MODIFICATION OF DESIGN AND INSERTION OF A TRANSPARENT CHAMBER FOR STUDYING THE MICROCIRCULATION IN THE SUBCUTANEOUS AREOLAR TISSUE OF THE RABBIT EAR

M. I. Reutov and Academician A. M. Chernukh*

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A modification of a transparent chamber implantable into the tissues of the rabbit's ear and intended for studying the microcirculation in the subcutaneous areolar tissue and the operation of its insertion are described. The design of the chamber has advantages over existing models: The optical properties are improved, assembly of the chamber is simplified, and by virtue of the new design the regenerating tissue can be used for later histological study. Sterilization of the chambers with γ rays is simple and reliable. Besides fixation of the chamber in the tissues of the rabbit's concha auriculae by means of Lavsan gauze, additional gluing of the gauze is suggested, to prevent penetration and development of secondary infection in the operation wound and to fix the chamber more securely in the tissues of the ear.

KEY WORDS: *microcirculation; implantable chamber; regeneration; biomicroscopy.*

Investigation of the regenerating network of microvessels in a transparent chamber under chronic experimental conditions is nowadays a promising method of studying the physiology and pathology of the microcirculation. The essence of the method is that observations can be

*Academy of Medical Sciences of the USSR.

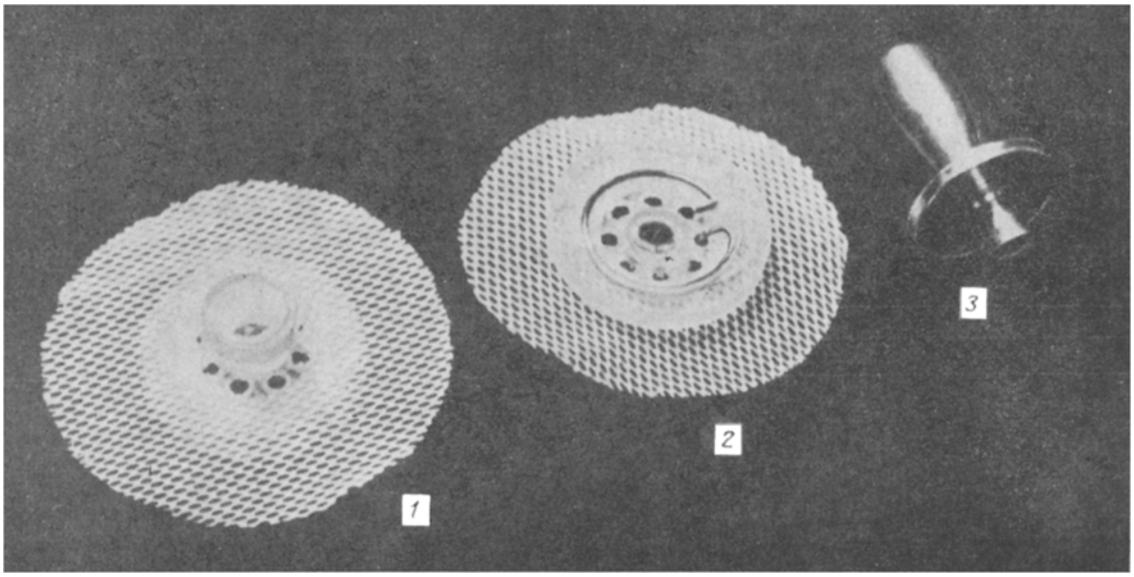

Fig. 1. Implantable chamber (1, 2) and die (3).

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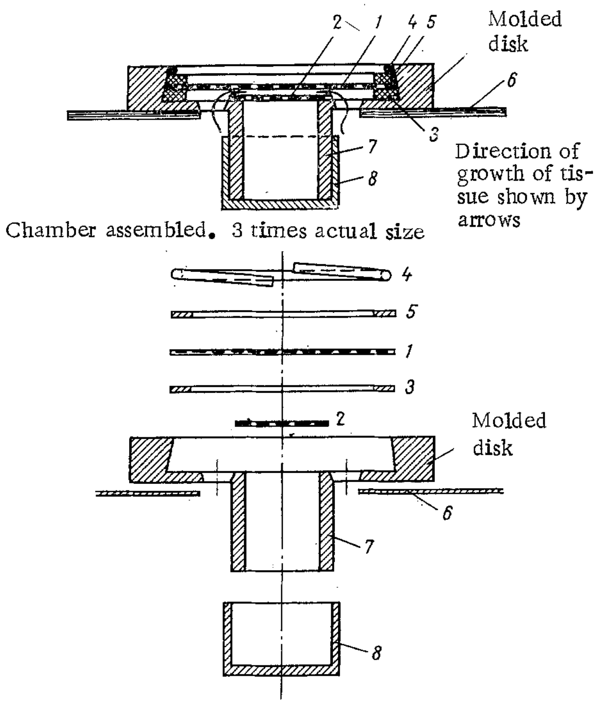

Details of chamber, 3 times actual size

Fig. 2. Diagram showing construction of implantable chamber. Explanation in text.

