mass. Joye et al (15) investigated the relationship between hematocrit and protein changes during arm exercise by analyzing local venous blood taken at the elbow near the contracting muscles. They suggested that the increase of both hematocrit and serum protein is not due exclusively to plasma concentration. There is no informations on mechanism of hematocrit and protein changes during exercise. Therefore, it would be of interest to follow the hematocrit and protein concentration change during various types of local muscle exercise.

In the present study we utilized an in-vivo preparation of the rabbit hindlimb and performed experiments similar to arm exercise in the human. We made simultaneous measurements of blood pressure, venous outflow, hematocrit, total plasma protein concentration and plasma crystalloid osmolality during several series of isometric contractions including short, prolonged and ischemic exercise, and the effect of norepinephrine.

Methods

Experiments were performed on healthy rabbits weighing 2.5－4.0 kg. After anesthesia with urethane (1g/kg, intraperitoneal), the femoral arteries and veins were exposed for cannulation, and the right sciatic nerve was divided for electrical stimulation of the peripheral end.

Following administration of sodium heparin (400 U/kg), we inserted a polyethylene catheter into left femoral artery for recording arterial pressure by strain gauge. The right femoral artery remained intact. Arterial input pressure was therefore the systemic pressure of the animal. A siliconized polyethylene cannula with an I. D. of 1 mm was introduced into the right peripheral end of femoral vein, and led to a reservoir through a photocell drop counter. The tip of venous tube was fixed at the heart

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position, which was taken as the zero reference level for pressure measurements. From the reservoir the blood was returned to the left femoral vein by a controllable roller pump. We excluded the skin blood flow from the lower leg with a ligature at the level of tarsus. The electric pulse signal from the photocell circuit was passed into an interval-voltage converter and its output was recorded as blood flow per minute. A few drop of blood were collected for analysis at several times during the experiment.

A short period of muscle exercise was produced by sciatic nerve stimulation for 1 minute. A light rhythmic contraction was caused by pulses of 5–12V, with a 0.5–2 msec duration and a frequency of 1–5 Hz whereas severe tetanic contraction was produced by increasing the frequency to 10–50 Hz at the same intensity and duration. Prolonged muscle exercise for 30 min was produced by sciatic nerve stimulation at 5 Hz for 30 sec each minute. To study the relationship between contraction force and blood flow, muscle tension force of the lower flexor muscles was recorded isometrically by connecting the Achilles tendon to a force transducer. The resting muscle was stretched to the length associated with maximum twitch tension.

Reactive hyperemia was produced by occluding and releasing the right femoral artery. A 1-min ischemic contraction was performed 2 or 3 min after onset of 5 min occlusion of the artery.

Hematocrit, protein concentration and plasma osmolality were determined before, during and after exercise. A few drops of venous blood were collected for analysis from the photocell counter system. The blood was introduced into a small glass tube (I. D. 1.0 mm, length 70 mm) and analyzed for hematocrit by centrifugation for 5 min at 12,000 rev/min. No correction was made for trapped plasma. The total plasma protein content (g/dl) of the sample was measured with a D-Z type (II) refractometer. The residual plasma after the analysis of hematocrit and protein concentration was used for the measurement of plasma crystalloid osmolality by a cryoscopic osmometer (H. Knauer & Co.). The change in percent plasma volume was calculated from the change in hematocrit according to a formula presented by van Beaumont (4).

In preliminary experiments, we observed no significant changes in hematocrit, protein concentration and osmolality in the carotid artery during exercise of right hindlimb and norepinephrine injection.

The effect of vasoconstriction on the hematocrit of venous blood from the muscle was studied by intravenous systemic injection of norepinephrine (5–10 μg/kg body weight). Changes in arterial pressure, venous outflow, hematocrit, protein concentration and osmolality were measured simultaneously. Vascular resistance unit (PRU) was calculated by dividing femoral arterial pressure by blood flow per 100 g of tissue (mmHg • 100g • min • ml^{-1}).

P_{O_2}, P_{CO_2}, and pH in venous outflow were measured with IL meter (IL Co.) after sampling in a covered space of photocell circuit.

Statistical comparison of results before and after exercise was carried out by Student's t-test on paired data.

Results

Short exercise: Two patterns of exercise hyperemia

The characteristics of exercise and postexercise hyperemia of skeletal muscle in rabbit hindlimbs

are illustrated in Fig. 1. Generally, two different types of blood flow increase during exercise were observed.

