Journal of Physiology (1990), **422**, *pp.* 227–243
With 8 figures
Printed in Great Britain

LOCAL OXYGEN SUPPLY AND BLOOD FLOW REGULATION IN CONTRACTING MUSCLE IN DOGS AND RABBITS

BY D. K. HARRISON*, S. BIRKENHAKE, S. K. KNAUF AND M. KESSLER

From the Institut für Physiologie und Kardiologie, University of Erlangen/Nürnberg, Waldstraße 6, 8520 Erlangen, FRG

(*Received 16 May 1989*)

SUMMARY

1. Multiwire surface electrodes were used to measure local hydrogen clearance curves and tissues P_{O_2} in the sartorius muscle in dogs under resting conditions and during stimulation of the muscle at 1, 2, 4, 8 and 20 Hz via the femoral nerve. Tissue oxygen supply was assessed by means of P_{O_2} histograms; evaluation of the initial slopes of the hydrogen clearance curves enabled the measurement of capillary blood flow.

2. In a further model, the analysis of hydrogen clearance curves measured in the femoral vein using intravascular needle electrodes in rabbits enabled the distribution of blood flow to be evaluated both under resting conditions and during direct 2 Hz stimulation of the vastus medialis muscle.

3. Increased oxygen consumption, induced by stimulation, caused increases in capillary flow which were not necessarily accompanied by augmentation of femoral artery flow.

4. P_{O_2} histograms provided no evidence of cellular anoxia even at the maximum level of oxygen consumption.

5. A two-compartment distribution of flow was measured under resting conditions, whereas only one compartment could be resolved during 2 Hz stimulation of the vastus medialis muscle in the rabbit experiments. A clear redistribution of flow was observed in the absence of any increase in total flow.

6. A model for oxygen-dependent regulation of capillary blood flow involving high-flow and normal-flow compartments is proposed.

INTRODUCTION

The heterogeneity of the regional blood flow to muscles of different types has been reported by Armstrong & Laughlin (1983) in studies using radioactive microspheres in the hindlimbs of rats. Even in resting muscles, sevenfold differences in flow were found, depending on the predominance of fibre type. The same authors reported that in some muscles (musculus plantaris in the rat) regional blood flow increased tenfold as the result of a short period of exercise whereas in others (soleus) an increase of

* To whom correspondence should be addressed at the Vascular Laboratory, Ninewells Hospital, Dundee DD1 9SY.

regional blood flow of only about 40% is observed. It would appear from these experiments that regulatory mechanisms were involved in controlling the blood flow between the muscles so that it was adequate to meet their respective metabolic requirements.

Considerable heterogeneities in red cell distribution and velocities have been reported by Groom, Ellis, Wrigley & Potter (1986) even within the same resting muscle (frog sartorius) using *in vivo* microscopy, but a reduction in the degree of heterogeneity has been observed during post-stimulation hyperaemia (Tyml, 1987).

The results of direct, surface electrode measurements of the distribution of capillary blood flow (microflow) and local oxygen supply in the resting sartorius muscle in dogs also indicated that considerable heterogeneities in both parameters were to be found within a single muscle (Harrison, Kessler & Knauf, 1990). However, even during extreme hypoxaemic conditions, the local oxygen supply was rigorously maintained by means of capillary blood flow regulation and reduction of oxygen consumption above a level at which cellular anoxia might occur.

One great advantage of the use of surface electrodes for measuring tissue P_{O_2} and microflow is that the techniques are non-invasive and another is that they can be used easily on moving organs, even the beating heart (Harrison, Günther, Vogel & Kessler, 1985). The following experiments were carried out to investigate how the capillary blood flow was related to the arterial flow and local oxygen supply when the oxygen consumption was increased as a result of contraction induced by electrical stimulation of the muscle.

Two series of experiments were performed. The first was carried out in mongrel dogs during stepwise increase of the stimulation frequency in a preparation similar to that already described (Harrison *et al.* 1990). In order to interpret the results of these experiments, a second series was carried out in rabbits where the distribution of blood flow within the whole muscle at rest and during stimulation at one frequency was investigated.

A preliminary report of some early data from these experiments has already been presented (Harrison, Birkenhake, Knauf, Hagen, Beier & Kessler, 1988). They are presented here in full, and a model for the regulation of capillary blood flow in skeletal muscle is proposed.

METHODS

The methods used for measuring and recording capillary blood flow (microflow) and tissue P_{O_2} are described in detail elsewhere (Kessler, Harrison & Höper, 1986; Harrison & Kessler, 1989*a*, *b*).

In addition, needle electrodes were constructed in order to measure intravascular H_2 clearances in rabbits. Their construction and properties are described elsewhere (Harrison, 1989). Briefly, 200 μm platinum wires were etched to a tip diameter of about 20 μm and then insulated with glass using a microprocessor-controlled puller designed and built in collaboration with the Institut für Antrieb und Technik, University of Erlangen/Nürnberg (Robisch, 1986). The electrodes were ground to the required diameter, palladinized and dip-coated with a collodion membrane. Each electrode was calibrated before and after every experiment.

The methods used for data acquisition and processing are described elsewhere (Harrison & Kessler, 1989*b*).

Animal preparation

Anaesthesia and surgical procedure in dogs

The anaesthesia and surgical procedure in the dog experiments has been described elsewhere (Harrison *et al.* 1990). It differed here only in two details. For obvious reasons, no muscle relaxants could be used for these experiments, so instead a deeper anaesthesia was maintained using piritrimide (Dipidolor, Janssen, 0·7 mg $kg^{-1}h^{-1}$) and flunitrezepam (Rohypnol, Roche, 7 $\mu g\ kg^{-1}h^{-1}$).

In addition to the surgical procedure already described, for the stimulation experiments the preparation of the femoral artery for application of the electromagnetic flow probe included separating the femoral nerve very carefully and suspending it lightly in a pair of hooked Ag–AgCl electrodes which were connected to a Model P Stimulator (Hugo Sachs Elektronik, Hugstetten/Briesgau, FRG).

Anaesthesia in rabbits

Rabbits very easily exhibit signs of fear and stress. For this reason the way in which the preliminary anaesthetic is administered is crucial to the success of the subsequent experiment. In these experiments the initial anaesthetic ketamine (50 mg kg^{-1} Ketanest, Parke Davis) and xylazine (5 mg kg^{-1} Rompun, Bayer) was injected intramuscularly. The light in the room was subdued and total quiet was maintained until the rabbit was unconscious. This initial anaesthesia was supported by placing the rabbit's head into a box through which flowed a mixture of 70% nitrous oxide and 30% oxygen which the animal could breathe spontaneously.

The rabbit was then tracheotomized and ventilated with 70% N_2O, 30% O_2 and 0·3% Isoflurane. The tracheotomy never took longer than 10 min. Immediately after being put onto the ventilator the rabbit was relaxed with 1–2 mg pancuronium bromide and maintained with further administrations of 0·5 mg of the relaxant every 45 min. Heart rate, ECG and arterial blood pressure were monitored continuously throughout the experiments to ensure adequacy of anaesthesia. Using these criteria, experience in our laboratory has shown that the use of 0·3% Isoflurane ensures adequate anaesthesia for our experimental conditions.

