INTRAVITAL METHOD OF STUDYING THE MICROCIRCULATION IN THE CREMASTER MUSCLE IN TRANSMITTED LIGHT

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A stand has been designed for albino rats to provide optimal conditions for optical observations on the vessels of the microcirculation of the cremaster muscle in transmitted light. Changes in the hemodynamics were recorded by a microfilming technique using the MKU-2 miniature motion picture camera manufactured by the Leningrad Optical Engineering Combine.

Several methods based on the use of incident light have been suggested recently for intravital microscopic studies of the peripheral circulation in skeletal muscles [2-4]. However, despite the use of modern opaque systems, incorporating a ring condenser increasing the depth of sharpness of the observed object, it is very difficult to obtain an image of high contrast of an untreated specimen.

To investigate the hemodynamics in the cremaster muscle of albino rats, Grant [2] used the tunica albuginea as a natural reflector of light. Majno et al. [3], to obtain better reflection of incident light, used a reflector as described by Fonviller [1]. They introduced a spatula into the sac of the tunica vaginalis

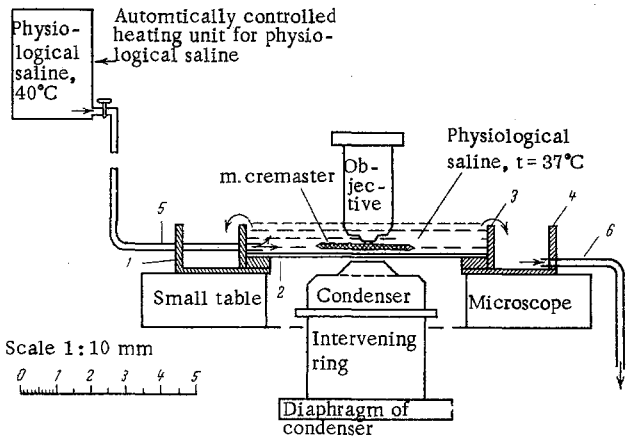

Fig. 1. Theoretical scheme of apparatus: 1) base of stand for animal; 2) transparent base of bath in which muscle is placed; 3) side of bath; 4) run off for excess physiological saline; 5) tube supplying physiological saline to bath; 6) outlet tube for physiological saline.

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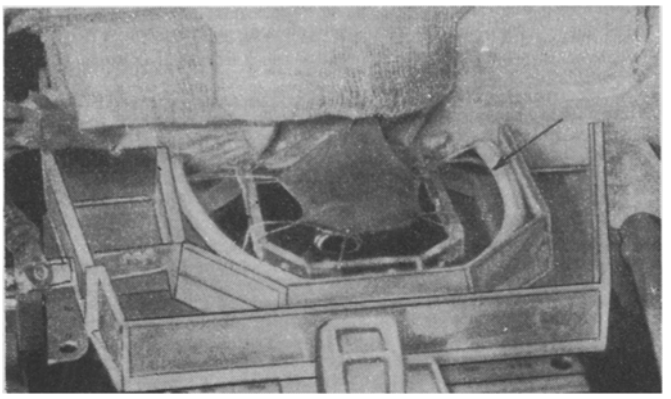

Fig. 2. Arrangement of muscle in bath containing physiological saline. Arrow points to heating system.

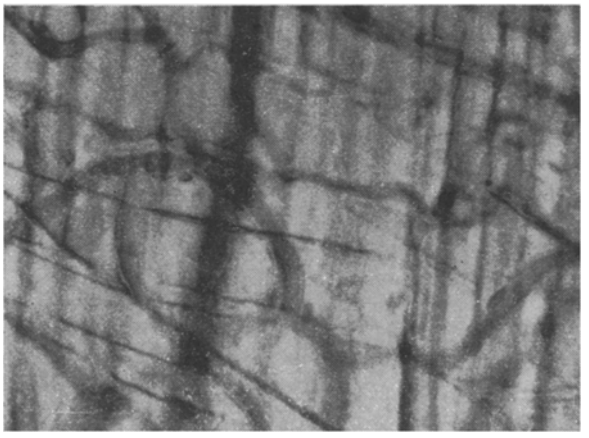

Fig. 3. Arteriole branching into precapillaries and capillaries (intravital photomicrograph, 150×).