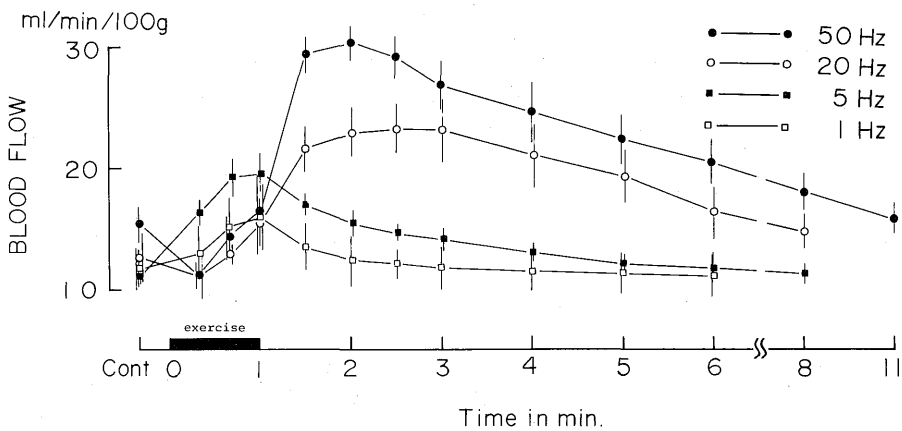

Fig. 1. Average time course of blood flow during and after exercise under a spontaneous arterial pressure at 80 mmHg.

When systemic arterial pressure was low, approximately 80 mmHg, light rhythmic exercise increased the blood flow immediately and it then rose to twice the resting (Fig. 1). Several tetanic contraction initially decreased the blood flow but, 15–20 sec later, flow increased gradually during tetanic contraction and continued to rise for 60 sec after contraction ceased. The recovery time of postexercise hyperemia was prolonged as the frequency of the stimulation increased: about 4 min at 5 Hz, 6–7 min at 10 Hz and 8–13 min at 20–50 Hz. The degree of postexercise hyperemia also depended upon the intensity of exercise.

When arterial pressure was spontaneously higher (average 117 mmHg), a rapid increase in flow occurred during both types of muscle contraction, and recovery time was shorter, about 2 min at 5 Hz and 4 min at 20 Hz.

During tetanic contraction, venous outflow initially decreased, but when force fell to 75% of control, flow slowly increased. The magnitude of initial decrease of venous outflow and the time to the inflection point depended largely upon the level of arterial pressure. When the spontaneous arterial pressure was higher than 130 mmHg, the initial decrease of venous outflow was less and the time to the inflection point was shorter.

Hematocrit measurement was taken frequently during the exercise and postexercise hyperemia as shown in Fig. 2 and Tables 1 and 2. With reference to the resting value, the maximal value of hematocrit was 107.8% at 5 Hz stimulation and 110.5% at 50 Hz stimulation. In one rabbit, the peak hematocrit was 120% of control during 50 Hz. These data showed a significant difference from the resting value ($p < 0.01$). The hematocrit increase occurred within a few seconds after the initiation of contraction by stimulation of 5 Hz or greater frequencies, and the rate and degree of hematocrit increase were in proportion to the severity of contraction. During postexercise hyperemia, however, hematocrit decreased progressively to the control level at the same rate as the decrease of hyperemia.

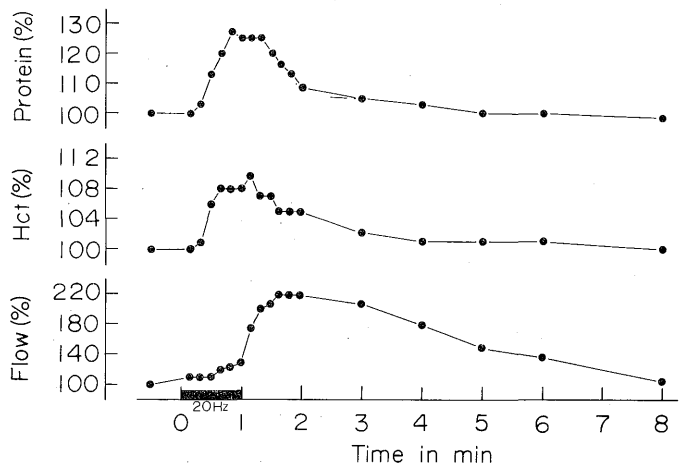

Fig. 2. Changes in venous outflow, hematocrit and total plasma protein concentration during and after exercise (20 Hz).

The total plasma protein concentration was determined as frequently as the hematocrit measurement (Fig. 2, Tables 1 and 2). The increase of protein concentration during exercise was in proportion to the frequency of contraction, but it was always larger than the rise of hematocrit. The maximal protein content was 115.4% at 5 Hz ($p < 0.01$), and 127.1% at 50 Hz ($p < 0.01$). An extreme example reached 146% at 50 Hz (protein value from 7.0 to 10.2 g/dl). During postexercise hyperemia, protein concentration decreased progressively to the control level in parallel with hematocrit and blood flow. Neither hematocrit nor protein concentration displayed a negative phase.

