AN IN VIVO METHOD OF OBSERVING NERVES IN FROG URINARY BLADDER

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Vital microscopy enhances the opportunities offered the histologic experimenter and aids in the correlation of morphological and physiological knowledge concerning the animal and human organism.

Real progress in achieving in vivo studies of the finer structures of the nervous system has been attained only recently. This circumstance explains the lack of adequate procedures for this investigative method.

In 1932 M. I. Gramenitsky [3] suggested microscopic observations on a living preparation of a distended frog heart using a penetrating light. Soon, from the laboratories of D. N. Nasonov and B. I. Lavrentyev there appeared a series of studies on the morphology of nerve cells (in the excitatory or phase of injury states) the material having been taken from frog intramural cardiac ganglia. Applying such stimulators as chemical, mechanical and electrical ones to the Gramenitsky preparation D. N. Nasonov, B. I. Lavrentyev and their pupils were able to follow experimentally the changes in the aggregate state of the neuroplasm, the nucleolus and the nucleus, as well as the pericellular apparatus.

In the later years, the penetrating-light method of vital microscopy has attained an increasing development. The technique has been developed by P. A. Fonviller [5], A. A. Vishnevsky and B. I. Lavrentyev [1, 2] and their students. This procedure differs from the Gramenitsky preparation in that it allows the study of the finer nerve structures while the circulation is intact, and also permits study of opaque or only slightly translucent organs so that the two methods can be used very satisfactorily to supplement each other.

Unfortunately, the penetrating-light method is effective for nerve studies only after a preliminary staining with a weak methylene blue solution, which acts as a stimulator. For this reason the Gramenitsky preparation is to be preferred, as it permits study of such fine structures as the pericellular apparatus without stains.

In developing the technique of vital microscopy for the study of the nervous system, we searched for a method which would combine the virutes of the two described methods. We believe we were successful in making such a preparation from the urinary bladder of the grass frog, which has been long used in vital microscopy of the circulation.

The preparation so made enabled us to make observations in penetrating light at all microscopic magnifications and with the preservation of the blood circulation, nervous connections and nerve fibers since the operated frog (with good care) can live for months.

To make these preparations, we took mainly larger grass frogs, male and female, weighing no less than 50 g, since the larger the animal, the less the necessity of resorting to a magnifying glass to control the course of the operation.

To immobilize the frogs, we used native curare-like diplastin* allowing 0.02 mg per g weight of animal. Such a dose weakens the skeletal musculature without impairing the respiration, over a period of some 4 hours.

* Russian trade name.

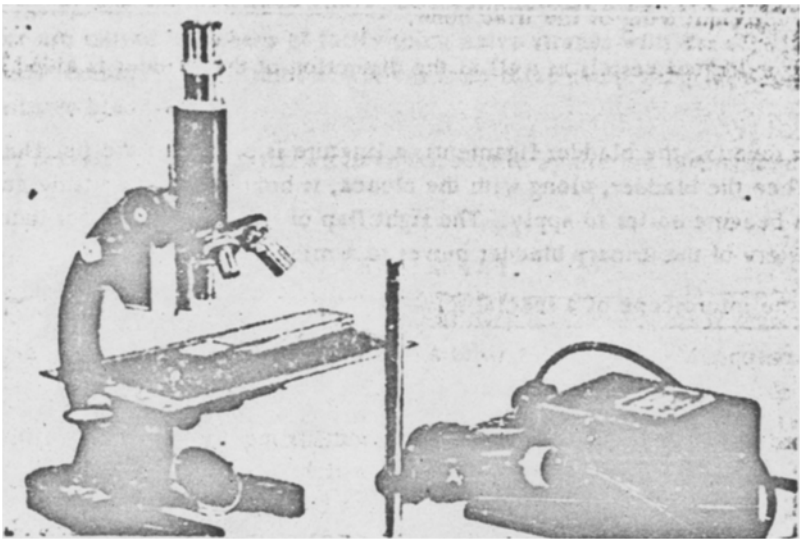

Fig. 1. General view of the special stage replacing the usual objective stage of the microscope.

If the experiment continued for a longer period, an additional half-dose injection would be given. Diplastin can be given intramuscularly or injected into the lymphatic sac.

Ringer solution was used for moistening the wound and skin surfaces.

The procedure consists of three stages: 1) preparation of the urinary bladder, 2) placement of a special stage on the microscope, 3) fastening the body of the frog and its urinary bladder to the special stage.

1. Preparation of the urinary bladder.

Essentially, this stage consists in extirpation of the wing of the iliac bone, free dissection of the corresponding flap of the urinary bladder and bringing it, along with the colon, outside the abdominal cavity. It is easier to work from the right, as the colon lies mostly on that side in the frog.

