

## HIGH FLOW DOES NOT ABOLISH THE REDUCED MAXIMUM OXYGEN UPTAKE IN DOG MUSCLE PERFUSED WITH 2, 3-DIPHOSPHOGLYCERATE-DEPLETED BLOOD

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*Abstract*: To clarify the limiting factors for maximum oxygen consumption ( $\dot{V}_{O_{2max}}$ ) in contracting skeletal muscle, we perfused isolated dog gastrocnemius muscle at a high flow rate with two kinds of blood which have different affinities for oxygen.  $\dot{V}_{O_{2max}}$  was decreased significantly in the perfusion with DPG-depleted blood and the reduction was shown to have resulted from a lower  $O_2$  extraction ratio.  $\dot{V}_{O_2}$  and  $O_2$  extraction were closely proportional to the reciprocal of resistance to blood flow. Furthermore, the different  $O_2$  affinities of the perfusion blood affected these relationships: the slopes for  $\dot{V}_{O_2}$  and  $O_2$  extraction against the reciprocal of resistance for DPG-restored blood were significantly higher than those for DPG-depleted blood. We assumed that red cell transit time is inversely proportional to the resistance to blood flow and that high  $O_2$ -affinity blood is inherently slow to unload  $O_2$  from red cells. The present results are well explained by our assumption. The results suggest that  $\dot{V}_{O_{2max}}$  may be determined by red cell transit time and  $O_2$  unloading from the red cell.

### Index Terms

erythrocyte, exercise, gastrocnemius, oxygen dissociation curve, oxygen uptake

### INTRODUCTION

High oxygen ( $O_2$ )-affinity of the blood has been reported to decrease the oxygen consumption ( $\dot{V}_{O_2}$ ) of organs with high metabolism like the heart and brain under fixed flow conditions or at constant  $O_2$  delivery<sup>1,19)</sup>, and to lead to an increase in blood flow to these tissues<sup>18)</sup>. It is not yet evident whether or not this increase could compensate for the tissue hypoxia induced by the elevated  $O_2$  affinity of blood.

Recently Honig et al<sup>7)</sup> have put forward the notion that oxygen consumption in contracting skeletal muscle is determined by red cell transit time and the  $O_2$  releasing time from red cells. If this notion holds true under conditions of maximum oxygen consumption ( $\dot{V}_{O_{2max}}$ ), the maximum rate of  $O_2$  uptake would be attained by the maximum transit time<sup>13)</sup> and the maximum rate of  $O_2$  off-loading from the erythrocyte. Determinants of mean circulation time are bulk flow and vascular bed volume. Assuming that vascular resistance reflects the vascular bed volume in the way that high bed volume decreases resistance and low bed volume increases resistance, and that mean circulation time is proportional to mean red cell transit time in the capillary, resistance to blood flow could relate to the transit time. When blood flow is maintained constant, the resistance is inversely proportional to mean red cell transit time.

Thus, lower resistance would allow the red cell to spend a longer transit time in the capillary. On the other hand, high  $O_2$  affinity blood has a low quasi-first order velocity constant<sup>2)</sup>, and this blood releases a lower amount of  $O_2$  in a given time. Therefore,  $\dot{V}_{O_{2max}}$  would be attained with lower resistance and higher  $O_2$  release kinetics. Because high flow induces shorter transit times in a maximally dilated vascular bed, it is doubtful whether high flow can abolish the reduction in  $\dot{V}_{O_2}$  that occurs with high  $O_2$  affinity blood.

The aim of the present experiment was to evaluate the influence of a high flow rate<sup>13)</sup> on  $\dot{V}_{O_{2max}}$  in muscle perfused with high  $O_2$  affinity blood. We perfused dog gastrocnemius muscle with DPG-restored and-depleted blood and measured  $O_2$  uptake, blood gas values, and resistance to blood flow. Our results indicate that  $\dot{V}_{O_{2max}}$  depends on resistance to blood flow, which is related to mean red cell transit time, and on the  $O_2$  affinity of the blood. The high flow did not allow the muscle to maintain  $\dot{V}_{O_{2max}}$  during perfusion with high  $O_2$  affinity blood.

