A METHOD OF MICROSCOPIC STUDY OF THE LIVING PERIPHERAL BLOOD CIRCULATION IN MICE

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It is well known that in the study of the physiology and pathology of the local circulation so-called "transparent cameras" are used. This method was first developed by Sandison [1] and perfected later by Clark and coworkers [2], while other investigators, particularly Williams [3], apart from students using it only in animal ears, adapted it for use in a portion of rabbit skin. All the cameras proposed by the various authors may be divided into two types. One is so constructed that the slit of the camera is built of a transparent material so that the tissues of the organ into which it has been inserted will grow into the visible field and so become the object of study; it follows that this camera type permits observation of regenerating tissues. The construction of the other camera type presupposes inclusion within the slit of the transparent camera of already formed tissues. To this second type of "transparent cameras" may be added the camera recently described by Algire [4] as being constructed specifically for insertion into skin folds of laboratory mice. This Alger Modification is of great interest as the mouse is a readily accessible animal, easily immobilized and quite conveniently adapted for microscopic observations.

However, the Algire camera has very serious defects: it is complicated, has many detailed parts, and has the especial drawback that its introduction into a skin fold is attended by marked trauma of the tissues and marked circulatory disturbance. This last effect is associated with the need for numerous sutures and holes in the skin for the passage of bolts which stretch the skin as a result of excessively high rim edges. All this led us to propose a modification of the "transparent camera" that avoided the defects just mentioned.

The camera consists of a zinc frame with a cover glass glued to it and having two assisting foundation bars (Fig 1). The dimensions as given in Fig. 1 are calculated for a mouse weighing 22-23 g; they may be altered to fit the size of the animal. The cover glass is given an edge to correspond to the frame dimensions and is glued to it with BF-2 glue.

The camera is introduced operatively under aseptic conditions; it can be done with use of narcosis (chloral hydrate solution 0.0003 g per g of animal) or without it.

The mouse is immobilized on a special table (which may be replaced by the camera plate) having five openings whose positions correspond to the paws and the middle of the tail when the mouse is prone on the table. Through each of these openings is passed a piece of doubled-up rubber tubing, or a rubber cord. The paws and tail of the mouse are inserted into the corresponding rubber loops, the free ends of which are drawn tightly to the undersurface of the table so that the mouse is firmly attached in a prone position to the table. The immobilized animal is covered with gauze sponges. Then the skin of the animal is depilated with the aid of taro starch in

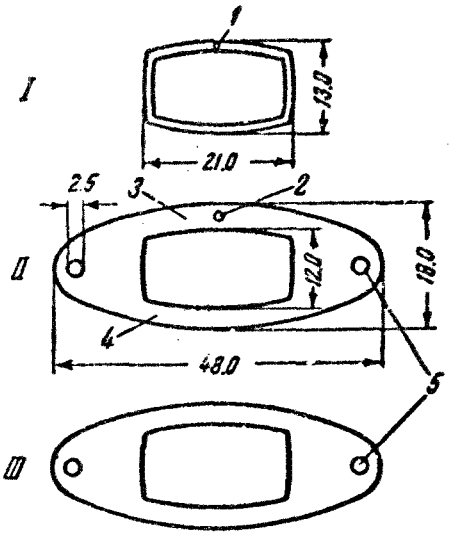

Fig. 1. I) Zinc frame to which is glued a cover glass, II) basic foundation bar, III) additional foundation resting bar. All dimensions in mm.

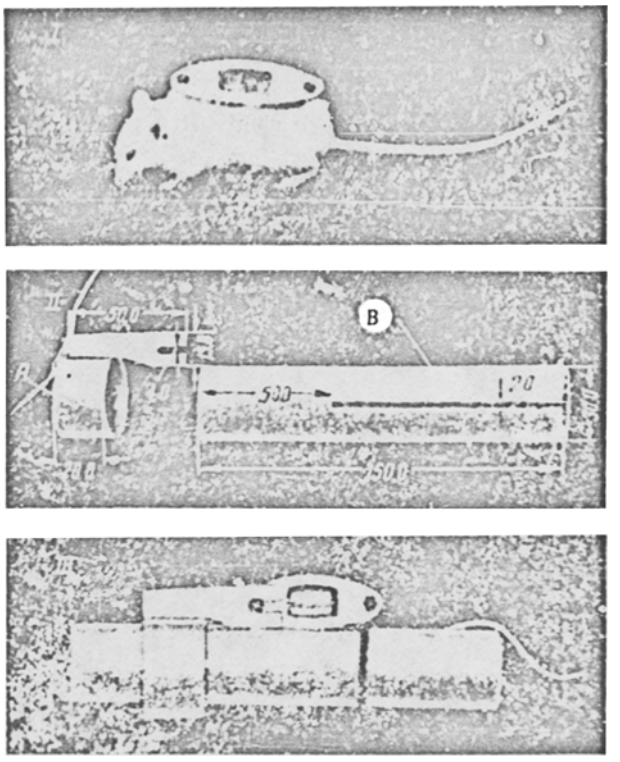

Fig. 2. I) Mouse with introduced camera, II) dis-assembled fixator. A) ring with fork; B) tube. III) mouse with camera inserted into fixator.