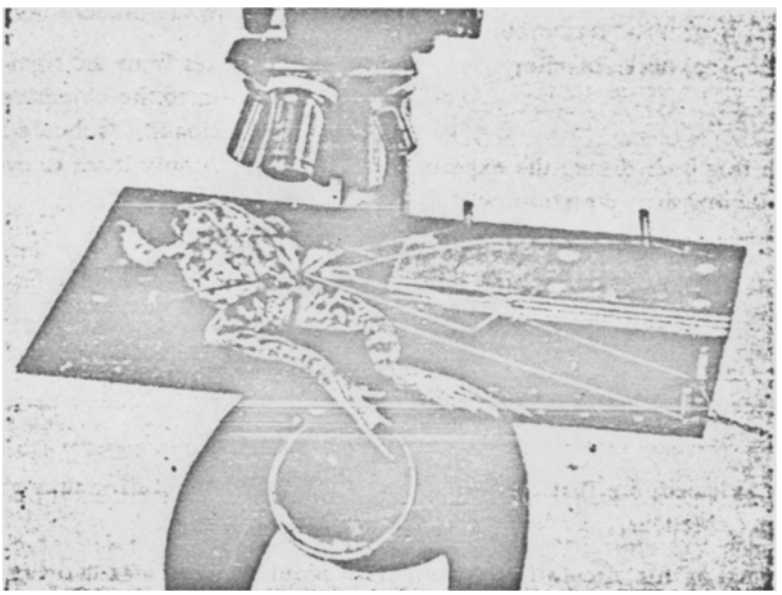

Fig. 2. General view of the complete preparation along with the needle used for intra-arterial infusions. k) Needle cannula.

Hemorrhage is avoided by the ligation of the spinolumbar vein and then the femoral artery before undertaking the extirpation of the right wing of the iliac bone.

The ligation of the indicated vessels as well as the dissection of the bladder is aided by the use of a magnifying glass.

Immediately after severing the bladder ligaments, a ligature is placed at the junction point of the large and small intestines. Then the bladder, along with the cloaca, is brought to the outside and filled with Ringer solution so that ligatures become easier to apply. The right flap of the urinary bladder then acquires a spherical shape while the spinal artery of the urinary bladder moves to a middle position.

2. Placement on the microscope of a special stage.

The ordinary microscopes M-9 and MBI-1 were used. We prefer the MBI-1 as the angle of its tube considerably simplifies the task of the investigator.

We replaced the ordinary objective stage, along with both its regulatory screws, with our special stage. The general view is as seen in Fig. 1. This stage consists of a right-angled board of about 2-3 mm thickness having small openings at the edges through which ligatures can be passed. In the center of the board is a round opening whose diameter must be no less than 4.5 cm. At the side of the aperture in the board there is fastened an immovable block having a groove for the objective slide (76 × 26 mm). This last is placed parallel to the board of the special table and is separated from it by a distance of 8 mm.

After the stage has been placed , lighting is arranged by the Köhler method [6].

3. Fastening the body of the frog and its urinary bladder to the special stage.

To fasten the body of the frog and to stretch the urinary bladder, no less than 4 ligatures must be placed: one each on the large bowel and the uper portion of the right shin, and two for the urinary bladder. The latter two are placed symmetrically, one on each side of the spinal artery, somewhat below the point where it branches into small arterioles.

The ligatures are placed by means of fine intestinal needles. After this is done, the Ringer solution is poured away from the bladder region.

The ligatures from the right shin and the colon of the frog are drawn to the objective glass so that the latter will rest on the cloaca. It should be borne in mind that inadequate fixation of the frog body during the experimental period inevitably leads to overstretching of the straightened urinary bladder and this, therefore, will disturb the circulation.

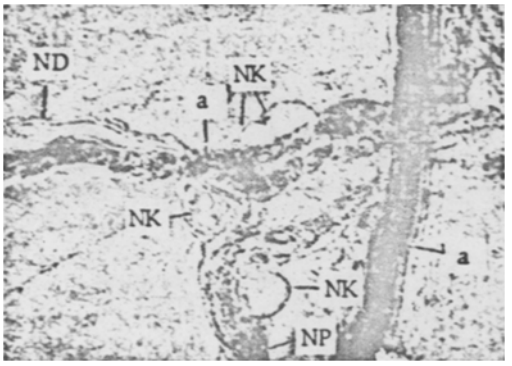

Fig. 3. Small ganglion in the nerve plexus of the urinary bladder of the grass frog. Film frame; obj. 20, ocular 10. a) Arteriole; np) nerve bundle; nk) nerve cell.

After careful placement of the ligatures fixing the body position of the animal, the bladder is straightened out, keeping in mind at all times that drying the urinary bladder surface will also diminish the flow of the blood in the vessels.

Chemical solutions may be introduced into the blood stream by means of a syringe attached to the No. 14 needle which has been placed into the lumen of one of the sciatic arteries. Figure 2 presents a general view of the preparation, the needle being in place.

As the preparation is viewed, the first thing observed is the blood circulating through the numerous vessels and capillaries of the urinary bladder.

Against the background of this circulation no staining is required as long as there is enough illumination that we may observe with sufficient clarity the intramural nerve plexus which consists of three gnaglia connected with each other by powerful nerve strands. This plexus lies on the dorsal wall of the urinary bladder between

the neck and the center of the flap being investigated. The ganglia vary in size: the smallest may contain only 6 nerve cells (Fig. 3), the average have 10-11, while the largest may have around 20 cells. The ganglia of the urinary bladder are united by means of fairly thick nerve strands with the sciatic-caudal and intramural plexi of the cloaca and rectum. In the other directions from these same ganglia, numerous strands of nerves entwine the entire urinary bladder.

The very finest nerve structures which we have uncovered by the use of this method we shall detail in subsequent communications.

SUMMARY

A special method for the in vivo study of the frog urinary bladder nerves is presented with illustrations. Subsequent communications will present additional material.

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