A CHAMBER FOR INTRAVITAL TISSUE MICROSCOPY IN EXPERIMENTS ON RATS

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The basic details of the design of a transparent chamber for intravital tissue microscopy in experiments on small laboratory animals are given.

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Various suggestions have been made [3-6] for special chambers intended for observations on the dynamics of the morphological changes taking place in the body tissues. Despite certain differences, as a rule the principle underlying the design of these chambers is the same: a narrow space bounded by parallel, flat, transparent plates, is formed, into which the animal's tissues grow, so that they can be examined under the microscope in transmitted light. Several models of such chambers have been made, both in the

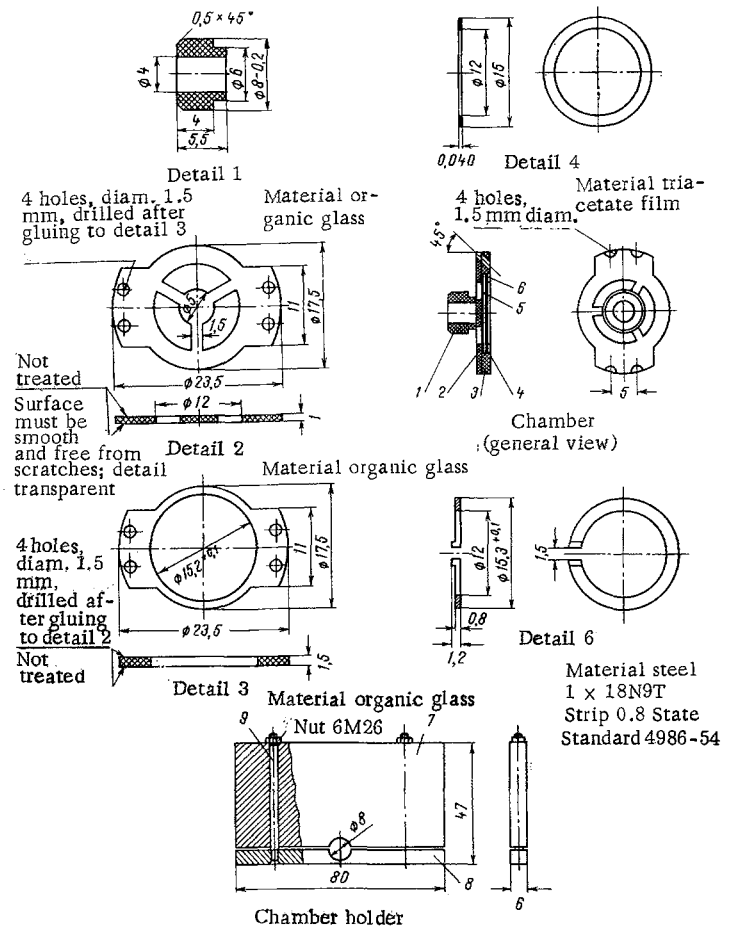

Fig. 1. Design of the chamber

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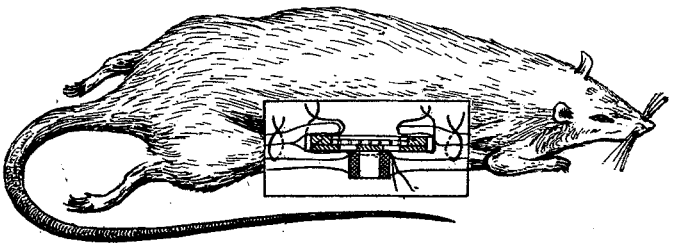

Fig. 2. Scheme showing chamber in situ in the rat's tissues.

Soviet Union [1] and elsewhere [2-6], adapted for experiments on rabbits and hens. However, these species of animals cannot be used for certain experimental oncologic investigations, and small laboratory animals sensitive to chemical carcinogens are preferred.

This paper describes a modification of a chamber with a variable inner compartment adapted for experiments on rats. It is a modification of Wood's design and of the chamber with a fixed compartment made previously at the Leningrad Institute of Oncology.

The chamber is made of organic glass.

Its base consists of a shaped circular plate (Fig. 1, detail 2), in the center of which is a transparent disk forming a stage, jointed by three cross-pieces to the inner edges of the plate. The collar (detail 1) is fixed beneath detail 2 by means of dichloroethane glue, and above it fixed detail 3, a shaped plate whose external outlines coincide with the external outlines of detail 2. The internal outline of this detail is circular. As a result of the fact that the internal outlines of details 2 and 3 do not coincide, a hollow open upward is formed, the base of which is the surface of detail 2, and its walls the inner edges of detail 3. To assemble the chamber before the operation of implantation, one or more rings or washers made of triacetate film (detail 4), a circular glass cover slip (detail 5, see Fig. 1, general view of chamber), a spacer, and a spring washer made of stainless steel (detail 6), fixing the cover slip and spacers in the hollow, are inserted in succession into the hollow. Because of these spacers placed beneath the cover slip, a space is formed between it and the stage of detail 2, the height of which can be varied by changing the number of spacers or their thickness. After implantation of the chamber (see below), the tissues fill the sector between the connecting bridges of detail 2 and invade the space in the compartment thus formed. To fix the chamber in the tissues two oblique holes are drilled on either side of the lugs on the combined details 2 and 3.

When the chamber is implanted in the tissues it is held on the stage of the microscope by a specially designed holder, consisting of a dismountable metal plate (details 7, 8) with a hole into which fits a projection from the chamber (detail 1). This projection is held tightly in the hole when the screws (detail 9) of details 7 and 8 are tightened.

The hair is carefully removed from the skin of the rat's flank. The operation is performed under strictly aseptic conditions.

The chamber is sterilized with formalin vapor and rinsed, and the spacers, cover slip, and spring fixing washer (sterilized by dry heat) are mounted in position.

Under ether anesthesia the skin is gathered up into a longitudinal fold, which is fixed by one suture close to the axilla and another close to the inguinal region. A circular hole is cut out of the dorsal layer of the skin fold, down to the inner surface of the ventral layer of skin. The dimensions of the hole correspond to the dimensions of the internal outlines of detail 3. Next, by means of scissors, a piece of subcutaneous cellular tissue and skin corresponding in size to the projection (detail 1) are removed from the inner surface of the ventral layer of skin at the center of the hole cut out of the dorsal layer. Antibiotics (penicillin, streptomycin, chloramphenicol) are introduced into the wound on the point of an ophthalmic scalpel.

The chamber is lowered into the bed thus prepared. It lies between the two layers of the skin fold, and the projection is brought out through the hole in the ventral layer. So that the tissues fit more snugly against the neck of the projection (detail 1), before the chamber is buried a catgut ligature is applied at a distance of 1-2 mm from the edge of the hole, its ends are brought out beneath the fold, and after the chamber has been implanted, it is drawn tight. The chamber is fixed in its upper part by four sutures (Fig. 2).

To protect the chamber from soiling and mechanical injury, an adhesive gauze dressing is applied to the skin flap. To stop the rats from biting the chamber, their incisors are removed.

When the chamber is examined under the microscope 7-8 days later, the tissues can be seen to have begun invading the space inside the chamber.

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