The small body weight and large surface area-to-volume ratio meant that the body temperature had to be carefully monitored at all times and the loss of body heat avoided at all costs. This was achieved by wrapping the animal in a heat-reflecting blanket as soon as the preparation was complete. As in the dog experiments, maintenance of the fluid balance through infusion of warm Ringer solution via a peripheral vein was essential. Any metabolic acidosis was corrected by administration of sodium bicarbonate and respiratory disturbances in acid–base status were corrected by appropriate adjustment of the ventilation frequency or volume.

Preparation for skeletal muscle experiments in rabbits

After completion of the tracheotomy, a catheter was inserted in the left femoral artery for monitoring arterial pressure, and another inserted in the jugular vein for monitoring central venous pressure, both using Statham pressure transducers. The arterial catheter also served for taking blood samples for blood gas, acid–base and haematocrit measurement.

A small area of the vastus medialis muscle was exposed and freed of fascia and a silicone disc applied to the surface in the same careful manner described previously. The muscle was then superfused with Ringer solution at 37 °C. Two small needle electrodes were inserted into the muscle about 1 cm apart, but at a considerable distance from the site of measurement, in order to allow direct electrical stimulation of the muscle.

The right femoral artery and vein were then carefully exposed so as not to disturb the muscle preparation, and a pre-calibrated H_2 needle electrode inserted into the vein above any branch point to the vastus medialis.

Unfortunately, due to the small distances between arterial branches, it was not possible to apply an electromagnetic flow-probe around the femoral artery without causing considerable changes in the distribution of flow within the limb. The silver–silver chloride reference electrode was sutured to adjacent connective tissue.

Experimental procedure

The protocol for the six stimulation experiments in dogs was as follows. Control measurements of P_{O_2} histograms, microflow (H_2 clearance), femoral artery flow, iliac aortic pressure, central venous pressure, blood gases, acid–base status, haematocrit, ECG and heart rate were carried out under normal, resting conditions.

After completion of these measurements the femoral nerve was stimulated with 0·5 ms pulses at a frequency of 1 Hz. The normal voltage used to stimulate the muscle was 1·3 V. Slightly higher voltages were used if no response to this stimulus was observed during a short test stimulation. Femoral artery flow, iliac artery and central venous pressures, together with tissue P_{O_2} were recorded during the onset of the stimulation and at each change of stimulation frequency. Control measurements of all parameters were then made under steady-state conditions.

The same procedure was carried out when the stimulation frequency was increased to 2, 4, 8 and 20 Hz. No recovery period was included between the increases.

In the rabbit experiments, initial control measurements were made as described above. In addition, arterial and venous H_2 clearances were recorded. The muscle was then stimulated directly with a mean voltage of 20 V at a frequency of 2 Hz with a pulse length of 0·5 ms and further control measurements were made. Each H_2 clearance was recorded for at least 45 min and usually for 1 h in order to be able to perform curve stripping accurately.

Both the dogs and rabbits were painlessly killed whilst still under anaesthesia at the end of each experiment.

Treatment of results

Component analysis

Component analyses of the intravenous H_2 clearance curves were carried out using the standard curve peeling method (see, for example, Fieschi, Isaacs & Kety, 1968). Total flow estimations (F) from the venous clearance curves were made using the stoichiometric method (height-over-area) of Zierler (1968) modified by Hutten (1970) for finite clearance curves. The formula for calculating F is given in eqn (1) (see Harrison & Kessler, 1989*b*), where $P_{H_2,\max}$ is the partial pressure of hydrogen measured at the peak of the curve and $t_{10\%}$ is the time taken for the P_{H_2} to fall to 10% of the peak value:

$$F = \frac{0{\cdot}9P_{H_2,\max}}{\int_0^{t_{10\%}} P_{H_2}(t)\,dt}. \quad (1)$$

For the two-component clearances recorded using the 30 s partial saturation technique, the distribution of flow was calculated from

$$W_1C_1 + W_2C_2 = F. \quad (2)$$

C_1 and C_2 are the flow values within the two compartments as measured by peeling the venous clearance curves. W_1 and W_2 are the weights of the components of flow (which represent the relative volume of the tissue compartment). Since for a two-compartment system $W_1 + W_2 = 1$, eqn (2) has a unique solution.

Local oxygen consumption

The relative local oxygen uptake rate (Harrison *et al.* 1990) was calculated from the P_{O_2} histograms and mean microflow (MF) measured in the same area of muscle. The class of the P_{O_2} histogram with the highest P_{O_2} values was assumed to represent the saturation of the blood ($S_{O_2,\max}$) at the arterial end of the capillaries. The mean venous P_{O_2} (and hence saturation $S_{O_2,\text{mean}}$) in the area of measurement was taken to be represented by the mean tissue P_{O_2}. The mean relative uptake rate in the area of muscle in which the P_{O_2} histogram and microflow values were measured ($\dot{V}_{O_2}$) was thus calculated from the equation

$$\dot{V}_{O_2} = 1{\cdot}34(\text{Hb})(S_{O_2,\max} - S_{O_2,\text{mean}})\text{MF}\,6 \times 10^{-4}. \quad (3)$$

where 1·34 is Hüfner's number (in ml g^{-1}), haemoglobin (Hb) was taken as 148 g l^{-1} (Altman & Dittmer, 1964) and 6×10^{-4} was a conversion factor so that the calculated consumption would be equivalent to ml min^{-1} 100 g^{-1}. However, since this calculation includes a number of assumptions, the local O_2 consumption will be expressed here in relative terms.

Student's t test for paired values was used to assess the statistical significance of the observed changes.

RESULTS

Table 1 summarizes the results of blood gas, acid–base and haematocrit measurements carried out under steady-state conditions at each stage of the stimulation experiments together with the mean arterial pressure. It can be seen that these parameters remained almost constant throughout the experiments and none of the changes was statistically significant.

TABLE 1. Arterial blood gas and acid–base status, haematocrit and mean arterial pressure (MAP) (±S.D.) during the stimulation experiments

Stimulation frequency (Hz)	P_{a,O_2} (mmHg)	P_{CO_2} (mmHg)	pH	Base excess (mM)	Haematocrit (%)	MAP (mmHg)
Control	122±17	36±1	7·36±0·03	−3·5±1·7	43±3	108±21
1 Hz	125±24	38±5	7·36±0·05	−2·5±2·7	43±6	112±9
2 Hz	120±7	36±1	7·37±0·03	−3·2±1·2	44±4	110±14
4 Hz	122±19	32±3	7·40±0·02	−3·2±0·6	44±4	110±9
8 Hz	124±23	36±4	7·41±0·04	−0·9±2·9	46±7	113±12
20 Hz	118±22	35±3	7·37±0·04	−3·5±1·2	46±7	112±15

Figure 1 shows P_{O_2} histograms recorded at each stimulation frequency in a typical experiment. The figure shows that the initial stages of stimulation (1 and 2 Hz) induced a shift of the histogram to the right (i.e. the minimum P_{O_2} in the tissue was raised) and the mean tissue P_{O_2} also increased. This pattern can also be clearly read from Table 2 which presents the range and frequency of the lowest class of the summated P_{O_2} histograms from all six experiments at each frequency of stimulation. Tissue P_{O_2} values below 5 mmHg (2%) were only found in one experiment, at 8 Hz stimulation. Table 2 shows that this represented only 0·3% of the collective histogram at 8 Hz. During this particular experiment the maximum microflow was also measured at this frequency of stimulation.

