METHOD OF INTRAVITAL MICROSCOPY OF BLOOD VESSELS OF THE FROG HYOGLOSSUS MUSCLE DURING AND AFTER STAINING

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A design is suggested for an illuminator for use in investigating blood vessels of the frog hyoglossus muscle in transmitted light. Simultaneous simulation of the two symmetrical hyoglossal nerves virtually prevents displacement of the muscle during its contraction and enables observations to be made on the blood vessels of the muscle actually during contraction.

To study reactions of blood vessels of skeletal muscles by intravital microscopy, Gaskell [3] and Heileman [4] used the flat hyoglossus muscle of the frog. To illuminate the muscle Gaskell cut it along one edge, turned it over, stretched it over a hole, and fixed it with pins. However, trauma of this nature must disturb the state of the vessels in the muscle. Heileman removed the frog's head and thus disturbed the state of the systematic circulation.

To avoid these disadvantages, the writers at first attempted to use a thin light conductor by bringing it from the free border of the muscle up to the base of the mandible. The light conductor consisted of a quartz rod [5-7] or a table type of light conductor [1], bent sharply and drawn out into a cone, the end of which was placed beneath the muscle.

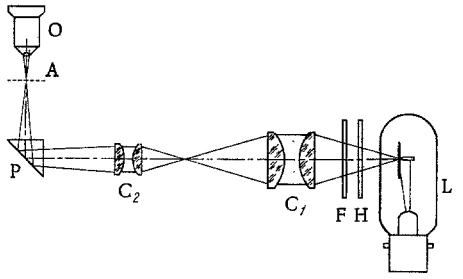

Fig. 1

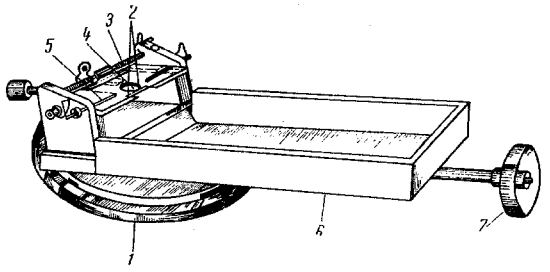

Fig. 2

Fig. 1. Diagram of illuminator. L) source of light; H) water heat filter; F) light filters; C_1, C_2) large and small condensers; P) prism; A) object to be examined; O) objective.

Fig. 2. Stage for frog incorporating chamber for microscopic examination of blood vessels of hyoglossus muscle: 1) stage of microscope; 2) transparent plastic slab; 3) spring; 4) hole for illumination and microscopic examination of muscle; 5) electrodes; 6) bath for frog's trunk; 7) knob for moving stage.

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To illuminate an optically dense object such as the muscle, light of very great brightness is required. A 100-W lamp with iodine cycle (KIM-12-100) therefore was used as the source of light. Experiments showed, however, that the light emerging from the end of the quartz or cable light conductor as a widening cone is scattered and largely absorbed by the surrounding tissues. Furthermore, when the end of the conductor is placed beneath the muscle, stretching of the muscle takes place with disturbance of the state of its blood vessels.

For this reason a special illuminator was designed and made (Fig. 1). It is similar in principle to that used previously for investigating the microcirculation in bone marrow [2].

To preserve the physiological state of the hyoglossus muscle it was illuminated from the mouth, after preliminary removal of all other muscles of the oral diaphragm.

The source of light was a zirconium lamp (DATs-50), with a point incandescent body (greatest diameter 1 mm) and giving great brightness. The following conditions were taken into consideration when designing the illuminator: 1) To obtain uniform illumination of the field of observation the diameter of the spot of light on the object must be twice the diameter of the incandescent body; 2) the diameter of the end of the illuminator must not exceed 8 mm so that it can easily be placed inside the frog's mouth; 3) the maximal intensity of illumination must be in the plane of the object.

The large condenser (C_1) of the illuminator doubles this diameter of the spot of light. To reduce the diameter of the outlet part of the illuminator a condenser (C_2) is used; this transforms the image of the source given by the condenser C_1, twice its natural size, into the focal plane of the objective. A prism P, turning the rays through 90°, is placed in the path of the light flux between the condenser C_2 and the plane of the object A.

The illuminator incorporates a water heat filter (H) and light filters (F), placed between the source of light and the condenser C_1. The water filter and a fan which circulates air around the jacket of the lamp prevent over-heating of the muscle.

To study the reactions of the blood vessels during and after contraction of the muscle a special stage is used (Fig. 2). This is fixed onto the stage of the microscope 1 so that it can be freely moved under the objective and any part of the muscle can be observed. The stage consists of a bath 6, filled with water or physiological saline, in which the frog's trunk is placed, and a chamber for the hyoglossus muscle. The purpose of the chamber is to prevent the muscle from drying and to prevent excessive bending of the muscle during contraction.

The chamber consists of two transparent plastic slabs 2, pressed together by a light spring 3, between which the hyoglossus muscle is placed. So that observations can be made on the blood vessels a hole 4 is made in the upper slab and a coverslip is glued to it. The space between the muscle and the upper slab is filled with physiological saline. To stimulate the hyoglossal nerves, two unipolar silver electrodes 5, enabling both hyoglossal nerves to be stimulated simultaneously, are mounted between the slabs. By stimulating the symmetrical nerves in this way displacement of the muscle during its contaction is practically eliminated, and in this way observations can be made on the blood vessels of the muscle and motion pictures taken of them actually during contraction.

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