The mean value of resting venous plasma osmolality taken at the femoral vein near the muscle was 321.3 ± 9.9 mOsm/kg ◦ H_2O in 15 rabbits. During both light and heavy exercise, the maximal osmolality was obtained at the end of 1-min exercise (Tables 1 and 2). The maximal value of venous osmolality was 109.1% at 5 Hz ($p < 0.01$), and 114.3% at 50 Hz ($p < 0.01$). The rate of increase and the length of recovery time were greater at 50 Hz than 5 Hz. Only a slight increase in osmolality was observed at 1 Hz, but was statistically insignificant.

Presented in Tables 1 and 2 are the data on transcapillary plasma fluid loss (-PV%) calculated according to van Beaumont's equation from the hematocrit value in venous outflow before, during and after muscle exercise. Maximal plasma fluid loss was 12.5% in the last part of 60 sec exercise at 5 Hz, and 16.3% at 50 Hz.

Table 1. Time course of exercise hyperemia evoked by 5 Hz stimulation and changes of venous Hct, plasma fluid loss, plasma total protein and plasma osmolality.

	Exercise (sec)			Postexercise (min)						
	control	40	60	0.5	1	2	3	4	5	7
Hct (%)	42.3 ± 0.7 (14)	44.9 ± 1.2 (7)	45.6 ± 1.2 (7)	44.3 ± 1.0 (9)	44.0 ± 0.9 (12)	43.8 ± 1.0 (12)	42.9 ± 1.1 (9)	42.7 ± 0.8 (11)	42.4 ± 1.0 (10)	42.3 ± 1.2 (9)
ΔPV%*	0	−10.0	−12.5	− 7.8	−6.7	−5.9	−0.9	−0.9	−0.4	0
Flow (ml/100g•min)	11.0	19.4	19.5	17.2	15.7	14.4	13.4	12.6	12.0	11.5
Net filtration (ml/100g•min)**	0	1.9	2.4	1.3	1.1	0.8	0.1	0.1	0.1	0
Protein (g/dl)	6.5 ± 0.3 (9)		7.5 ± 0.4 (8)		7.2 ± 0.5 (8)	7.1 ± 0.5 (8)	6.9 ± 0.4 (9)	6.6 ± 0.4 (8)	6.5 ± 0.3 (9)	
Plasma osmolality (mOsm)	324.6 ± 4.1 (14)	334.6 ± 4.3 (14)	354.1 ± 5.1 (14)		350.6 ± 9.3 (12)	346.8 ± 8.6 (13)		338.6 ± 6.6 (14)		332.5 ± 7.0 (11)

Mean ± S. E. (n)

* calculated from van Beaumont equation: $\Delta PV(\%) = [100/(100 - \text{Hct cont.})] \times \{[100 - (\text{Hct cont.} - \text{Hct exer.})] / \text{Hct exer.}\}$

** calculated from: Net filtration (ml/100g•min) = ΔPV(%) × Flow (ml/100g•min)

Table 2. Time course of exercise hyperemia evoked by 50 Hz stimulation and changes of venous Hct, plasma fluid loss, plasma total protein and plasma osmolality.

	Exercise (sec)			Postexercise (min)							
	control	40	60	0.5	1	2	3	4	5	7	10
Hct (%)	42.0 ± 0.7 (11)	44.5 ± 1.1 (6)	46.4 ± 1.0 (11)	45.5 ± 1.3 (6)	44.3 ± 0.9 (11)	43.4 ± 0.8 (11)	42.9 ± 0.8 (10)	42.6 ± 0.7 (11)	42.2 ± 0.8 (10)	42.2 ± 0.7 (11)	41.8 ± 0.7 (11)
ΔPV%*	0	−9.7	−16.3	−15.9	−8.9	−5.2	−3.6	−2.4	−0.8	−0.8	+0.4
Flow (ml/100g•min)	15.5	14.4	16.5	29.5	30.6	27.3	24.7	22.4	20.5	17.8	15.8
Net filtration (ml/100g•min)**	0	1.4	2.7	4.7	2.6	1.4	0.9	0.5	0.2	0.1	0.1
Protein (g/dl)	5.9 ± 0.3 (11)		7.5 ± 0.7 (11)	7.4 ± 0.7 (7)	7.0 ± 0.4 (10)	6.5 ± 0.4 (11)	6.5 ± 0.4 (9)	6.3 ± 0.4 (10)	6.3 ± 0.4 (10)	6.2 ± 0.3 (10)	6.2 ± 0.3 (8)
Plasma osmolality (mOsm)	319.2 ± 4.4 (13)	342.2 ± 5.9 (13)	364.9 ± 6.0 (13)		352.2 ± 5.9 (13)	340.8 ± 6.4 (13)		330.9 ± 5.3 (13)		324.5 ± 5.1 (13)	

Mean ± S. E. (n)

* calculated from van Beaumont equation: $\Delta PV(\%) = [100/(\text{Hct cont.} - \text{Hct exer.})] \times \{[100 - (\text{Hct cont.} - \text{Hct exer.})] / \text{Hct exer.}\}$