carried out in transmitted light, and still and motion pictures can be taken of the microvessels in the subcutaneous areolar tissue of the rabbit ear, growing into the narrow closed space formed by two transparent disks. The advantages of this method over biomicroscopy in acute experiments are that the various physiological and pathophysiological microcirculatory responses and morphological changes of the microvessels are accessible for microscopic observation over a long period of time (3-4 months) under the most favorable optical and physiological conditions. These advantages are secured by inserting a special miniature transparent chamber into the tissues of the rabbit's ear. The mica chamber first suggested by Clark et al. [1] was later improved and its design was modified by several investigators. However, even models of these chambers described in recent years [2, 3], as the present writers have found, have required further improvement. This applies in particular to the design, the optical properties of the materials used, and the method of assembly of the chambers. In order to improve the chamber and the method of its insertion, the investigation described below was carried out.

DESIGN AND ASSEMBLY OF THE CHAMBER

The main element in the design of the chamber developed by the writers (Fig. 1: 1, 2) as in all models described previously, is a narrow closed space between two parallel transparent disks (Fig. 2: 1, 2). After insertion of the chamber a network of microvessels of the subcutaneous areolar tissue grows into this space. The regenerating network of microvessels can be observed for 21-23 days after insertion of the chamber. Unlike models described previously, the carrying part of the chamber consists of a molded disk, cast in a special press mold from polycarbonate. The making of a single part instead of three separate components by casting has greatly simplified the technique of assembly of the chamber as a whole, and the material from which the disk is made, namely polycarbonate (macrolon), has physicochemical properties which satisfy all the requirements of an allograft. The molded disk is circular, and on it rests a ring in the central part of which there is a tube (Fig. 2: 7), forming the optical field of the chamber. Around the tube in the disk eight holes are arranged concentrically, through which the microvascular network grows into the space.

The order of assembly of the chamber is also shown in Fig. 2. The closed space (20 μ) is formed by two glass disks of different diameters and 150 μ thick (Fig. 2: 1, 2) and a polyethylene circular washer placed between them (Fig. 2: 3). The disk of smaller diameter is secured in the center of the molded disk and the disk of larger diameter, which is removable, is fixed by a spring clip (Fig. 2: 4) beneath which is placed a polyethylene packing ring (Fig. 2: 5). A piece of Lavsan gauze is glued to the molded disk on the side of the

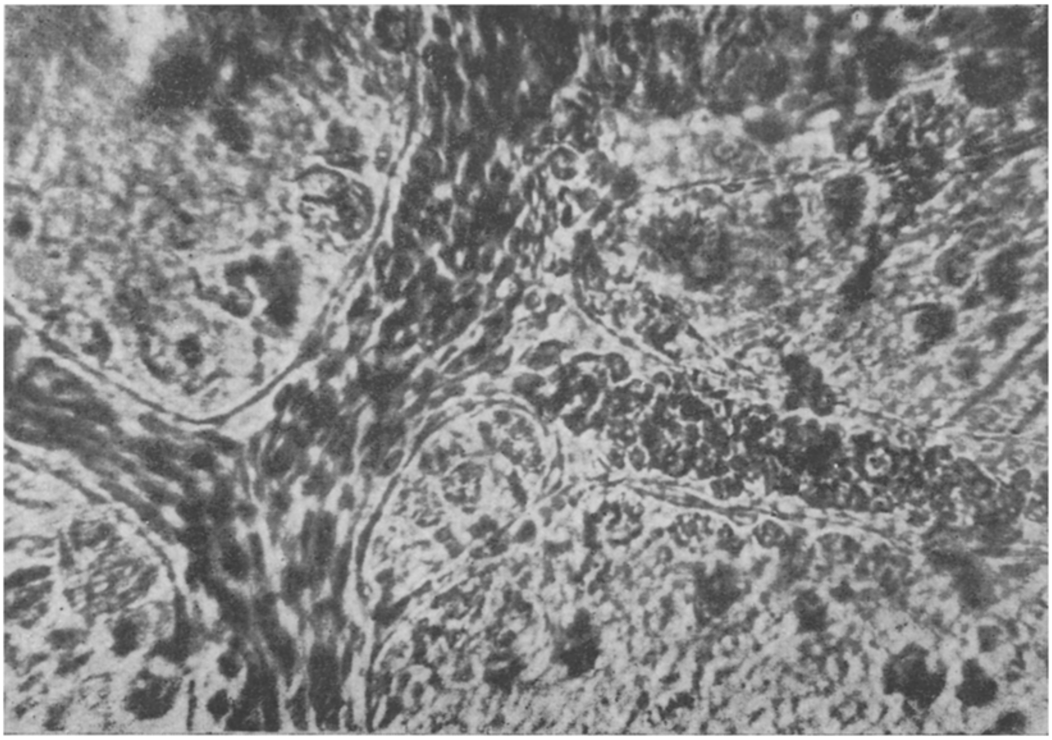

Fig. 3. Microvessels of subcutaneous areolar tissue of rabbit's ear on 43rd day after implantation of chamber. Intravital microscopy, 240×.

tube (Fig. 2: 6). It is placed between the skin and cartilage of the concha auriculae and holds the chamber securely in the rabbit's ear as a result of the subsequent ingrowth of connective tissue. As the illustrations show, the tube of the molded disk is equipped with a removable hood (Fig. 2: 8), which serves for additional fixation of the chamber in the tissues of the ear and also prevents soiling of the optical part of the chamber. The hood is removed during biomicroscopy.