of the scrotum in albino rats through the peritoneal cavity. The scrotal skin was divided and retracted. Since, in order to study the microcirculation in the organs, it is extremely important to know the cellular composition of the wall of the intramural blood vessels, and also their state, a method of intravital microscopy of the microcirculation of the cremaster muscle in transmitted light was developed. By working with transmitted light, satisfactory contrast and good depth of sharpness of an untreated specimen can be obtained, and the resolving power of ordinary and immersion objectives can be utilized to the full.

Wistar albino rats weighing 150-180 g were anesthetized with Nembutal (5 mg/100 g body weight, intramuscularly). The skin of the scrotum was divided on the left for a distance of 3-3.5 cm from the lower pole of the testis and retracted. The subcutaneous cellular tissue, which is poorly developed in the scrotal region of rats, was separated by blunt dissection from the cremasteric fascia covering the cremaster muscle. A monolayer specimen of the muscle was then formed. To do this, the cremasteric fascia, cremaster muscle, internal spermatic fascia, and the parietal layer of the tunica vaginalis testis were divided in one stage by a lateral incision so as not to disturb the blood supply to the muscle as a whole. Vessels running toward the testis were ligated, after which they were freely returned to the peritoneal cavity. This concluded the first stage of preparation for the experiment.

After the preparation of the monolayer specimen of the cremaster muscle, the animal was placed in a special stand fixed to the stage of the microscope. The design of the stand for the animal was adapted for the MBB-1 microscope, on the base of which a type MKU-2 miniature motion picture camera, manufactured by the Leningrad Optical Engineering Combine, was mounted. The theoretical scheme of the experiment is shown in Fig. 1. An essential feature of the design of the stand for the animal is that the organ to be studied must be in the focal plane of the condenser; otherwise it would be impossible to obtain a satisfactory depth of focus, with a consequent loss of resolving power of the objective. By means of the intervening ring C placed between the condenser and its diaphragm, the condenser could be raised above the upper plane of the microscope stage so that it almost came up to the transparent base of the bath (Fig. 1) in which the muscle was placed.

The stand for the animal (Fig. 2) was made of transparent plastic and consisted of two compartments A and B, separated by a vertical wall having a piece cut out. The animal was placed on the left side in compartment A. The cremaster muscle was passed through the notch in the vertical wall into the compartment B, designed for observation on the specimen. Compartment B consists of a bath with thin, transparent glass base, 1 mm thick (Fig. 1, 2), in which the muscle is stretched out, and a system of channels

(Fig. 1, 4), leading away the physiological saline bathing the muscle. The muscle was stretched by 4 or 5 ligatures as shown in Fig. 2. Ringer's solution with gelatin (1 g gelatin to 100 ml solution), warmed to 40°C in a special unit with automatic heating control, was supplied to the bath along the tube 5. As a result of the loss of heat in the system the temperature in the bath was maintained at 36.5-37°C. A heating system (Fig. 2), connected with an ultrathermostat working on an external circuit, was placed in the bath. As the physiological saline entered the bath, any excess overflowed freely over its side and was led away via the outflow system (Fig. 1, 6). The experimental conditions were close to the normal physiological conditions and they enabled the steady circulation of blood in the muscle to be studied for 6-8 h.

To study the character of the blood flow in the muscle capillaries, miniature motion pictures were taken with a Konvas-avtomat camera at filming speeds varying from 10 to 48 frames/sec.

With the method described it is possible to study the normal hemodynamics in a skeletal muscle (Fig. 3), the cell composition of the walls of blood vessels in a small part of the circulatory system, and the responses of the vessels to various drugs.

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