Table 2 also presents the changes in microflow (calculated from semilogarithmic plots of the curves) and femoral blood flow, both expressed as percentages relative to the initial control value, for all frequencies of stimulation. At 1 Hz stimulation the mean microflow increased by 18% but was not accompanied by any increase in arterial flow. Further progressive increases in capillary blood flow were observed at stimulation frequencies of 2, 4 and 8 Hz to 27, 36 and 60% above control respectively. The maximum mean microflow, 73% above control, was to be found at the 20 Hz stimulation frequency. None of the changes in femoral artery blood flow were of statistical significance. However, a slight tendency to increase can be seen upon raising the stimulation frequency to 4 Hz and even more at 8 Hz. There also seemed to be no further increase in arterial flow at the 20 Hz stimulation frequency.

Although Table 2 shows that the capillary blood flow tended to increase with the frequency of stimulation, the nature of the model (i.e. the aim of causing the least possible trauma to the muscle) precluded the absolute quantification of the work the muscle was doing. In order to obtain an estimate of this parameter locally, the oxygen uptake rate was calculated using eqn (3) for each stimulation frequency. Figure 2 shows the relative consumption at each stage, referred to the maximum

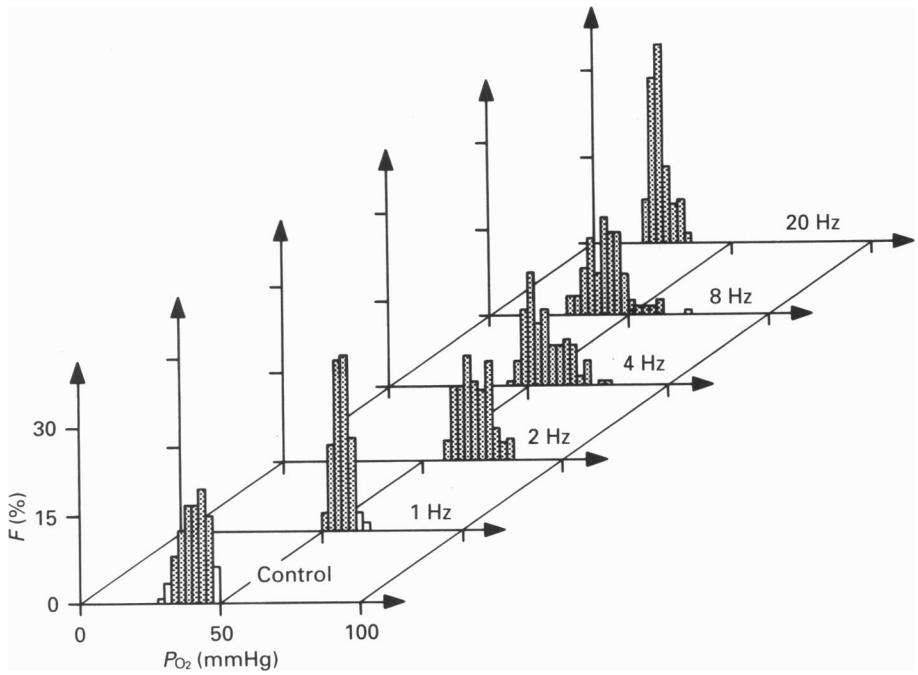

Fig. 1. Typical P_{O_2} histograms measured in the sartorius muscle under control conditions and during stimulation at 1, 2, 4, 8 and 20 Hz. Each histogram is composed of 104 individual measurements.

($\dot{V}_{O_2, max}$) for each experiment. It is immediately apparent that the frequency at which $\dot{V}_{O_2, max}$ was observed varied considerably. It was observed once each at 1, 2, 4 and 8 Hz, and twice at 20 Hz. In addition, 99% $\dot{V}_{O_2, max}$ was also measured once at 8 Hz and 90% once at 20 Hz. Figure 3 shows the mean O_2 consumption relative to the mean $\dot{V}_{O_2, max}$ for the series of experiments and also the changes in microflow (see Table 2). It shows that the mean O_2 consumption increased with the frequency of stimulation (with the exception of 2 Hz). On the other hand, the variability displayed in Fig. 3 hints that stimulation frequency may not necessarily be the indicative parameter for local work.

Figure 4 shows the relationship between percentage changes in microflow and femoral artery flow (referred to control values) and the calculated local relative consumption (as a percentage of $\dot{V}_{O_2, max}$). The values for the abscissa were extracted from the data for control consumption values 25 (±5)% of $\dot{V}_{O_2, max}$, 50 (±5)% of $\dot{V}_{O_2, max}$ and $\dot{V}_{O_2, max}$. The mean minimum tissue P_{O_2} values recorded in the histograms are also plotted. The figure shows an increasing divergence between capillary blood flow and femoral artery flow together with the maintenance of a minimum P_{O_2} at increasing O_2 consumption. This reinforces the pattern evident in Table 2 that an increased oxygen demand is primarily accommodated by a decrease in tissue P_{O_2}, to a certain regulated level, and a simultaneous increase in capillary blood flow without any significant increase in femoral artery flow.

Analyses of the relationships between relative $\dot{V}_{O_2}$, microflow, arterial blood flow

and lowest tissue P_{O_2} values within each of the six experiments revealed that the only significant ($P < 0{\cdot}05$) positive linear correlations were between $\dot{V}_{O_2}$ and microflow (mean regression coefficient, $r_{mean} = 0{\cdot}76 \pm 0{\cdot}17$). A negative, but not significant, linear correlation ($r_{mean} = -0{\cdot}58 \pm 0{\cdot}27$) between $\dot{V}_{O_2}$ and tissue P_{O_2} was also found.

TABLE 2. Mean and minimum tissue P_{O_2} values from all histograms ($N = 6$, $n = 624$) together with changes in capillary blood flow (MF, $N = 6$, $n = 40$) and femoral artery flow (AF, $N = 6$)

Stimulation frequency (Hz)	$P_{O_2,min}$ (f%)	Mean P_{O_2} (mmHg)	MF (percentage of control)	AF
Control	20–25 (1·6)	43·4	100±40	100±53
1 Hz	20–25 (0·9)	48·3	118±34*	101±19
2 Hz	25–30 (0·5)	50·5	127±21‡	103±49
4 Hz	5–10 (0·3)	43·7	136±66†	113±44
8 Hz	0–5 (0·4)	46·0	160±69‡	133±54
20 Hz	5–10 (0·1)	39·3	173±120‡	132±43

* $P < 0{\cdot}01$; † $P < 0{\cdot}002$; ‡ $P < 0{\cdot}001$. Changes in arterial flow not significant.