### MATERIALS AND METHOD

Twenty-seven mongrel dogs (weight 5.2 to 11.1 kg) were anesthetized with pentobarbital sodium (30 mg·kg<sup>-1</sup> i. v.) with additional doses given as necessary. The animals were ventilated with room air through a cuffed endotracheal tube by means of a respirator. The left gastrocnemius-plantaris muscle group was surgically prepared as described previously by Stainsby *et al.*<sup>14)</sup>. After the popliteal artery and vein were exposed, heparin (1,000 IU·kg<sup>-1</sup>) was administered intravenously. A cannula inserted into the popliteal vein served as a sampling port for venous blood. To prevent collateral circulation, the portion proximal to the insertion of lateral and medial head of gastrocnemius was ligated. All venous outflow from the gastrocnemius muscle was directed through the popliteal vein with the other vessels ligated and cut. The muscle was covered with a sheet of Saran Wrap and a thermostatically controlled IR-lamp was used to keep the surface of the muscle at 37 °C. The achilles tendon was severed close to the calcaneus and attached to a force transducer. The length of the muscle was adjusted at 10 g force · g muscle weight<sup>-1</sup> <sup>6)</sup>. The following equation was used to assess the weight of the muscle (M in g) from the body weight (BW in kg) :

$$M = 3.83 \times BW - 1.04 \quad (r = 0.866) \quad (1)$$

Bone pins were set in the distal end of the femur and anterior aspect of the tibia and then fastened to stationary metal posts to prevent the leg from moving during contractions. The sciatic nerve was sectioned and placed on a stimulating electrode. An arterial cannula, which was connected to a dual-tube heat exchanger (37 °C), was inserted into the popliteal artery. After cannulation, the muscle was perfused for 30 min under resting conditions and the flow rate was kept at about 10 ml·min<sup>-1</sup>·100 g<sup>-1</sup>.

The muscle was then stimulated via the sciatic nerve (4 V, 0.2 ms duration) to contract isometrically at a rate of 4 Hz, and perfused by a peristaltic pump so as to maintain a perfusion pressure of 120-140 mmHg at 3 min of contraction. The perfusion rate was set at the value ascertained at 3 min of contraction throughout the 15 min of contraction. The perfusion blood in the reservoir was stirred gently with a teflon-coated magnetic stirrer. The perfusion pressure was measured using a mercury manometer and blood flow determined by weighing the venous effluent collected over a 15-sec period in a tared glass vessel.

*Blood for perfusion* Normal O<sub>2</sub> affinity blood was prepared by incubating CPD-stored human red cells (storage for 1 week in 14 experiments and for 3 weeks in 3 experiments) with 8 mM inosine, 8 mM pyruvate and 6 mM phosphate at 37 °C for 10–15 min. Blood stored in CPD for 3 weeks (4 °C) was used as high O<sub>2</sub> affinity blood. The red cells were washed three times with Ringer-Krebs-Bulbring solution containing 4 % polyvinylpyrrolidone equilibrated with a 95 % O<sub>2</sub>/5 % CO<sub>2</sub> gas mixture. Finally, the red cells were suspended in the washing solution. The washing solution was passed through a filter of 1 μm pore size prior to use. The haematocrit was adjusted to 30 %. The base excess was adjusted to zero with 7 % bicarbonate. Blood-gas equilibration was performed using a bubble oxygenator for 15 min at 37 °C. The gas phase above the equilibrated blood in the reservoir was replaced by N<sub>2</sub> gas. The blood gas values of the equilibrated blood in the reservoirs at 4 °C did not change during the course of the experiment. Epinephrine and norepinephrine were added to the perfusate so that each was in a final concentration of 3 ng•ml perfusate<sup>-1</sup>. Our pilot experiment indicated that catecholamines were required for the development of normal tension in muscle perfused with an RBC suspension devoid of plasma<sup>12</sup>). We also added inosine to the perfusates (0.4 mM), because the residual inosine with which the red cells were incubated would induce vasodilation during perfusion in the case of the DPG restored blood.

Samples of venous blood were collected at rest and at 3, 5, 8, 10, 13 and 15 min of contraction. Arterial samples were drawn directly from the reservoir. All samples had their So<sub>2</sub> measured with an OSM-2 (Radiometer), and their pH, Pco<sub>2</sub>, and Po<sub>2</sub> with a blood gas analyzer (Radiometer, BMS-Mk 2).  $\dot{V}_{O_2}$  was calculated as follows:

$$\dot{V}_{O_2} = \text{flow} \times (\text{Ca}_{O_2} - \text{Cv}_{O_2}) \quad (2)$$

where Ca<sub>O<sub>2</sub></sub>-Cv<sub>O<sub>2</sub></sub> is arterio-venous O<sub>2</sub> content difference. The blood oxygen content in ml/dl (Co<sub>2</sub>) was calculated as follows:

$$\text{Co}_2 = 1.39 \times [\text{Hb}] \times \text{So}_2 + 0.0031 \times \text{Po}_2 \quad (3)$$

where [Hb] is haemoglobin concentration in g/dl, and So<sub>2</sub> is fractional O<sub>2</sub> saturation. Resistance to blood flow was calculated as mean arterial pressure divided by blood flow. Haemoglobin concentration was measured by cyanmet-Hb method. Haematocrit (Ht) was measured with a microhematocrit centrifuge. P<sub>50</sub> was determined by the single point method<sup>9</sup>). Lactate concentration was measured enzymatically.

