granules, is in keeping with the observation that the enzymatic activity of lysosomes is not changed after vital staining with NR[15]. NR has a potent vasodilator action in relatively low doses. The results of our hindlimb-perfusion experiments show that the inhibition of noradrenaline-induced vasoconstriction by NR is the probable mechanism for the vasodilation. NR has a special capacity to accumulate in the granules of the APUD endocrine cells[16], which also bind catecholamines and their precursors[17,18]. It is conceivable that NR has an affinity to other catecholamine-binding sites, too. Thus, NR can displace noradrenaline from its binding sites in vascular smooth muscle cells.

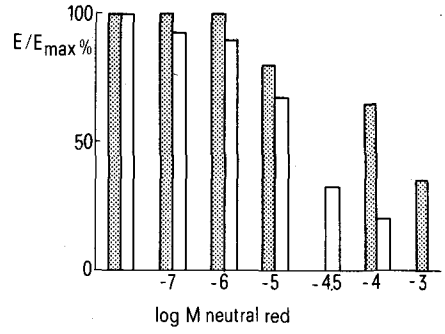


Fig. 2. Effect of neutral red on the perfusion pressure of isolated hindlimbs. White columns represent the effects of neutral red concentrations on the perfusion pressure produced by $10^{-5.5}$ M/l L-noradrenaline. Dotted columns represent the effects of neutral red concentrations on the perfusion pressure produced by 90 mM/l KCl.

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2 This work was supported by the German Research Foundation within the SFB 90 'Cardiovasculäres System'.
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## Skeletal muscle capillary densities during reactive hyperemia

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*Summary.* Reactive hyperemia was induced in hindlimbs of rats by occlusion of the femoral artery. Using fluorescein dye as a peripheral vascular marker, we observed that there was an increase in the number of flowing capillaries supplying the muscle fibres following release of the occlusion. The results indicate that the number of flowing capillaries is not dependent on the duration of occlusion (2-10 min).

Reactive hyperemia is defined as the increase in blood flow which occurs immediately following the release of complete arterial occlusion. Krogh[1], using a method of intravitam injection of India ink, observed that reactive hyperemia in skeletal muscle was associated with an increase in the number of flowing capillaries. More recently, Burton and Johnson[2], using a dual slit photometric system to measure red blood cell velocity profiles, suggested that the increase in blood flow following occlusion release is due to an augmentation of blood flow through previously flowing capillaries rather than an increase in the number of flowing capillaries. In the experiments reported here, we measured capillary densities following arterial occlusion in the musculature of rats using fluorescein dye as a peripheral vascular marker. In addition, we investioated the relationship between occlusion duration and capillary density since both the magnitude of flow response and duration of hyperemia have been shown to be related to the duration of cessation of flow[3,4].

*Materials and methods.* Adult male rats (500-750 g) were anesthetized with sodium pentobarbital (i.p., 35 mg/kg) and one of the external jugular veins was cannulated with polyethylene tubing. The gracilis muscle of each hindlimb was exposed. The femoral artery of the left hindlimb was dissected free and occluded for either 2, 6, 8 or 10 min. The right hindlimb served as the control since no elevation in blood flow through the contralateral vessel during reactive hyperemia has been reported[5]. At the end of the occlusion period, blood flow was returned to the left hindlimb. 15 sec after release of the arterial occlusion, a 6.0% solution of sodium fluorescein (300 mOsmols; 0.005 ml/g b.wt) was injected via the jugular cannula. 8 sec after the completion of injection, the gracilis muscle of each hindlimb was frozen in situ by the use of copper plated forceps precooled in liquid nitrogen (−196 °C). These timed intervals before freezing were determined to be optimal for all durations of occlusion suggesting that the time to peak capillary response was similar for all occlusion intervals under these conditions. Following freezing, each muscle was quickly excised from the animal and placed in separate dewars of liquid nitrogen. The frozen muscles were removed from the liquid nitrogen and placed in a cryostat (−30 °C; International Model CTV Microtome Cryostat). A tissue block (6×2×1 mm) was cut from the center of the muscles and mounted for sectioning so that the axial ends of the muscle were cut. After sectioning, the tissue sections (6-12 µm) were freeze-dried at pressures between $10^{-4}$ and $10^{-5}$ Torr for 14-16 h (Thermovac cryopump, TEFD-G2). Following

Capillary densities during reactive hyperemia

| Duration of occlusion | Number of animals | Capillary density (capillaries containing fluorescein per mm$^2$) | | Ratio of capillary densities (hyperemic value: control value) |
|---|---|---|---|---|
| | | Control muscle | Hyperemic muscle | |
| 2 min | 5 | 347 ± 19 | 614 ± 55* | 1.8:1 |
| 6 min | 5 | 403 ± 45 | 682 ± 67* | 1.7:1 |
| 8 min | 5 | 378 ± 42 | 716 ± 26* | 1.9:1 |
| 10 min | 5 | 377 ± 39 | 621 ± 30* | 1.7:1 |

Values in the 3rd and 4th columns are the mean ± SEM. The asterisks represent statistical difference from control values ($p < 0.05$).

drying, the tissue sections were mounted on glass slides with benzene and sealed with mounting cement.