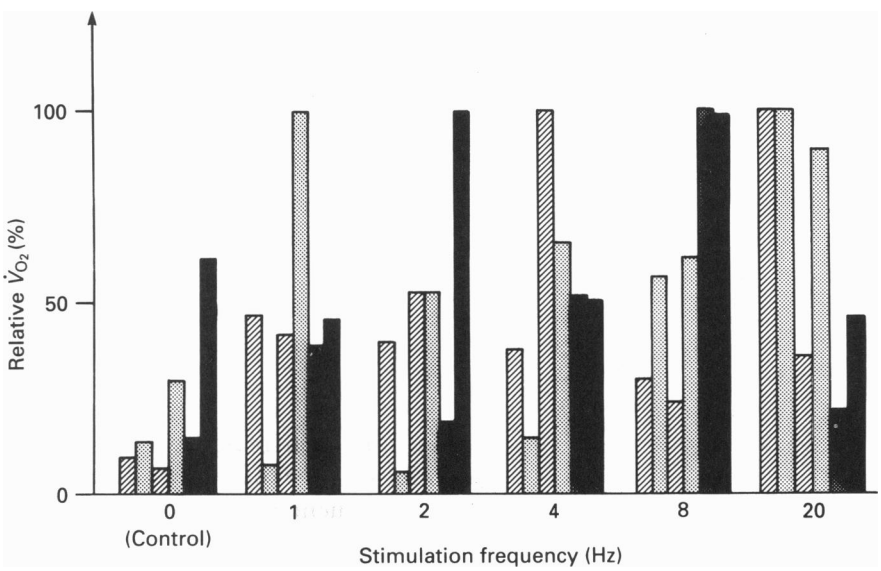

Fig. 2. Relative O_2 uptake rate expressed as percentage of maximum for each experiment at each stimulation frequency. It can be seen that $\dot{V}_{O_2,max}$ was even achieved at 1 Hz in one experiment.

Interestingly, no correlations between microflow and femoral artery flow or femoral artery flow and $\dot{V}_{O_2}$ were found.

Intravascular H_2 clearances

The large discrepancies between femoral artery and capillary blood flows observed in earlier hypoxia experiments (Harrison *et al.* 1990) and the above stimulation

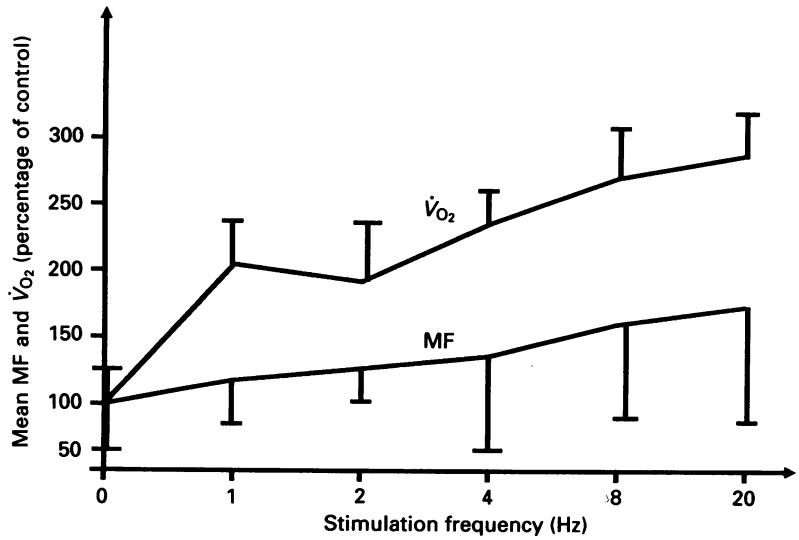

Fig. 3. Mean relative O_2 uptake rate, calculated from all experiments, expressed as percentage of initial control value (±S.D.) for each frequency of stimulation. The mean changes in microflow (MF) from Table 2 are also plotted.

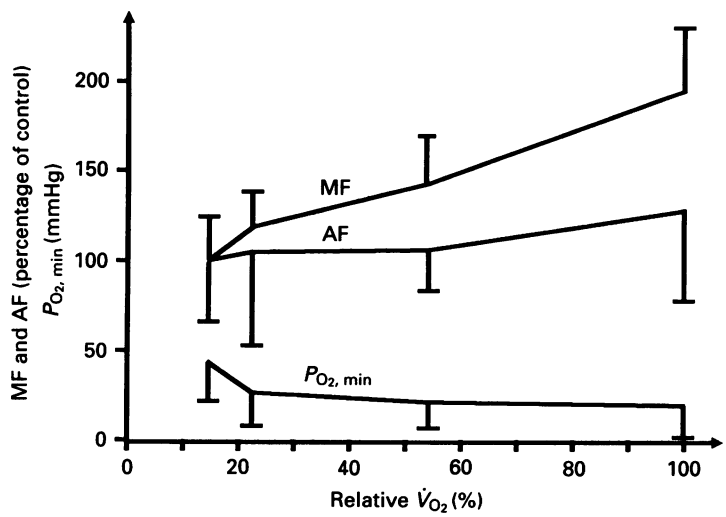

Fig. 4. Mean changes in microflow (MF) and femoral artery flow (AF) expressed as percentage of control value plotted together with mean minimum tissue P_{O_2} as function of relative O_2 consumption (percentage of $\dot{V}_{O_2, max}$).

experiments led to the postulation of the existence of high-flow capillary channels which could not be detected at the surface of the muscle by the hydrogen electrode due to its small catchment depth of only 32 μm (Harrison & Kessler, 1989*a*).

The purpose of the second series of investigations, which was carried out in the

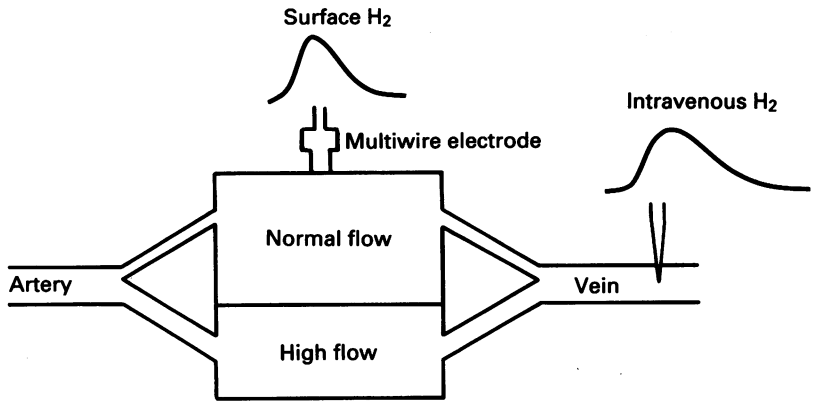

Fig. 5. Schematic representation of the principle behind the experimental set-up for the rabbit experiments. A flow component not 'seen' by surface H_2 clearance measured with the multiwire electrode would be detected in the intravenous clearance measured with the needle electrode.