** calculated from: Net filtration (ml/100g•min) = ΔPV(%) × Flow (ml/100g•min)

Prolonged exercise

During 30-min contraction of hindlimb muscle induced by interval stimulation (30 sec exercise per min) of the sectioned sciatic nerve at 5 Hz, peripheral vascular resistance (PRU) decreased rapidly to 45% of the control and then increased gradually to 48% at the end of 30-min contraction ($p < 0.01$) (Table 3). Maximal tension force developed in the initial stage of exercise decreased to 65% at the final stage of exercise. Hematocrit, protein concentration and osmolality rose to 104.2%, 111.3% and 106.6% within 5 minutes after onset of exercise ($p < 0.01$), respectively, and then all of them decreased gradually. Venous Po_2 decreased to 74.1% at 2 minutes after the start of exercise, and at the end

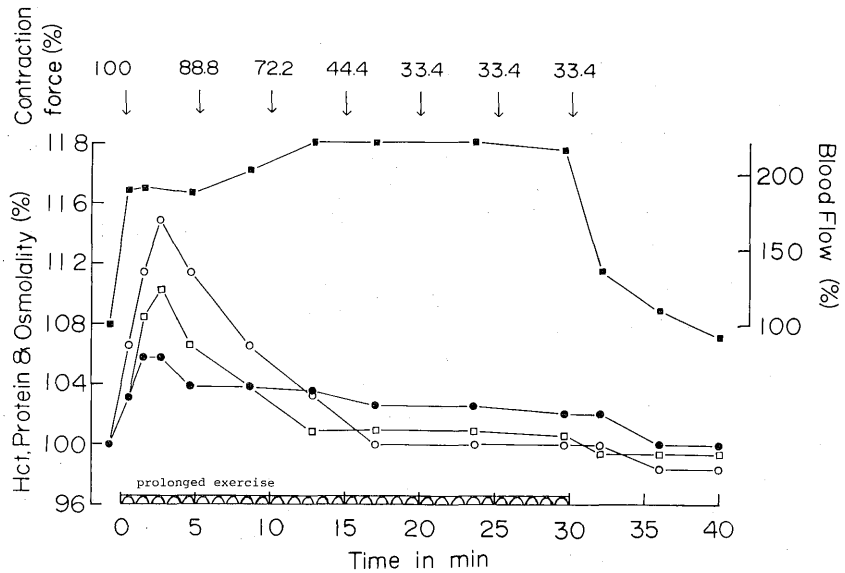

Fig. 3. A typical example of prolonged exercise hyperemia and changes of hematocrit, total plasma protein concentration, osmolality and tension force of lower flexer muscles.

Table 3. Changes of vascular resistance, blood gases and tension force before, during and after prolonged exercise.

			Exercise					Recovery
	n	Control	2′	5′	10′	20′	30′	10′
Resistance (mmHg·100g·ml^{-1})	13	12.8 ± 0.9	5.8 ± 0.5	5.9 ± 0.6	5.9 ± 0.6	6.0 ± 0.5	6.1 ± 0.5	11.0 ± 0.6
Hct (%)	13	41.8 ± 1.0	43.5 ± 1.1	43.6 ± 1.1	43.2 ± 1.1	42.5 ± 1.1	42.1 ± 1.1	41.3 ± 1.0
Total plasma protein (g/dl)	13	5.9 ± 0.2	6.6 ± 0.2	6.5 ± 0.2	6.3 ± 0.2	6.0 ± 0.2	5.9 ± 0.2	5.8 ± 0.2
Plasma osmolality (mOsm/Kg·H_2O)	13	335.4 ± 2.3	353.1 ± 4.5	357.8 ± 5.4	349.1 ± 4.0	341.6 ± 4.0	339.8 ± 4.0	336.8 ± 3.2
PO_2 (mmHg)	7	46.5 ± 1.5	34.5 ± 2.5	33.4 ± 2.5	32.4 ± 2.9	31.7 ± 2.3	31.5 ± 1.8	44.7 ± 2.9
PCO_2 (mmHg)	7	30.3 ± 2.4	47.7 ± 4.3	52.2 ± 4.7	50.9 ± 4.4	46.5 ± 2.7	42.6 ± 3.3	29.9 ± 2.8
H^+ (nM)	8	44.3 ± 2.2	61.3 ± 3.2	71.0 ± 5.1	67.6 ± 4.5	60.9 ± 4.1	56.4 ± 3.6	47.2 ± 3.8
Tension force (g) (M. gastrocnemius)	6		153.3 ± 24.4	145.0 ± 23.8	135.8 ± 23.0	121.2 ± 21.9	102.2 ± 21.8	

of 30-min exercise its level reached 67.5% ($p < 0.01$). Venous P_{CO_2} and H^+ increased and reached maximum at 5 minutes ($p < 0.01$). In preliminary experiments we observed no changes of blood constituents and gases in the sample of carotid artery during muscle exercise of hindlimb. Fig. 3 showed a typical example of prolonged exercise in which hematocrit, protein concentration, osmolality and tension force decreased at 5 min after onset of 30-min exercise, whereas the increased exercise hyperemia was maintained approximately constant.