The assembled chambers have been sterilized by radiation (γ rays) and gas (ethylene oxide, "OB" mixture) methods.

RESULTS OF TRIALS OF THE CHAMBER IN CHRONIC EXPERIMENTS

Altogether 40 chambers were implanted for different periods (up to 4 months) in the conchae auriculae of 20 chinchilla rabbits weighing 2.0-2.5 kg. The implantation operation was performed as described by Wood et al. [3] with certain modifications on animals anesthetized with urethane (1 g/kg, 25% sterile solution, intraperitoneally). After removal of the hair from the whole surface of the concha auriculae the latter was treated with Roccal disinfectant. To implant the chamber the least vascularized area of the concha auriculae measuring 0.5 × 0.5 cm was chosen at the boundary between the upper and middle thirds, laterally to the middle auricular artery. Using a special drill (a thin-walled steel tube) a hole was bored in the chosen area of the concha auriculae (beneath the tube of the chamber) around which the skin was dissected on its anterior surface within a radius sufficient to apply the Lavsan gauze. Considering the contractility of the dissected skin, the piece excised beneath the molded disk was one-fifth less in diameter than the disk itself. In that way the subsequent formation of an undesirable skin defect around the molded disk was avoided. To trace the precise boundary of the excised portion a special instrument (a die; Fig. 1: 3) was used, and the piece of skin was cut out around the outline of the impression made by the die. This completed the preparation of the concha auriculae for implantation of the chamber. The chamber without the hood was implanted with the tube in the hole made in the concha auriculae from the side of the excised skin, after which the hood was firmly replaced on the tube and the gauze was fitted under the dissected skin and straightened. In that way the molded disk lay on the cartilage of the concha auriculae and the gauze was placed between the cartilage and the dissected skin. The free edge of the skin was glued to the molded disk and gauze with "Cyacrin" glue. This increased the reliability of fixation of the chamber in the tissues of the ear and prevented infection from penetrating between the free edge of the skin and the molded disk. The operation of implantation was completed by painting 10% chloramphenicol emulsion on the skin around the chamber.

Biomicroscopic investigations showed that a regenerating network of microvessels was observed in all the implanted chambers 21-23 days after the day of the operation, and it completely filled the space in the chamber at the end of the fourth week. No case of ejection of the chamber or necrosis of the tissues around the chamber was observed. The optical properties of the chamber proved to be highly satisfactory and they remained essentially unaltered throughout the period of implantation (Fig. 3). The designed features of the chamber also enabled the regenerating tissue to be used for subsequent histological study.

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RHEOGRAPHY OF THE STOMACH

B. I. Mazhbich and É. I. Beloborodova

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A practical method of rheography is suggested for studying the state of the human gastric circulation. The theoretical aspects of the use of a bipolar system of electrodes (external and internal) with essentially different sizes of contact surface are examined with respect to the stomach. A scheme and brief description of the simple apparatus are given. Control experiments were carried out on animals to confirm the validity of the basic assumptions. Mean values of some rheographic indices frequently used in clinical practice, based on rheograms of the stomach of 30 healthy subjects, are given.

KEY WORDS: *rheography; stomach; animals; man; sensitive probe.*

Rheography [17], electroplethysmography [4], and impedance plethysmography [19] are widely used at the present time in clinical investigations, mainly to assess the state of the regional circulation: in the limbs [3, 18, 20], the skull [1, 5, 16], the thorax [11], and the region of the liver [6] and other organs [10]. However, the method of rheography, with electrodes placed on the surface of the human body, frequently cannot be used to assess the state of the circulation in the internal organs, with the consequent need for introducing electrodes inside the lungs [7, 8], kidney [15], and rectum [2].

Todorov [12], who placed one electrode freely inside the lumen of the stomach and the other on the skin of the epigastrium, concluded that the motor activity of the stomach can be assessed in this way but not the state of its circulation. Yet assessment of the state of the circulation in the stomach is a very important matter in clinical practice.

The object of this investigation was to attempt to use the method of rheography to investigate the state of the gastric circulation. To record a rheogram of the stomach wall reproducible under identical conditions reliable electrical contact must be ensured with the

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Laboratory of Physiology of the Circulation and Department of Physiology of Visceral Systems, Institute of Physiology, Academy of Medical Sciences of the USSR, Siberian Branch, Novosibirsk. Second Department of Internal Medicine, Tomsk Medical Institute. (Presented by Academician of the Academy of Medical Sciences of the USSR D. D. Yablokov.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 84, No. 7, pp. 118-121, July, 1977. Original article submitted December 20, 1976.