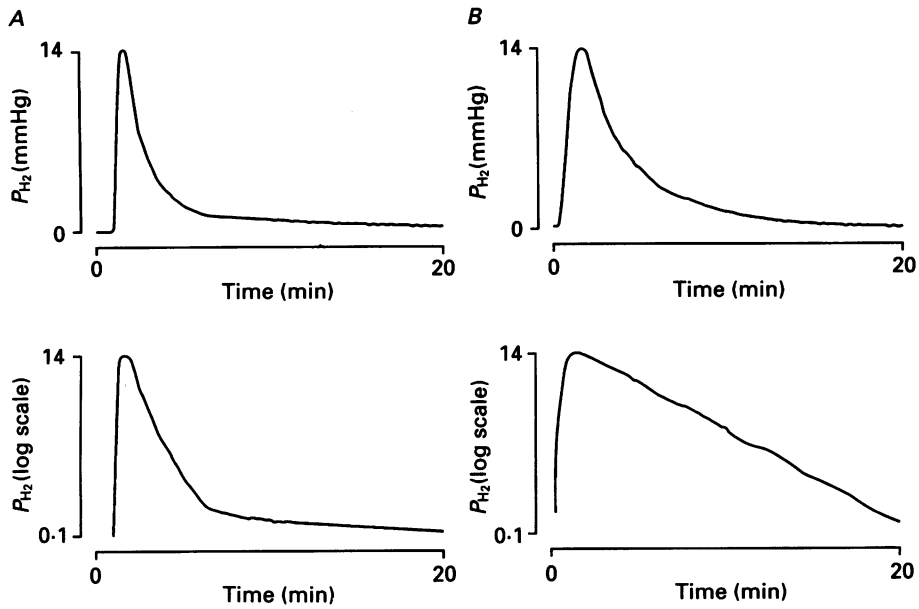

Fig. 6. Semilogarithmic plots of intravenous H_2 clearance curves measured with the muscle at rest (*A*) and with the muscle contracting at a frequency of 2 Hz (*B*). In *A* the slopes of the two components of flow are shown; in *B* only one component could be resolved.

rabbits, was to try and identify, functionally, whether such channels exist. The principle behind this model was the identification of any components of flow bypassing the catchment volume of the surface electrode by measuring hydrogen clearance curves intravenously (see Fig. 5). Furthermore, it should also be possible

TABLE 3. Results of component analyses of intravenous H_2 clearance curves measured at rest in rabbits (1–8) and dogs (9–14)

	Compartment 1		Compartment 2	
Experiment	Flow (%) (W_1C_1/F)	Tissue (%) (C_1)	Flow (%) (W_2C_2/F)	Tissue (%) (C_2)
1	86	38	14	62
2	86	39	14	61
3	56	24	44	76
4	64	32	36	68
5	94	51	6	49
6	91	24	9	76
7	42	9	58	91
8	91	9	9	91
9	78	29	22	71
10	62	32	38	68
11	86	43	14	57
12	87	33	13	67
13	92	50	8	50
14	81	48	19	52
Mean (±S.D.)	78±16	33±13	22±16	67±13

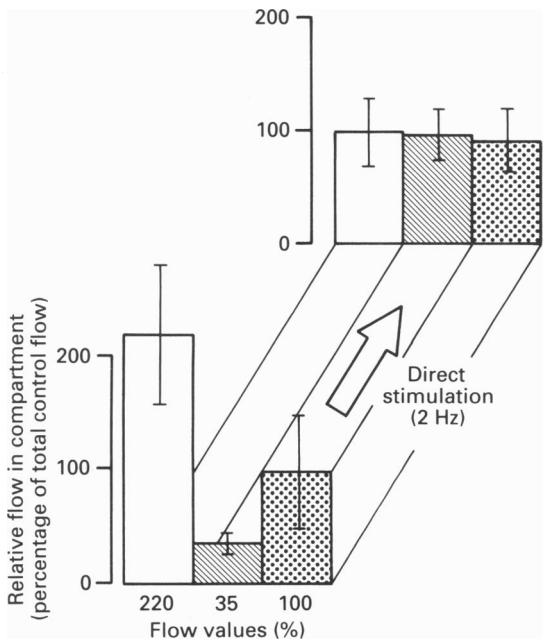

Fig. 7. Changes in the distribution of blood flow measured in eight experiments as a result of 2 Hz stimulation of the vastus medialis muscle. The flow in the two compartments is expressed as percentage of total flow under control conditions. It can be seen that the total flow actually fell slightly (not significant) and a considerable redistribution to a more homogeneous pattern of flow occurred as a results of stimulation.

to quantify any changes in heterogeneity of flow occurring as a result of stimulating the muscle.

A schematic drawing of the model, which shows the locations of the electrodes, is shown in Fig. 5. The larger box at the surface of the muscle represents the compartment in which the surface tissue P_{O_2} and microflow measurements are made. The smaller box represents a compartment not 'seen' by the surface sensors, but whose flow would comprise a component of a hydrogen clearance curve measured intravenously.

Figure 6 shows a semilogarithmic plot of a 30 s partial saturation venous clearance at rest (*A*) and during stimulation of the muscle (*B*). The components of the curves are indicated in the diagrams and it can be seen that two components could be detected following the 30 s partial saturation but that only one could be detected during stimulation of the muscle.

Eight clearances measured at rest after a 30 s partial saturation and analysed using eqns (1) and (2) revealed (Table 3) that two components of flow can be detected with the vastus medialis muscle at rest in rabbits using the 30 s partial saturation technique; $77 \pm 19\%$ of the flow supplies $28 \pm 14\%$ of the tissue and $23 \pm 19\%$ supplies $72 \pm 14\%$.

Table 3 also shows the results of six intravascular measurements under resting conditions in two dogs. These results revealed that $81 \pm 11\%$ of flow supplied $39 \pm 9\%$ of tissue and $19 \pm 11\%$ supplied $61 \pm 9\%$ of tissue. The differences between the two species were not significant, and on average the distribution was that of $78 \pm 16\%$ of flow supplying $33 \pm 13\%$ of tissue and $22 \pm 16\%$ supplying $67 \pm 13\%$.

Figure 7 shows that, when the vastus medialis muscle in rabbits ($n = 5$) or the sartorius ($n = 3$) in dogs was stimulated, the relative flow in the larger compartment increased from 33% of the total flow to 96%. Simultaneously, the flow in the other compartment reduced from 220% of the total flow to 99%. A small mean decrease of 10% in total flow was found during stimulation.