Ischemic exercise

Venous outflow from the skeletal muscle decreased rapidly to 50–60% of the resting at the onset of femoral artery occlusion and remained approximately constant until 5-min occlusion was released. The peak flow after release of occlusion was 150% above control.

Fig. 4 demonstrates a typical example of ischemic exercise on venous outflow, hematocrit and protein concentration. A 1-min muscle contraction during 5-min occlusion produced only a slight increase in verous outflow and large increase in hematocrit and protein concentration. Table 4 shows the maximal changes of hematocrit, protein concentration and osmolality immediately after the release of 5-min occlusion (control) and ischemic exercise (5 and 50 Hz). During 5-min occlusion, for the first, hematocrit tended to decrease to 98.6 ± 0.6% ($n = 10$, $p < 0.2$) at 1 minute after the initiation of occlusion, and then increased gradually to 102.4 ± 0.4% ($p < 0.01$) until the occlusion was released.

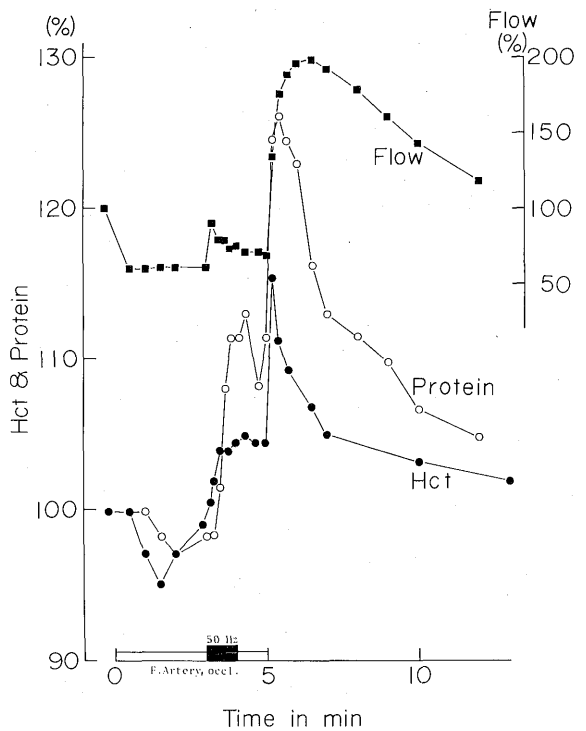

Fig. 4. A typical example of ischemic exercise hyperemia and changes of hematocrit, and total plasma protein concentration during and after ischemic contraction by 50 Hz.

Immediately after release of 5-min occlusion hematocrit increased rapidly to 105.3 ± 0.4% ($p < 0.01$). The blood flow and hematocrit returned to the preocclusion levels within 2 munutes after release. The hematocrit immediately after a 1-min ischemic exercise was 105.9 ± 1.5% ($n = 5$, $p < 0.01$) at 5 Hz, and 107.9 ± 2.2% ($n = 8$, $p < 0.01$) at 50 Hz stimulation. After the release of 5-min occlusion with ischemic exercise hematocrit reached 108.8 ± 1.7% ($n = 5$, $p < 0.01$) at 5 Hz and 111.3 ± 2.1% ($n = 8$, $p < 0.01$) at 50 Hz. During post-ischemic exercise hematocrit progressively returned to the control levels within 10 minutes. Maximal plasma loss calculated (-PV%) was 7.8%, 13.1% and 15.9% during the control occlusion, 5 and 50 Hz ischemic exercise, respectively. Maximal increase of protein concentration was 102.3 ± 0.6% ($n = 7$, $p < 0.01$) at the end of 5-min control occlusion, 113.2 ± 1.5% ($n = 5$, $p < 0.01$) during 5 Hz and 121.5 ± 3.0% ($n = 6$, $p < 0.01$) during 50 Hz ischemic exercise. Maximal plasma osmolality was 102.5 ± 0.5% ($n = 7$, $p < 0.01$) at the end of 5-min control occlusion, 110.9 ± 1.4% ($n = 5$, $p < 0.01$) during 5 Hz and 113.2 ± 2.4% ($n = 6$, $p < 0.01$) during 50 Hz ischemic exercise.