For statistical analyses, the unpaired t-test was used.

## RESULTS

Table 1 presents haematological and blood gas data for the blood used for perfusion. The difference between the P<sub>50</sub> of DPG-restored blood and that of DPG-depleted blood was 6.5 torr. In the present experiment, we did not measure 2, 3-diphosphoglycerate in red blood cells, but we did estimate the DPG to Hb molar ratio from the P<sub>50</sub> values according to the relationship between DPG and P<sub>50</sub> for CPD-stored blood<sup>11</sup>). The DPG to Hb molar ratios for normal and high affinity blood were 1.2 and 0.6, respectively.

Resting O<sub>2</sub> uptake for DPG-restored and -depleted blood was 0.31 ± 0.10 and 0.38 ± 0.16 ml • min<sup>-1</sup> • 100 g<sup>-1</sup>, respectively. Resting blood flow for DPG-restored and -depleted blood was 9.13 ± 2.22 and 10.50 ± 6.20 ml • min<sup>-1</sup> • 100 g<sup>-1</sup>, respectively.

Table 1. Haematological and blood gas data from perfusion blood equilibrated with bubble oxygenator

	DPG-restored blood#	DPG-depleted blood##
P <sub>50</sub> (torr)	29.6±1.7	23.1±1.5*
DPG/Hb molar ratio	1.2	0.6
Ht (%)	32.3±1.4	32.4±1.2
Hb (g/dl)	12.7±0.5	12.8±0.5
MCHC (g/dl)	39.3±0.7	39.6±0.7
Sao <sub>2</sub> (%)	98.5±0.5	99.3±0.3
pH	7.401±0.024	7.395±0.023
Paco <sub>2</sub> (torr)	38.3±4.4	41.4±4.5
Pao <sub>2</sub> (torr)	89.9±4.2	90.0±3.2
Cao <sub>2</sub> (ml/dl)	17.7±0.6	18.1±0.6

Values are means ± SD. #; CPD-stored human red cells (for 1 or 3 weeks) incubated with 8 mM inosine and pyruvate, and 6 mM phosphate at 37 °C. ##; 1 mM adenine-CPD stored human red cells (for 3 weeks, 4 °C). P<sub>50</sub>, P<sub>o2</sub> at half oxygenation of blood; DPG, 2, 3-diphosphoglycerate; MCHC, mean cell haemoglobin concentration; Sao<sub>2</sub>, arterial oxygen saturation; Paco<sub>2</sub> and Pao<sub>2</sub>, arterial Pco<sub>2</sub> and Po<sub>2</sub>; Cao<sub>2</sub>, arterial O<sub>2</sub> content. \*Significantly different from DPG-restored blood (P<0.05).

Table 2. Correlation between the reciprocal of resistance and oxygen uptake

Expt. No.	DPG-restored blood			Expt. No.	DPG-depleted blood		
	r	slope	Y-intercept		r	slope	Y-intercept
1	0.985	7.658	0.687	1	0.977	3.321	4.878
2	0.934	6.842	4.414	2	0.807	6.354	1.314
3	0.925	11.530	-0.080	3	0.987	4.940	4.791
4	0.965	4.318	7.191	4	0.977	7.365	0.762
5	0.861	21.040	-27.370	5	0.942	5.869	3.614
6	0.939	7.387	-4.128	6	0.959	6.675	4.126
7	0.947	10.550	-3.593	7	0.990	10.959	1.323
8	0.956	10.890	-1.036	8	0.852	7.814	3.628
9	0.974	14.870	-11.790	9	0.929	2.247	6.067
10	0.843	13.440	-5.568	10	0.944	8.314	-1.187
11	0.968	20.400	-20.070	11	0.975	14.445	-10.777
12	0.956	13.730	-6.791	12	0.953	5.021	4.176
				13	0.934	3.067	4.480
				14	0.981	4.820	3.725
				15	0.980	3.186	7.080
Median	11.888*	-5.678*		Median	6.293*	2.533*	
	0.947 ±4.956	±9.504			0.959 ±3.240	±4.257	

Values are means ± SD, except for correlation coefficient (r), indicated with medians. Expt. No., experimental number. r, correlation coefficient between the reciprocal of resistance and oxygen uptake. Each regression line was calculated from data taken at six different times of contraction in each experiment. \*; Difference between DPG-restored blood and DPG-depleted blood was significant (p<0.05).

