INTRODUCTION

Use of myoglobin (Mb) as an indicator of intracellular PO₂ has been dormant for half a century, chiefly because of difficulty in differentiating Mb from hemoglobin (Hb) when both are illuminated. We recently devised a microspectrophotometer with which light can be collected exclusively from either Hb or Mb. Spatial resolution is 2-5 μ. Since freezing arrests chemical reaction, saturation measurements on a large cell population can be interpreted as though all the measurements had been made simultaneously. In this way the purely spatial uniformity of O₂ delivery can be evaluated. Measurements can be made at several loci within one cell, at various loci in a cell cluster, or in cells selected at random from grossly different regions of the muscle. The method offers the further advantage that the contribution of local capillary recruitment to O₂ delivery can be evaluated.

METHODS

Dogs, 20-25 Kg body wt., were anesthetized with pentobarbital, 30 mg/Kg. Both gracilis muscles were isolated, taking care to preserve all 3 sets of vessels and the obturator nerve. The muscle surface was covered with Saran® and maintained at 37°C. Venous blood could be returned to the heart or diverted to a tared flask for flow and O₂ measurements. VO₂ (O₂ consumption) was measured by use of the Fick principle. Immediately thereafter the Saran® covering was removed, and a 5 cm cube of copper, cooled to -196°C, was applied to the muscle at 0.09 Kg/cm² by use of an air-driven piston. In a representative experiment loci 600 μ from the surface reached 0°C in 470 msec, and -43°C in 1 sec. If VO₂ is 2.5 μl/g.min,
Q10 is 2, and [Mb] is 0.5 mM. VO₂ during cooling changes saturation by 10⁻⁵ per cent. The amount of O₂ physically dissolved is so small that the increase in O₂ affinity of myoglobin during cooling introduces a similarly trivial error. Though loci up to 2 mm deep are probably suitable in the resting state, measurements were made between 200 and 600 μ from the surface.

The muscle was transferred to liquid N₂ while still in contact with the copper block, and stored at -196°C. No change in the frequency distribution of saturation could be detected in muscles stored for 9 mo. The microscope cold stage consisted of a brass heat sink containing liquid N₂, to the base of which a brass column was attached. A well in this column was filled with 95% ethyl alcohol, regulated at -110°C ± 3°. At this temperature no change in the spectrum of fully desaturated Mb was observed over a 3 hr. period. The sample was positioned in the well so that a freshly cut muscle surface was just above the alcohol surface. Cells were observed in cross-section.

The specimen was viewed by reflected light with a Leitz orthoplan microscope and metallurgical head. Polarizers (extinction υ 1:5000) attenuated reflection from the film of viscid alcohol which coated the specimen. The alcohol prevented frost formation. A 1000 W Xenon lamp and monochromator (300 nm blaze) provided quasi-monochromatic light at wave lengths set ± 0.1 nm by a control circuit and stepping motor. A block diagram of the system is shown in Fig. 1.