DISCUSSION

In the first series of experiments, the initial response as a result of stimulating the muscle at a frequency of 1 Hz (see Table 2) was obviously a local one since an increase in capillary flow could be seen in the absence of any change in femoral artery blood flow. The discrepancy between capillary blood flow and arterial flow persisted at each stage of stimulation and was maximal at 20 Hz.

The results of local O_2 consumption calculations and the subsequent correlations of microflow, arterial blood flow and P_{O_2} values showed that capillary blood flow increased with O_2 consumption, whereas tissue P_{O_2} tended to decrease to a level which always remained above that which might indicate the presence of cellular anoxia ($> 1\%$ of P_{O_2} values below 5 mmHg) (Starlinger & Lübbers, 1973; Chance, 1988).

Tamura, Hazeki, Nioka & Chance (1989) have recently compared the redox state of cytochrome $a+a_3$ with the saturation curves for myoglobin and haemoglobin. They show that cytochrome $a+a_3$ is still more than 90% oxidized at a myoglobin

saturation below 5% (i.e. an O_2 concentration of about 1 μM) but the respiratory function of this enzyme can be maintained even at 0·1 μM.

Since, overall, only 0·3, 2·0 and 0·1% of P_{O_2} values were recorded below 10 mmHg at 4, 8 or 20 Hz stimulation respectively, the tissue P_{O_2} appeared to be kept even above the level where myoglobin buffering may have become significantly involved (see Harrison *et al.* 1990).

It is to be noted that the tissue P_{O_2} values recorded at 8 Hz stimulation in these experiments were considerably higher than those estimated from myoglobin saturations at 6–8 Hz stimulation of the dog gracilis muscle as reported by Gayeski, Connett & Honig (1987). This may, in part, be attributed to their use of sodium pentobarbitone as the only anaesthetic. This has been described as being unphysiological compared with the awake state (Lund, 1985) and as having a detrimental effect upon peripheral capillary blood flow (Kazmers, Whitehouse, Zelenock, Cronenwett, Lindenauer & Stanley, 1984). Pentobarbitone was used solely as *introductory* anaesthesia in our dog experiments.

A further difference between experimental conditions was the central mean arterial pressure which was 108 mmHg in the series described here but 180 mmHg in a typical experiment reported by Gayeski, Connett & Honig (1985). The experiments were also quite different with regard to the invasiveness of the preparation (Honig, Odoroff & Frierson, 1982; Connett, Gayeski & Honig, 1984) and tissue oxygen measurement technique (Gayeski & Honig, 1978, 1986).

A model to describe the intracellular P_{O_2} distribution based on these authors' results was developed by Groebe & Thews (1986) assuming a maximum extracellular P_{O_2} of about 30 mmHg and thus large diffusion gradients between the red blood cells and the interior of the muscle fibre. Their model demonstrated that, despite low levels of P_{O_2}, the distribution of oxygen within the cell remained homogeneous due to facilitated diffusion and the O_2 buffering power of myoglobin.

High flow capillaries

The results of the intravenous hydrogen clearance measurements indicate a considerable heterogeneity of flow within the resting skeletal muscle. The results imply that the mean flow through the high-flow compartment is six times higher than in the normal one.

One explanation might be the existence of arterio-venous (or non-nutritive) shunts. However, the existence of such hypothetical structures has yet to be proved in skeletal muscle (Hammersen, 1971; Myrhage & Eriksson, 1984; Oude Vrielink, Slaaf, Tangelder & Reneman, 1986).

A simple analysis of laminar flow according to Hagen-Poiseuille shows that vascular resistance is inversely proportional to the fourth power of the radius of the vessel and directly proportional to its length. Thus, a small population of capillaries of relatively large diameter would carry an over-proportional amount of flow. This would even be true for a Gaussian distribution of capillary diameters.

Studies by Potter & Groom (1983) using corrosion casts in rat gastrocnemius muscle revealed a bimodal distribution of capillary diameter with modes at 5·5 and 7·5 μm. Although the population of large diameter capillaries represented only 13%, according the Hagen-Poiseuille law they would carry 71% of the flow. The remaining 29% would flow through 87% of the capillaries.

The proportions of the flows differ slightly in magnitude from those found in this study but the agreement is surprisingly close. There is, therefore, morphological evidence for the existence of high-flow capillary channels consistent with the flow distributions measured in this study.

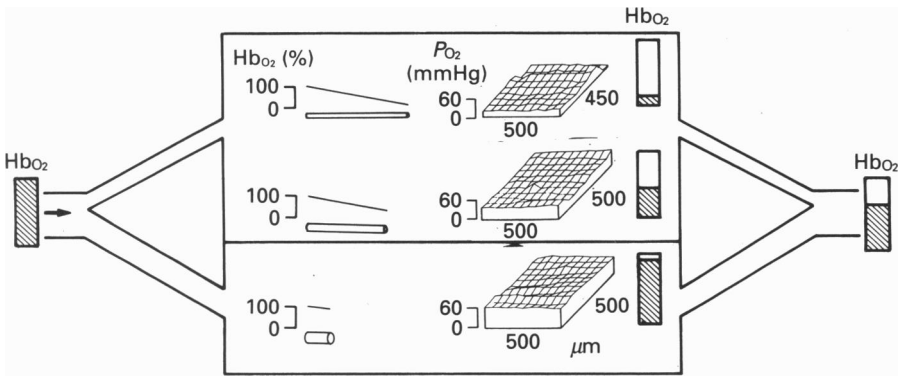

Fig. 8. Schematic representation of capillary flow distribution in skeletal muscle based on the present study incorporating typical P_{O_2} topograms recorded by Beier (1987). For details see text. Hb_{O_2}, haemoglobin saturation.

The role of flow heterogeneity in oxygen supply to skeletal muscle

Previous studies (Beier, 1987; Harrison *et al.* 1990) have shown that a considerable degree of heterogeneity of tissue P_{O_2} values can be found between capillary supply units. The existence of a small number of high-flow channels necessarily adds to this heterogeneity, but these supply channels have a particularly important function.

Figure 8 combines the 'topogram' structure of local P_{O_2} distribution in skeletal muscle, as measured by Beier (1987), with the flow distributions measured in the present studies using the hydrogen clearance technique. The capillary radius is included schematically. The large tissue compartment contains capillaries of different lengths and radii which give rise to the various levels of P_{O_2} (represented by the two topograms) measured in different supply units. The appropriate levels of haemoglobin saturation (Hb_{O_2}) along the capillaries are indicated. The longer, narrower capillaries contribute more towards gas exchange than the shorter, wider ones. This is particularly true for the high-flow capillaries in the second compartment which release relatively little oxygen to the tissue. The levels of Hb_{O_2} for the blood emerging at the venous end of the compartments and that of the mixed venous blood are illustrated.

The resultant levels of saturation demonstrate how the high-flow compartment causes the venous P_{O_2} to be higher than the mean tissue P_{O_2} (measured in the bulk of the tissue) and explains the findings of other workers, not only in skeletal muscle (Kunze, 1967; Lund, 1979) but also in the liver (Görnandt & Kessler, 1973), duodenum (Rink & Kessler, 1973) and brain (Lübbers & Wodick, 1971).