such fashion that there remains along the course of the backbone a narrow strip of hairs to which is glued (with BF-2 glue) the upper edge of one of the foundation bars (Fig. I, 3). After a few minutes, when the glue has dried, the assistant grasps with a Pean clamp from the right side this foundation bar and places it, with the attached skin fold in a vertical position. With an eye scalpel, in the outer wall of the depilated skin, a square opening is made in which the scalpel is introduced and the fascia of the intact skin are then carefully removed. We recommend irrigation of the internal layer of the depilated skin with a penicillin solution. Then, through the aperture in the skin we introduce the zinc frame with the glued on cover glass doing this in such a manner that that side of the frame to which the glass is glued faces the intact wall of the depilated skin. The zinc frame is fixed to the foundation bar with a stitch passed throught the upper side of the frame (1) and also the center of the upper edge of the foundation bar (2). With the aid of a second stitch, the skin forming the lower edge of the cut-out window is drawn tightly to the foundation bar (the needle with the thread is passed through the lower edge of the aperture under the introduced camera, and is then drawn above the lower border of the foundation bar (4) through the intact skin, after which the needle is introduced from the outside into the skin at the same border of the foundation bar and is drawn out by the side where it was first introduced; the free ends of the thread are tied into a knot and so the skin on the side of the aperture is found drawn to the skin on the opposite side). Following this, the skin at the edges of the aperture is trimmed to correspond to the camera dimensions; to the foundation bar, with the aid of two bolts having openings at the bar edges (5), the second additional foundation bar is mounted, and thus the skin fold with the camera becomes fixed in a vertical position. As the fascia on the side of the intact skin have been removed, the camera comes into direct contact with the blood vessels.

It can be seen that in our proposed method we are offering not a camera occupying a certain volume of space, but a transparent glass sheet, fixed into an aperture and replacing the removed skin with the aid of two bars vertically supporting the depilated skin. For this reason, the term "camera" should be accepted in our case as having a special connotation.

Microscopic observations are made without the aid of narcosis; the mouse with the attached camera (Fig. 2, I)

is immobilized with the aid of a special fixator as represented in Fig. 2, II. This fixator consists of a tube with a prolonged slit in its wall (II, B) and rings with forks fastened on it (II, A); the ring with the attached fork is placed on the tube in such a manner that the lower edge of the fork fits into the slit of the tube. The mouse is placed within the lumen of the fixator and the camera is placed vertically and moved along the slit of the tube, while, in order to immobilize the fork totally as it lies on the fixator, we have placed it between the head of the bolt and the bar; at the end opposite the camera, where it protrudes from the slit of the fixator, the rubber ring lying over the fixator is snugly fitted (Fig. 2, III). Microscopic observations are most conveniently made when the object table has been removed from the instrument; the camera in that case lies directly on the condenser.

We prepared 80 mice in the manner just described, and continued skin observations for 30 days and more. The camera permits picture-taking at various magnifications. In the small and large vessels the blood circulation was visible; in the capillaries and the arteries the walls were seen clearly; it is more difficult to demonstrate venous vessel walls.

The method described can be used for the study of the blood supply and growth of skin grafts placed in the skin folds as, for example, malignant tumors. Skin transplants can be performed with the aid of a thick needle supplied with an obturator. A bit of the tissue to be transplanted is placed within the lumen of the needle and then the needle is introduced on the window side and the end is pushed between the skin and glass plate and then, about in the middle of the latter, the piece of tissue is pushed out with the obturator. Then, to the outer surface of the foundation bar, BF-2 glue is used to fasten a cover glass over the window on the bar so that, in this manner, the skin is contained between two transparent sheets. In such a case, we are dealing with a camera whose slit actually consists of the fold of mouse skin.

SUMMARY

A modified transparent camera for observation is described. The operation of placing the camera is technically simple and excludes any damage to blood circulation in the skin. Observations through the camera may last for 30 days and more. Microphotography with various magnifications might be accomplished through this camera. The blood stream and the vascular walls are clearly seen. The camera might also be used in studies of tissues transplanted into skin folds.

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