Table 4. Maximal percent changes of hematocrit, total plasma protein concentration and osmolality immediately after release of 5-min occlusion (non-exercise) and ischemic exercise (5 and 50 Hz).

	non-exercise	5 Hz	50 Hz
Hematocrit	105.3 ± 0.4 n = 10 p < 0.01	108.8 ± 1.7 n = 5 p < 0.01	111.3 ± 2.1 n = 8 p < 0.01
Protein	102.3 ± 0.6 n = 7 p < 0.01	113.2 ± 1.5 n = 5 p < 0.01	121.5 ± 3.0 n = 6 p < 0.01
Osmolality	102.5 ± 0.5 n = 7 p < 0.01	110.9 ± 1.4 n = 5 p < 0.01	113.2 ± 2.4 n = 6 p < 0.01

Effects of Norepinephrine and Severe Hypoxic Hypoxia

Intravenous injection of norepinephrine (5–10 μg/kg) decreased venous outflow to 70% within 2 minutes after the injection of norepinephrine. The maximal vascular resistance reached 200% of resting value (Fig. 5). At this time, hematocrit was 97.5 ± 0.2% ($n = 9$, $p < 0.01$) of control. Plasma total protein concentration showed a slight increase after the injection of norepinephrine (102%). The effect of intravenous epinephrine injection (7–15 μg/kg) on vascular resistance, hematocrit and total plasma protein concentration was approximately the same result as that of norepinephrine.

Fig. 6 shows the increase of local blood flow from the muscle during severe hypoxia (below 5% oxygen), accompanied by marked increase of systemic blood pressure. However, there was no change in hematocrit, protein concentration and osmolality in the venous blood.

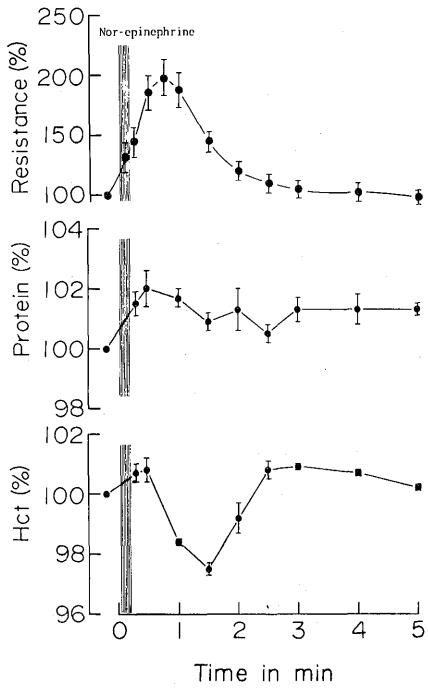

Fig. 5. Vascular resistance, hematocrit and total plasma protein concentration in the resting muscle before, during and after intravenous injection of norepinephrine.

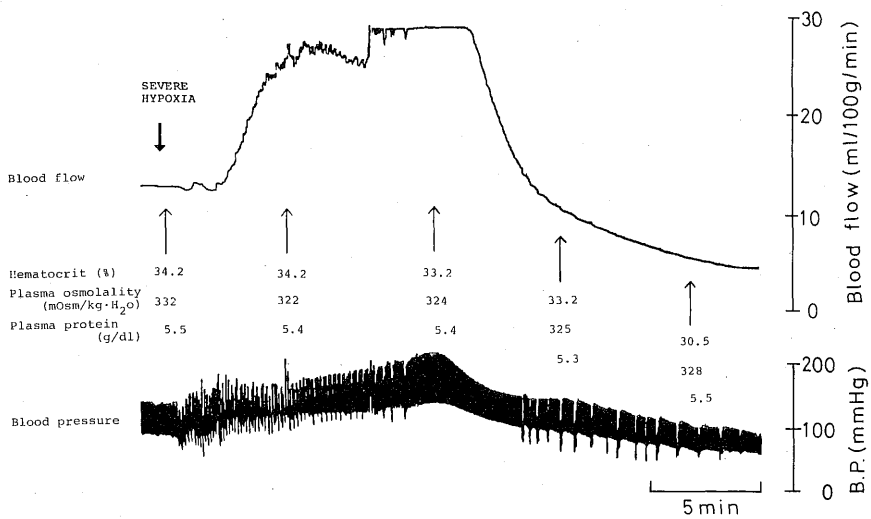

Fig. 6. Effect of severe hypoxic hypoxia on local blood flow, hematocrit, total plasma protein concentration and osmolality. As compared with the increase of venous outflow, hematocrit and protein indicated no change.

Discussion

Utilizing the non-isolated hindlimb preparation of the rabbit, the results confirm that, as in other species local blood flow increases in proportion to the severity of muscle exercise and hematocrit, protein concentration and osmolality of venous blood from the muscle rise. Although the increase of hematocrit and protein concentration during muscle exercise, thereby suggesting hemoconcentration, paralleled with the venous outflow in the light and short exercise, the same phenomenon was not observed in the heavy and prolonged exercise, ischemia, ischemic exercise and severe hypoxic load. Furthermore, the percent increase of protein concentration is always higher than hematocrit in outflow from the various exercising muscle. No satisfactory explanation of these phenomena has yet been proposed.