Figure 8 is a block diagram of the 'flow compartments' and is obviously simplified. Anatomically, the two compartments may not be so clearly separated. However, the high-flow supply units probably provide a local flow reserve everywhere in the muscle.

During stimulation, oxygenated blood was probably diverted *from* the high-flow compartment *to* the normal capillaries in order to meet the increased local oxygen demand. The intravenous hydrogen clearances confirmed that during stimulation the slope of the fast component of the curve was reduced and that of the slower component increased until, in almost all cases, they became indistinguishable from each other. Early results obtained independently from analysis of sodium fluorescein clearance curves in resting muscle and during stimulation further reinforce this interpretation (Harrison, Frank, Birkenhake, Hagen, Dümmler, Appelbaum & Kessler, 1987).

The two compartments in Fig. 8 are analogous to two parallel electrical resistances. If Ohm's law is applied for the changes in flow (current) induced by stimulation, it can be shown that both the resistance of the large compartment must decrease and that of the high-flow compartment must increase if the changes in flow pattern observed in these experiments are to be explained.

The opposite occurs in the case of hypoxaemia (Harrison *et al.* 1990). This centrally induced oxygen deficiency appears to induce a primary decrease in resistance in the arterioles feeding the high-flow capillaries. The latter may well be more subject to global or regional regulatory control than the more numerous low-flow capillaries.

In both cases an active regulation of flow must take place in *both* compartments.

Regulation of flow between the two compartments may be activated by signals emanating from one or more cells threatened by oxygen deficiency and transmitted by way of the capillary endothelium to the nearest smooth muscle cell (Höper & Kessler, 1981; Kessler, Höper & Lübbers, 1981; Kessler, Höper, Harrison, Skolasinska, Klövekorn, Sebening, Volkholz, Beier, Kernbach, Rettig & Richter, 1984) inducing a redistribution of the flow within the normal capillary network and causing an initial levelling out of the P_{O_2} topogram. Should a large number of cells by affected, such as would happen as a result of an increased oxygen consumption in the working muscle, signals converge 'upstream' and induce a redistribution of flow from the high-flow compartment.

It is interesting that the highest mean capillary blood flow (Fig. 4) was measured at the maximum rate of oxygen uptake. The minimum P_{O_2} values shown in Table 2 and Fig. 4 indicate that some threshold value may be the set point for the local regulation of capillary blood flow. This threshold lies considerably above the critical P_{O_2} for cytochrome $a+a_3$. In addition, the apparent (but not statistically significant) increase in mean collective arterial flow at $\dot{V}_{O_2,max}$, when the lowest P_{O_2} values were observed (Fig. 4), may have been a result of the convergence of signals being conducted even further upstream. This response indicates that an additional, centrally controlled increase in flow could also be induced if the local redistribution of flow was insufficient to meet the oxygen demands of the muscle. This would especially be the case if more than one muscle, or groups of muscles, are activated simultaneously.

In this context, it should be borne in mind that these results were obtained under experimental conditions involving peripheral stimulation of the nerve, or direct stimulation of the muscle itself under anaesthesia. The results therefore only reflect the degree of *local* regulation of blood flow. Under physiological conditions, it is probable that the central motor system also programs an increase in arterial blood

flow if the planned movement involves groups of muscles and, as is well known, at a certain point (e.g. fight or flight) hormones are involved. However, ultimately the reaction is that of the muscle being able to cope with a sudden increase in oxygen consumption which, for the first few seconds, may not be accompanied by an increase in regional flow. In this situation local regulatory mechanisms are of primary importance.

Even during conditions of oxygen lack, this most efficient local system for the regulation of capillary blood flow in skeletal muscle ensures that the tissue oxygen supply is always sufficient to supply the needs of cells at the critical end of the supply chain.

The authors are grateful to Drs H. Günther and H. Vogel, N. Hagen, A. Kellermann and A. Rebhan for their scientific and technical assistance. D.K.H. was supported by the Alexander von Humboldt Foundation, Bonn during the first stage of this research.

REFERENCES

ALTMAN, P. L. & DITTMER, D. S. (1964). *Biology Data Book*, p. 239. Federation of American Societies for Experimental Biology, Washington, DC.

ARMSTRONG, R. B. & LAUGHLIN, M. H. (1983). Blood flows within and among rat muscles as a function of time during high speed treadmill exercise. *Journal of Physiology* **344**, 189–208.

BEIER, I. (1987). Die Verteilung des Sauerstoffpartialdruckes an der Oberfläche des Musculus gracilis der Ratte. M.D. Dissertation. University of Erlangen/Nürnberg.

CHANCE, B. (1988). Early reduction of cytochrome c in hypoxia. *FEBS Letters* **226**, 343–346.

CONNETT, R. J., GAYESKI, T. E. J. & HONIG, C. R. (1984). Lactate accumulation in fully aerobic, working, dog gracilis muscle. *American Journal of Physiology* **246**, 120–128.

FIESCHI, C., ISAACS, G. & KETY, S. S. (1968). On the question of heterogeneity of the local blood flow in grey matter of the brain. In *Blood Flow through Organs and Tissues*, ed, BAIN, W. H. & HARPER, A. M., pp. 226–231. Livingstone, Edinburgh.

GAYESKI, T. E. J., CONNETT, R. J. & HONIG, C. R. (1985). Oxygen transport in rest-work transition illustrates new functions for myoglobin. *American Journal of Physiology* **248**, H914–921.

GAYESKI, T. E. J., CONNETT, R. J. & HONIG, C. R. (1987). Minimum intracellular pO_2 for maximum cytochrome turnover in red muscle *in situ*. *American Journal of Physiology* **252**, H906–915.

GAYESKI, T. E. J. & HONIG, C. R. (1978). Myoglobin saturation and calculated pO_2 in single cells of resting gracilis muscles. *Advances in Experimental Medicine and Biology* **94**, 77–84.

GAYESKI, T. E. J. & HONIG, C. (1986). O_2 gradients from sarcolemma to cell interior in red muscle at maximal VO_2. *American Journal of Physiology* **251**, H789–799.

GÖRNANDT, L. & KESSLER, M. (1973). Tissue pO_2 in regenerating liver tissue. In *Oxygen Supply* ed. KESSLER, M., BRULEY, D. F., CLARK, L. C., LÜBBERS, D. W., SILVER, I. A. & STRAUSS, J., pp. 288–289. Urban & Schwarzenberg, München/Berlin/Wien.

GROEBE, K. & THEWS, G. (1986). Theoretical analysis of oxygen supply to contracted skeletal muscle. *Advances in Experimental Medicine and Biology* **200**, 495–514.

GROOM, A. C., ELLIS, C. G., WRIGLEY, S. M. & POTTER, R. F. (1986) Architecture and flow patterns in capillary networks of skeletal muscle in frog and rat. In *Microvascular Networks: Experimental and Theoretical Studies*, ed. POPEL, A. S. & JOHNSON, P. C., pp. 61–76. Karger, Basel.