Table 3. Correlation between the reciprocal of resistance and oxygen extraction ratio

DPG-restored blood				DPG-depleted blood			
Expt. No.	r	slope	Y-intercept	Expt. No.	r	slope	Y-intercept
1	0.988	0.174	0.026	1	0.924	0.076	0.162
2	0.744	0.138	0.160	2	0.809	0.183	0.038
3	0.438	0.168	0.220	3	0.846	0.123	0.173
4	0.936	0.111	0.359	4	0.963	0.176	0.074
5	0.760	0.449	-0.565	5	0.892	0.165	0.126
6	0.765	0.154	-0.069	6	0.936	0.231	0.105
7	0.909	0.341	-0.131	7	0.964	0.263	0.155
8	0.958	0.390	-0.001	8	0.836	0.213	0.099
9	0.941	0.369	-0.259	9	0.846	0.054	0.159
10	0.715	0.502	-0.197	10	0.960	0.198	-0.029
11	0.942	0.661	-0.718	11	0.955	0.321	-0.142
12	0.853	0.263	-0.095	12	0.910	0.090	0.189
				13	0.948	0.089	0.118
				14	0.963	0.086	0.129
				15	0.673	0.036	0.223
Median	0.310*	-0.106*		Median	0.154*		0.105*
	0.853	±0.164	±0.294		0.924	±0.083	±0.093

Values are means ± SD, except for correlation coefficient (r), indicated with medians. Expt. No., experimental number. r, correlation coefficient between the reciprocal of resistance and oxygen extraction. Each regression line was calculated from data taken at six different times of contraction in each experiment. Oxygen extraction ratio was calculated by dividing arterio-venous oxygen difference by arterial oxygen content. \* ; Difference between DPG-restored blood and DPG-depleted blood was significant (P<0.05).

Tables 2 and 3 summarize the relation of oxygen uptake and O<sub>2</sub> extraction to resistance to blood flow. The median correlation coefficients for DPG-restored and -depleted blood were 0.947 and 0.959 for the relation of  $\dot{V}_{O_2}$  to the reciprocal of resistance (R), and 0.853 and 0.924 for the relation of O<sub>2</sub> extraction to R<sup>-1</sup>, respectively. The slope and Y-Intercept for DPG-restored blood were significantly different from the values for DPG-depleted blood (P<0.05).  $\dot{V}_{O_2}$  is directly proportional to the reciprocal of resistance, but the relationship was affected by the O<sub>2</sub> affinity of the blood. The higher slope of  $\dot{V}_{O_2}$  and O<sub>2</sub> extraction against R<sup>-1</sup> for DPG-restored blood seems to account for the higher amount of O<sub>2</sub> released from the low O<sub>2</sub>-affinity red cell.

Blood flow and O<sub>2</sub> delivery were fairly constant throughout the perfusion time (Fig. 1). Maximal oxygen uptake ( $\dot{V}_{O_{2max}}$ ) at 3 min for DPG-depleted blood was 12.17±2.49 and that for DPG-restored blood 14.22±2.49, the difference being statistically significant (P<0.05; Fig. 2). During 15 min of contraction, the  $\dot{V}_{O_2}$  for DPG-depleted blood remained significantly different from that for DPG-restored blood. After 15 min,  $\dot{V}_{O_{2max}}$  had decreased to 34 % for DPG-restored blood and 54 % for DPG-depleted blood. Resistance to blood flow at 3 min for DPG-restored and -depleted blood was 0.687±0.203 and 0.725±0.120, respectively, though the difference was not statistically significant. An increase in resistance during the contraction was observed in both types of perfusion and the difference between DPG-restored and -depleted blood became significant after 8 min (Fig. 3). After 15 min of contraction, the % increase in

resistance was 30 % for DPG-restored blood and 87 % for DPG-depleted blood. As for DPG-restored blood prepared with CPD-blood stored for 3 weeks, resistances at 3 min and 15 min were  $0.760 \pm 0.305$  and  $0.903 \pm 0.323$  ( $n=3$ ), respectively. DPG-restored blood invariably

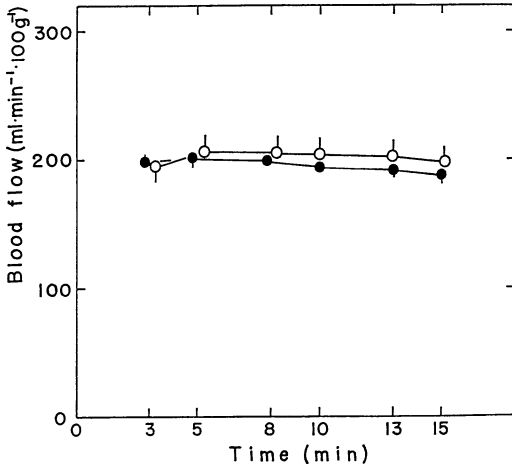


Fig. 1. Blood flow in canine gastrocnemius-plantaris muscle perfused with peristaltic pump at constantly high perfusion rate. Values are mean  $\pm$  SE. Open circles, DPG-restored blood; closed circles, DPG-depleted blood.