The increase in hematocrit and plasma protein concentration during whole body and arm exercise have been reported earlier by Kaltreider et al (16) and Joye et al (15). Schlein et al (23) also demonstrated the increase of hematocrit, protein concentration and potassium in the isolated, in situ gracilis muscle preparation of the dog. Filtration of plasma fluid into the tissue space considered the cause of these changes (1, 2, 4, 7, 15, 16, 21).

To answer the question of whether the increase in hematocrit and protein concentration are specific to muscle contraction or results from an increased blood flow in the muscle tissue, we quantified the relationship between the magnitude of hematocrit and protein concentration, the increase in blood flow, and the severity of muscle contraction in various conditions. There was always found a proportional relationship between the severity of contraction and the increase in venous hematocrit and protein concentration suggesting that the hematocrit and protein changes are closely related to muscle contraction.

The mechanism of plasma fluid loss in the contracting muscle is probably multifaceted (3, 6, 11, 12, 19). Dilatation of the precapillary vessels presumably induced by metabolites will elevate the capillary filtration pressure and will also increase the number of patent exchange vessels (11, 17, 23). Compression intensities of postcapillary vessels by contraction modes may elevate the capillary filtration pressure. Ischemia produces a slight increase in hematocrit and protein concentration, and ischemic contraction has a greater effect. The other mechanism of plasma fluid movement is presumablly the osmotic gradient produced by the metabolites, mainly lactate and HPO_4^{--}, to the interstitial space and capillary (11, 17, 18, 19, 23). All these factors will facilitate the fluid shift into the extravascular space. The increased tissue fluid will moderate the increase in the tissue osmolality and at the same time promote the lymph flow from the muscle (14). These mechanism undoubtedly aid in the homeostasis of the tissue, especially in the dynamic homeostasis of the prolonged exercising tissue.

That the plasma fluid filtration is not the only mechanism for hematocrit change in venous outflow is suggested by our experiment demonstrating maximal effect of norepinephrine injection. During injection of norepinephrine intravenously, systemic blood pressure increases rapidly. This, of itself, suggests the movement of plasma fluid toward tissue. As a matter of fact, protein in venous outflow increases, whereas the hematocrit decreases significantly. Norepinephrine (1.5–3.0 μg base/min) decreases forelimb weight markedly initially and then more slowly with time (11). It is therefore inferred that the rapid decrease in venous hematocrit during norepinephrine injection is resulted from the decreased diameter of arterio-venule system (9, 10) which tends to increase the ratio of plasma

layer thickness to the vessel diameter and/or from the constriction of precapillary sphincter which tends to obstruct more red cell passing through the capillaries. The same rheological hypothesis may be also introduced to the mechanism of venous hematocrit increase during 5-min occlusion without exercise. It is assumed that the hematocrit increase comes partly from the decrease of plasma layer thickness associated with lowering of the flow rate by occlusion and the increase of vessel diameter caused by low oxygen tension and metabolites.

The hematocrit changes in venous outflow may also be discussed from a hypothesis of the functional dual blood flow in the local tissue. In our prolonged exercise, we observed a gradual decrease of hematocrit, protein concentration and osmolality from the maximal increase in a few minutes after the onset of 30-min exercise, while the venous outflow increase and the decreased PO_2 were kept approximately constant. As demonstrated in Fig. 6, a marked increase of local blood flow was observed in our experiment without any change of hematocrit and protein concentration. The data also indicated that the resistance of venous circuit is not the cause of hematocrit and protein concentration increases. A postulate may be made that blood flows through the macrovascular arterio-venous shunts, microvascular nutritive and non-nutritive channels (13). The nutritive capillary channels may open during exercise. The arterio-venous shunt and the non-nutritive channels may open during rapid increase of venous return. In the excised muscle preparation, Morganroth et al (20) suggested the unknown chemical factor as for the vasodilation during prolonged exercise after they discussed many factors including low PO_2, osmolality and metabloites.

It has been demonstrated that physical exercise (22) and arm exercise (15) produce greater increase in total plasma protein concentration than increase of hematocrit. These observations were confirmed repeatedly in our experiment of rabbit hindlimb preparation. Although the movement of protein molecules in microvascular systems have been reported by some investigators (5, 22), the reason for the greater increase during muscular exercise is not yet clear.

In conclusion, it is appeared that short and ischemic exercise sets largely the nutritive capillary flow channel to work, which is associated with fluid movement, and prolonged one activates gradually the joining of non-nutritive flow channel in proportion to the degree of muscle work severity or of muscle fatigue.

Conclusion

The effects of muscular exercise on the muscle blood flow, venous hematocrit, plasma protein concentration and osmolality were studied on intact hindlimb of rabbits anesthetized with urethane.