HAMMERSEN, F. (1971). *Anatomie der terminaler Strohmbahn*. Urban & Schwarzenburg, München/Berlin/Wien.

HARRISON, D. K. (1989). Regulation der Kapillardurchblutung und Sauerstoffversorgung des Skelettmuskels – ein neues Modell. Habilitationsschrift. University of Erlangen/Nürnberg.

HARRISON, D. K., BIRKENHAKE, S., KNAUF, S., HAGEN, N., BEIER, I. & KESSLER, M. (1988). The role of high flow capillary channels in the local oxygen supply to skeletal muscle. *Advances in Experimental Medicine and Biology* **222**, 623–630.

HARRISON, D. K., FRANK, K. H., BIRKENHAKE, S., HAGEN, N., DÜMMLER, W., APPELBAUM, K. &

KESSLER, M. (1987). Direct comparison of fluorescent tracer and hydrogen washout techniques in skeletal muscle. In *Microcirculation: an Update*, vol. I, ed. TSUCHIYA, M., ASANO, M., MISHIMA, Y. & ODA, M., *Excerpta Medica*, 755. Elsevier, Amsterdam.

HARRISON, D. K., GÜNTHER, H., VOGEL, H. & KESSLER, M. (1985). Oxygen supply and microcirculation of the beating dog heart after haemodilution with Fluosol DA 20%. *Advances in Experimental Medicine and Biology* **191**, 445–452.

HARRISON, D. K. & KESSLER, M. (1989*a*). A multiwire hydrogen electrode for *in vivo* use. *Physics in Medicine and Biology* **34**, 1397–1412.

HARRISON, D. K. & KESSLER, M. (1989*b*). Local hydrogen clearance as a method for the measurement of capillary blood flow. *Physics in Medicine and Biology* **34**, 1413–1428.

HARRISON, D. K., KESSLER, M. & KNAUF, S. K. (1990). Regulation of capillary blood flow and oxygen supply in skeletal muscle in dogs during hypoxaemia. *Journal of Physiology* **420**, 431–446.

HÖPER, J. & KESSLER, M. (1981). pO_2 and sodium dependent mechanism regulating liver blood flow. In *Oxygen Transport to Tissue*, ed. KOVACH, A. G. B., DORA, E., KESSLER, M. & SILVER, I. A., *Advances in Physiological Science*, vol. 25, pp. 163–164. Akademiai Kiado/Pergamon, Budapest/London.

HONIG, C. R., ODOROFF, C. L. & FRIERSON, J. L. (1982). Active and passive capillary control in red muscle at rest and in exercise. *American Journal of Physiology* **243**, H 196–206.

HUTTEN, H. (1970). Untersuchung nichtstationärer Austauschvorgänge in gekoppelten Konvektions-Diffusions-Systemen, p. 41. Steiner, Akadamie der Wiss. u. d. Literatur, Wiesbaden.

KAZMERS, A., WHITEHOUSE, W. M. JR., ZELENOCK, G. B., CRONENWETT, J. L., LINDENAUER, S.M. & STANLEY, J. C. (1984). Early derangements of arteriovenous anastomosic and capillary blood flow in the canine hindlimb induced by supplemental pentobarbital anesthesia. *Journal of Surgical Research* **36**, 102–107.

KESSLER, M., HARRISON, D. K. & HÖPER, J. (1986). Tissue oxygen measurement techniques. In *Microcirculatory Technology*, ed. BAKER, C. H. & NASTUK, W. L., pp. 391–425. Academic Press, New York.

KESSLER, M., HÖPER, J., HARRISON, D. K., SKOLASINSKA, K., KLÖVEKORN, W. P., SEBENING, F., VOLKHOLZ, H. J., BEIER, I., KERNBACH, C., RETTIG, V. & RICHTER, H. (1984). Tissue O_2 supply under normal and pathological conditions. *Advances in Experimental Medicine and Biology* **169**, 69–80.

KESSLER, M., HÖPER, J., LÜBBERS, D. W. & JI, S. (1981). Local factors affecting regulation of microflow, O_2 uptake and energy metabolism. In *Oxygen Transport to Tissue*, ed. KOVACH, A. G. B., DORA, E., KESSLER, M. & SILVER, I. A., *Advances in Physiological Science*, vol. 25, pp. 155–162. Akademiai Kiado/Pergamon, Budapest/London.

KUNZE, K. (1967). Das Sauerstoffdruckfeld im normalen und pathologisch veränderten Muskel. Habilitationsschrift, Giessen.

LÜBBERS, D. W. & WODICK, R. (1971). Sauerstofftransport im Warmblüterorganismus. *Umschau in Wissenschaft und Technik* **13**, 486–492.

LUND, N. (1979). Studies on skeletal muscle surface oxygen pressure fields. Dissertation No. 71. Linköping University Medical Dissertations, Linköping.

LUND, N. (1985). Skeletal and cardiac muscle oxygenation. *Advances in Experimental Medicine and Biology* **191**, 37–43.

MYRHAGE, R. & ERIKSSON, E. (1984). Arrangement of the vascular bed in different types of skeletal muscles. In *Skeletal Muscle Microcirculation*, ed. HAMMERSEN, F. & MESSMER, K., pp. 1–14. Karger, Basel.

OUDE VRIELINK, H. H. E., SLAAF, D. W., TANGELDER, G. J. & RENEMAN, R. S. (1986). Does capillary recruitment exist in young rabbit skeletal muscle? *International Journal of Microcirculation: Clinical and Experimental* **5**, 220.

POTTER, R. F. & GROOM, A. C. (1983). Capillary diameter and geometry in cardiac and skeletal muscle studied by means of corrosion casts. *Microvascular Research* **25**, 68–84.

RINK, R. & KESSLER, M. (1973). Signs of hypoxia in the small intestine of the rat during hemorrhagic shock. *Advances in Experimental Medicine and Biology* **37A**, 469–475.

ROBISCH, J. (1986). Automatisierung einer Glas-Zieheinrichtung. Diplomarbeit. Lehrstuhl für Elektrische Antriebe und Steuerung. University of Erlangen/Nürnberg.

STARLINGER, H. & LÜBBERS, D. W. (1973). Polarographic measurements of the oxygen pressure

performed simultaneously with optical measurements of the redox state of the respiratory chain in suspensions of mitochondria under steady-state conditions at low oxygen tensions. *Pflügers Archiv* **341**, 15–22.

TAMURA, M., HAZEKI, O., NIOKA, S. & CHANCE, B. (1989). *In vivo* study of tissue oxygen metabolism using optical and nuclear magnetic resonance spectroscopies. *Annual Reviews of Physiology* **51**, 813–834.

TYML, K. (1987). Red cell perfusion in skeletal muscle at rest and after mild and severe contractions. *American Journal of Physiology* **252**, H485–493.

ZIERLER, K. L. (1965). Equations for measuring blood flow by external monitoring of radioisotopes. *Circulation Research* **16**, 309–321.