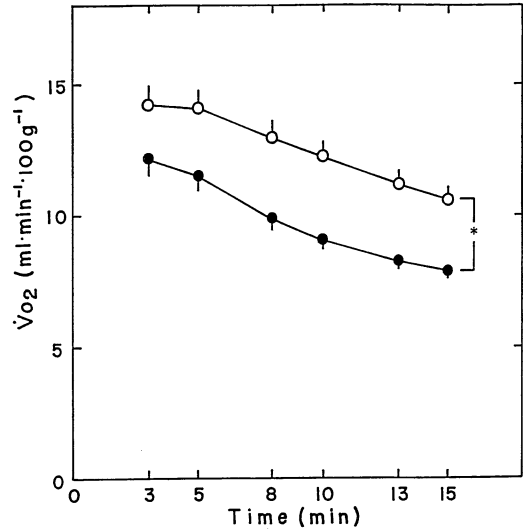


Fig. 2. The influence of DPG-depleted (closed circles) and restored blood (open circles) on  $O_2$  uptake during muscle contraction. The % decrease in  $O_2$  uptake is 54 % in DPG-depleted blood and 34 % in DPG-restored blood during the 15 min contraction period (\*:  $P < 0.05$ ). Values are mean  $\pm$  SE.

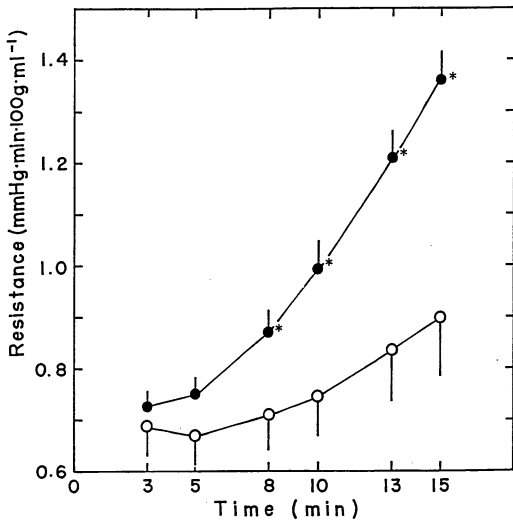


Fig. 3. The increase in resistance to blood flow during contraction. Resistance values for DPG-depleted blood (closed circles) are significantly higher than those for DPG-restored blood (open circles) after 8 min of contraction (\*:  $P < 0.05$ ). Values are mean  $\pm$  SE.

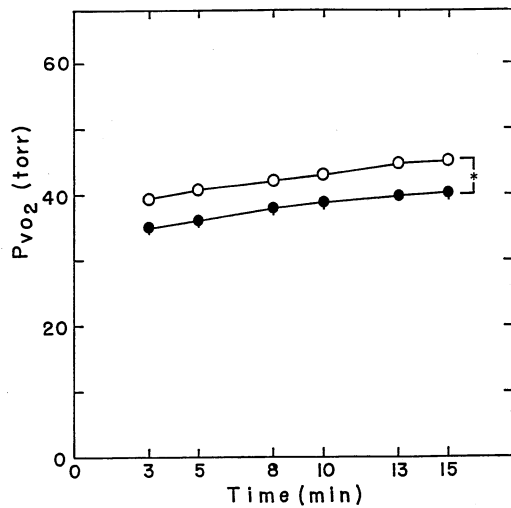


Fig. 4.  $P_{O_2}$  in venous effluent ( $P_{vo_2}$ ) during contraction; the difference between DPG-depleted (closed circles) and -restored (open circles) blood is statistically significant (\*:  $P < 0.05$ ). Values are mean  $\pm$  SE.

showed a higher oxygen partial pressure in venous effluent ( $P_{vO_2}$ ) than DPG-depleted blood (Fig. 4). The difference in  $O_2$  extraction between DPG-restored and -depleted blood was statistically significant ( $P < 0.05$ ; Fig. 5).  $\dot{V}O_{2max}$  at 3 min contraction for DPG-restored and -depleted blood was directly proportional to  $P_{vO_2}$  under the conditions of our experiments (Fig. 6). Fig. 7 shows the relationship between  $\dot{V}O_2$  and resistance in maximally stimulated muscle. Lower resistance induced a higher  $\dot{V}O_2$  and so  $\dot{V}O_{2max}$  was attained at minimum resistance.