Photomicrographs of hyperemic and control tissue sections were taken by fluorescence microscopy. Capillary densities were determined from the negatives of photomicrographs by profile projection. Only capillaries which contained fluorescein dye were considered to be 'flowing' during the blood flow response. Countings were done on 3 different exposures for each determination. These countings were averaged and the capillary density was recorded as capillaries per mm$^2$. Some experiments were counted 'blindly' to avoid bias. Mean values and SEM were calculated for the hyperemic and control capillary densities for each occlusion interval. The ratio of flowing capillaries in the hyperemic muscle versus those flowing in the contralateral muscle was determined. An unpaired analysis (Student's t-test) was used to compare the different groups statistically. The significance level for all analyses was $p < 0.05$.

*Results.* The capillary densities of hyperemic and control muscles at the different occlusion intervals are shown in the table. The hyperemic muscles contained almost twice as many capillaries with fluorescein as compared to their respective control muscles. The density of capillaries was not statistically correlated with the duration of the arterial occlusion. The ratio of capillary densities (hyperemic vs control) was approximately 1.8:1 for all periods of occlusion (see last column, table).

*Discussion.* The goal of the present study has been to determine if reactive hyperemia in skeletal muscle is associated with an alteration in the number of flowing capillaries supplying the muscle fibres. Capillary densities were determined in the gracilis muscle of the rat following release of arterial occlusion. The rat gracilis muscle was selected for these studies because of its ease of isolation, its blood supply, and accessibility for freezing. Using fluorescein dye as a peripheral vascular marker, we observed that reactive hyperemia in this muscle was characterized by an almost 2-fold increase in the number of flowing capillaries as compared to the contralateral control muscle. Furthermore, the results indicate that the number of flowing capillaries during the postocclusion hyperemic phase is not dependent on the duration of occlusion (occlusion duration: 2-10 min).

Krogh[1], using India ink injection method, observed that reactive hyperemia was due mainly to changes in the number of flowing capillaries supplying the tissue. More recently, Gentry and Johnson[6] and Burton and Johnson[2] have shown that reactive hyperemia in cat sartorius muscle appears to be due to an augmentation of blood flow in capillaries already flowing rather than an increase in the number of flowing capillaries. Burton and Johnson[2] also pointed out that the ratio of flowing capillaries in hyperemic muscle to those supplying the control muscle was about 1.3:1. They suggested that this was not indicative of an increase in capillary number during reactive hyperemia, since the capillaries flowing during the control phase had only short intervals of zero flow. The results of this study show that the ratio of flowing capillaries in the hyperemic muscle to those in the control muscle was approximately 1.8:1. Although the reasons for this difference in ratios are not apparent, it may be due in part to differences in animal species[7]. It is also possible that the experimental conditions may have contributed to the difference in the capillary response. For example, Klitzman and Duling[8] have observed that the capillary hyperemic response in hamster cremaster muscle is importantly related to the oxygen supplied to the tissue. At 5% $O_2$, the ratio of flowing capillaries during contraction to those at rest was 2.0:1 (calculated from their results); when 0% $O_2$ was perfused, the ratio was 1.3:1. The metabolic behavior of the muscle fibres may also be an important determinant of the capillary response during postocclusion hyperemia. Gray[9] has observed that the vascular bed of white skeletal muscle is characterized by vasomotion and many momentarily flowing capillaries in the resting state, whereas the blood flow in red muscle is much more steady and uniform. The rat gracilis muscle is a mixed muscle, containing both white and red fibres. It is therefore possible that the composition of muscle fibres in this tissue may have contributed to the capillary reactive hyperemia response.

These experiments also indicate that the capillary response during reactive hyperemia is not dependent upon the duration of arterial occlusion. The ratio of flowing capillaries in the hyperemic muscle to capillaries flowing in the control was approximately 1.8:1 at all occlusion intervals (2-10 min). This observation may reflect the fact that the peak blood flow of reactive hyperemia is relatively little increased with prolonged periods of arrest of the circulation. Myhre[4] has observed that the maximal flow after 30-sec arterial occlusion was 89.5% of the maximal flow after 5-min occlusion. Similarly, Johnson et al.[10] have observed that relatively short occlusions (10-15 sec) are sufficient to produce near maximal capillary flows.

Our findings support the hypothesis that skeletal muscle reactive hyperemia is characterized by an increase in the number of flowing capillaries supplying the muscle fibres compared to the resting state. The duration of the occlusion period (2-10 min) did not significantly alter the magnitude of the capillary reactive hyperemia.

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