1). With 1-min exercise, the increase of muscle blood flow and hematocrit depended upon the work severity. With reference to the resting value, the maximal hematocrit was 107.8% at 5 Hz stimulation and 110.5% at 50 Hz ($p < 0.01$). The maximal increase of total plasma protein concentration was 115.4% at 5 Hz and 127.1% at 50 Hz ($p < 0.01$). The maximal plasma crystalloid osmolality was 109.1% at 5 Hz and 114.3% at 50 Hz ($p < 0.01$).

2). In the prolonged exercise, the elevated venous outflow was approximately constant until the end of 30-min exercise, whereas hematocrit, protein, osmolality and tension force reached maximal levels a few minutes after the onset of exercise and thereafter decreased gradually.

3). When blood flow was reduced to 50–60% of the normal value by femoral artery occlusion, hematocrit tended to decrease at 1 minute after the initiation of occlusion, and then increased gradual-

ly above the control. One minute exercise during the occlusion caused hematocrit, protein concentration and osmolality to rise to approximately the same levels as during unrestricted flow. The maximal hematocrit immediately after release of occlusion reached 108.8% at 5 Hz and 111.3% at 50 Hz ($p < 0.01$).

4). Intravenous injection of norepinephrine decreased the hematocrit to 97.5% ($p < 0.01$), whereas the protein concentration increased to 102.0%.

These results support the view that the hematocrit increase in venous blood from the exercising muscle is mainly due to loss of plasma water and depends upon the severity of muscle work. The more complicated mechanism including the modes of muscle exercise, functional dual blood flow and rheological regulation of microvascular system may be necessary to explain the fact that the change of hematocrit value is less proportional to the change of muscle blood flow.

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各種の運動負荷による局所筋血流，hematocrit，plasma protein および osmolality の変動について

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著者らはこれまで筋持久力の問題を組織微小循環生理の側面より実験的に分析してきたが，とくに微小循環網と細胞・線維間の組織水動態（流れ，貯留）には深い関心をもっている。すなわち，毛細血管から組織への水供給や組織から毛細血管・リンパ系への水回収等この組織水はO_2・CO_2の拡散を律速する拡散因子の1つとして運動時でも重要な役割を果すと思われるからである。本論文はこのような仮説を実験的に検討したいという動機を有しており，この水移動には循環系を考慮にいれた血液組成すなわち hematocrit や plasma protein の情報量がモデルになると考えたわけである。また，筋組織からの静脈血 plasma osmolality は毛細血管――組織間液の電解質情報をうる手段として活用をすすめたのである。本研究ではウレタン麻酔下のウサギ後肢筋を生体内標本のかたちで利用しているためにリンパ系の関与を前提にしている。結果は次の通りである。

1). 1分間の運動（short exercise，坐骨神経の電気刺激）では運動強度の増大によりヘマトクリットおよび筋血流が増加した。このときの流出静脈血における最大ヘマトクリットは control に比して5 Hz 刺激強度で 107.8％，50 Hz で 110.5％に増加した（$P < 0.01$）。蛋白濃度は5 Hz で 115.4％，50 Hz で 127.1％の最大値を得た（$P < 0.01$）。同一サンプルの浸透圧は5 Hz で 109.1％，50 Hz で 114.3％に増加した（$P < 0.01$）。

2). 30分間の運動（prolonged exercise）では筋血流の増大とその定常状態は維持されたが，いっぽう，ヘマトクリット，蛋白濃度，浸透圧および筋張力（g）はともに定常でなかった。すなわち，これらは運動開始後2～3分の最大値を境いにその後漸減し，運動終了後はすみやかに control 値に復帰した。

3). 筋の虚血時運動（ischemic exercise）によるヘマトクリットは開始後の最初の1分間で減少する傾向にあるがその後運動前値をうわまわる経時的漸増が確認された。虚血性運動の終了後でかつ大腿動脈の閉塞解除直後のヘマトクリットは5 Hz で control の 108.8％，50 Hz で 111.3 に達した（$P < 0.01$）。

4). ノルエピネフリンの静注で筋血流は減少したが，同時にヘマトクリットも control の 97.5％に減少した（$P < 0.01$）。いっぽう，蛋白濃度は 102.0％を示し，この理由は不明である。（先行実験として，総頸動脈部位よりの同時サンプルで得た動脈血ヘマトクリットは下肢の一連の実験中に変動しないことを確認している）。

これらの結果より，造血器官を内包しない局所の筋運動の場合も，ヘマトクリットの増加現象が短時間に反復して観察されるところから，血液の濃縮すなわち毛細血管から周辺組織へ水移動が行なわれたものと判断する。いっぽう，短時間の激運動や長時間運動では筋血流と上記血液組成に経時変化の対応がみられなかったが，これについては筋運動の様式や筋疲労，血管レオロジー，二相血流路などの影響を考えるものである。末梢組織における微小循環と細胞間液層の動力学が局所と全身のホメオスティシス，performance，安全，疲労などにどのようなかたちで役割を演じているかについては今後さらに検討をすすめていくつもりである。