Developed tension for DPG-restored blood averaged  $15.13 \pm 2.34 \text{ kg} \cdot 100 \text{ g muscle}^{-1}$  at 3 min of contraction, and fell to  $10.78 \pm 1.53 \text{ kg} \cdot 100 \text{ g}^{-1}$  at 15 min. Developed tension for DPG-depleted blood was  $12.42 \pm 2.41 \text{ kg} \cdot 100 \text{ g}^{-1}$  at 3 min and  $8.12 \pm 1.45 \text{ kg} \cdot 100 \text{ g}^{-1}$  at 15 min.

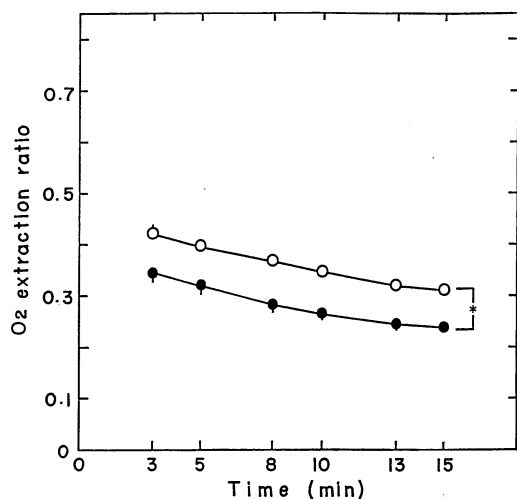


Fig. 5. The decrease in  $O_2$  extraction ratio during the contraction.  $O_2$  extraction ratio = (arterio-venous  $O_2$  content difference)/arterial  $O_2$  content. The  $O_2$  extraction ratio for DPG-depleted blood (closed circles) is significantly lower than that for DPG-restored blood (open circles, \* :  $P < 0.05$ ). Values are mean  $\pm$  SE.

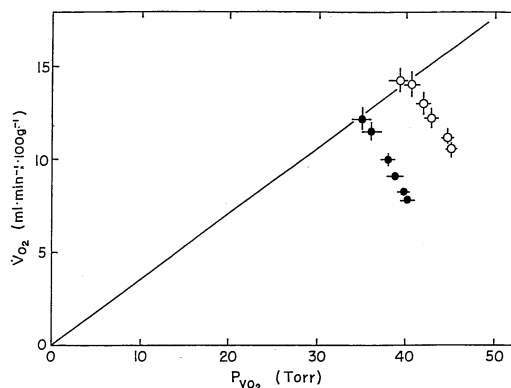


Fig. 6.  $P_{vO_2}$ -dependency of  $\dot{V}O_{2max}$  at 3 min contraction in 4 Hz twitch contracting gastrocnemius muscle perfused with DPG-restored (open circles) and -depleted blood (closed circles). Values are mean  $\pm$  SE.

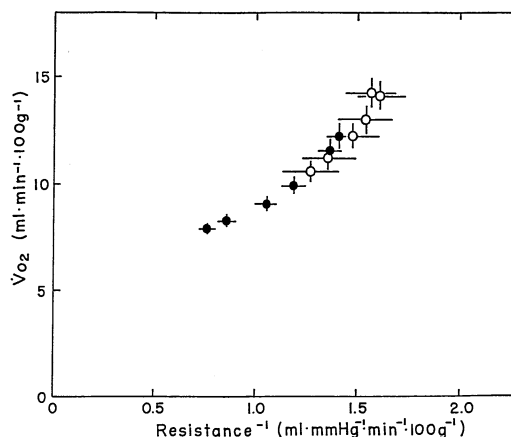


Fig. 7. The relationship between  $\dot{V}O_2$  and the reciprocal of vascular resistance (perfusion pressure divided by flow) in maximally stimulated muscle. Open circles, DPG-restored blood; closed circles, DPG-depleted blood. Values are mean  $\pm$  SE.

Developed tension for DPG-depleted blood was significantly lower than that for DPG-restored blood throughout the 15 min contraction ( $P < 0.05$ ). Lactate release for the perfusion with DPG-restored blood was  $146.5 \pm 78.0$  at 3 min and  $81.04 \pm 47.49 \mu\text{mol} \cdot \text{min}^{-1} \cdot 100 \text{g}^{-1}$  at 15 min. The release for the perfusion with DPG-depleted blood was  $150.65 \pm 64.81$  at 3 min and  $143.16 \pm 114.17 \mu\text{mol} \cdot \text{min}^{-1} \cdot 100 \text{g}^{-1}$ . Thus, lactate release at 3 min was comparable for the two perfusions, but the release at 15 min for DPG-depleted blood was significantly higher than that for DPG-restored blood ( $P < 0.05$ ).

## DISCUSSION

In the present experiment, the maximum rate of  $\text{O}_2$  uptake ( $\dot{V}_{\text{O}_{2\text{max}}}$ ) was  $14.22 \text{ml} \cdot \text{min}^{-1} \cdot 100 \text{g}^{-1}$  in 4 Hz contracting skeletal muscle at 3 min of contraction at a high flow rate of  $195 \text{ml} \cdot \text{min}^{-1} \cdot 100 \text{g}^{-1}$ . This value of  $\dot{V}_{\text{O}_{2\text{max}}}$  reached the maximum rate seen in twitch contracting gastrocnemius-plantaris muscle<sup>3)</sup> and was comparable to the  $\dot{V}_{\text{O}_{2\text{max}}}$  in canine gracilis muscle reported previously<sup>4,8)</sup>. Since the present purpose is to evaluate the influences on  $\dot{V}_{\text{O}_{2\text{max}}}$ , it is important for oxygen uptake to reach a maximum rate.

The present results indicate that  $\dot{V}_{\text{O}_2}$  for both DPG-restored and -depleted blood is proportional to the reciprocal of resistance to blood flow and that this relationship is different for blood of different oxygen affinities. Our assumption is that  $\dot{V}_{\text{O}_2}$  in contracting skeletal muscle is limited by red cell transit time and  $\text{O}_2$  off-loading kinetics. The former is related to bulk flow and vascular bed volume. First, we should discuss the relationship between  $\dot{V}_{\text{O}_{2\text{max}}}$  and vascular resistance. Horstman *et al.*<sup>8)</sup> have indicated that  $\dot{V}_{\text{O}_{2\text{max}}}$  is attained when the vascular bed is maximally dilated. Thus, vascular bed volume could be assumed to be inversely proportional to the vascular resistance. When blood flow is maintained constant, mean red cell transit time is proportional to the reciprocal of resistance. The longest transit time is attained at the minimum resistance to blood flow. The present experimental data support this idea.

Secondly, we should consider the relation of  $\dot{V}_{\text{O}_{2\text{max}}}$  to the  $\text{O}_2$ -affinity of the blood. The  $\text{O}_2$  releasing rate of high  $\text{O}_2$ -affinity blood is lower than that of low-affinity blood<sup>2)</sup>. For a given transit time of red cells through capillaries, the  $\text{O}_2$  extraction ratio of high  $\text{O}_2$  affinity blood might be lower as compared to that for normal  $\text{O}_2$  affinity blood. In the present experiment, the  $\text{O}_2$  extraction for DPG-restored blood was significantly higher than that for DPG-depleted blood at a comparable resistance for the first 8 min of contraction. This suggests that there is indeed a difference between the  $\text{O}_2$  unloading kinetics of red cells with high and normal  $\text{O}_2$  affinity. However, there might be some morphological changes in red blood cells induced by the decrease in DPG or ATP content, which in turn could contribute to a decrease in  $\dot{V}_{\text{O}_{2\text{max}}}$ . Further studies are needed to evaluate the influence of  $\text{O}_2$  unloading kinetics from red cells on  $\dot{V}_{\text{O}_{2\text{max}}}$ .

It is still possible that elevated  $\text{O}_2$  affinity of blood could decrease  $\dot{V}_{\text{O}_2}$  as a result of a decrease in  $\text{PvO}_2$ . First,  $\text{PvO}_2$  reflects end-capillary  $\text{Po}_2$  so that when  $\text{PvO}_2$  decreases below a critical level, an anoxic region arises in the "lethal corner" of Krogh's cylinder. Stainsby *et al.*<sup>15)</sup> indicated that the critical  $\text{PvO}_2$  for a 1 Hz contracting muscle was lower than that for resting muscle. Thus, the critical level at  $\dot{V}_{\text{O}_{2\text{max}}}$  could be much lower than the value estimated at 1 Hz. The  $\text{PvO}_2$  for DPG-depleted blood perfusion was 34.1 torr at 3 min and it did not reach the critical  $\text{PvO}_2$ . Horstman *et al.*<sup>8)</sup> measured  $\text{PvO}_2$  at  $\dot{V}_{\text{O}_{2\text{max}}}$  as 12 torr. In our case with high

blood flow,  $O_2$  delivery was about  $34 \text{ ml} \cdot \text{min}^{-1} \cdot 100 \text{ g}^{-1}$ , so that even at an  $O_2$  extraction of 0.45,  $\dot{V}O_2$  comes out at  $15 \text{ ml} \cdot \text{min}^{-1} \cdot 100 \text{ g}^{-1}$ .  $PvO_2$  would be 28 torr (pH 7.25,  $P_{50}$  23 torr) when  $SvO_2$  is 50 %. Therefore,  $PvO_2$  at  $\dot{V}O_{2\text{max}}$  is higher than the critical  $PvO_2$ . Secondly,  $\dot{V}O_{2\text{max}}$  in skeletal muscle is determined by the diffusion process from capillary to tissue. Hogan et al.<sup>5,6)</sup> proposed the hypothesis that  $\dot{V}O_{2\text{max}}$  is directly proportional to  $PvO_2$ . In the  $\dot{V}O_2$ - $PvO_2$  relationship (Fig. 6),  $PvO_2$  directly determined  $\dot{V}O_{2\text{max}}$  at 3 min contraction. When we estimate diffusion conductance ( $\dot{V}O_{2\text{max}}/PvO_2$ ) at 3 min of contraction, the values for DPG-restored and -depleted blood were  $0.362$  and  $0.348 \text{ ml} \cdot \text{min}^{-1} \cdot 100 \text{ g}^{-1} \cdot \text{torr}^{-1}$ , respectively. The conductance ( $\dot{V}O_2/PvO_2$ ) at 15 min was  $0.235$  for DPG-restored blood and  $0.196$  for DPG-depleted blood. The diffusion conductance is mainly determined by the surface area of the perfused capillaries. At 3 min of contraction, resistance was similar for the two perfusions, while at 15 min, resistance for the DPG-restored blood was lower than for the DPG-depleted blood. The data on vascular resistance seem to reflect the data on diffusion conductance. When diffusion conductance varies,  $\dot{V}O_{2\text{max}}$  also depends on the conductance. Thus, a  $PvO_2$ -dependency of  $\dot{V}O_2$  in maximally stimulated muscle could be obscured at 15 min contraction.

In the present experiment, lactate release at 3 min of contraction was not significantly different from that for DPG-depleted blood. This result is consistent with the observation reported by Hogan et al.<sup>6)</sup> But the present value was higher than their value ( $39$ - $52 \mu\text{mol} \cdot \text{min}^{-1} \cdot 100 \text{ g}^{-1}$ ). We added epinephrine to the perfusate to induce a development of normal tension in muscle perfused with plasma-free red cell suspension<sup>12)</sup>, and perfused at a flow rate of  $200 \text{ ml} \cdot \text{min}^{-1} \cdot 100 \text{ g}^{-1}$ . When these facts were taken into account, the value of the mean lactate release and the change in lactate release, especially in the perfusion with DPG-restored blood, were comparable to the previous results of Stainsby et al.<sup>16)</sup> Lactate release for DPG-restored blood at 15 min of contraction was significantly different from that for DPG-depleted blood. This is partly explained by accelerated anaerobic metabolism due to ischemic hypoxia induced by a microembolisation for DPG-depleted red cells. On the contrary, an increase in lactate release from contracting muscle was not observed under an affinity hypoxic condition due to low  $P_{50}$ . This result suggests that these two hypoxia induce a different mechanism for lactate release.

Resistance to blood flow increased by 87 % for DPG-depleted blood and 30 % for DPG-restored blood during 15 min of contraction. The increase in resistance was probably caused by a decrease in the number of perfused capillaries due to microembolisation and/or some factors closing capillaries. Although blood viscosity was not measured in vitro, resistance at 3 min of contraction was comparable for each perfusion, so that viscosity was presumably similar for both blood types. Thus, blood viscosity may not be related to the increase in resistance to blood flow. We performed three perfusions with DPG-restored blood which was prepared by incubating CPD blood stored for 3 weeks, and the increase in resistance was of the same extent as that seen for 1 week-stored CPD blood. Consequently, the cause of the increase in resistance is not related to the storage period of the blood, but is related to some factors affected by DPG and ATP in red blood cells. Morphological change in red cells occurs in the decrease in DPG and ATP content<sup>17)</sup>. In the present experiment DPG content in DPG-depleted blood decreased to half of normal blood. The morphological factors not only decrease deformability of red cells but also reduce  $O_2$  releasing velocity<sup>10)</sup>.

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