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AN INTRAVITAL METHOD FOR THE EXPERIMENTAL STUDY OF THE PULMONARY CAPILLARIES

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In recent decades considerable attention has been paid to the study of the microcirculation as a possible means of explaining the mechanism of disturbances of the activity of various organs and systems. Progress has been made with the study of such living organs as the liver, kidney, muscle, spleen, nerve tissue, mesentery, retrobuccal pouch, and so on [5]. However, some vitally important organs, such as the lungs, are difficult to investigate. A study of the microcirculation of the lungs could shed light on the pathogenesis and treatment of many pathological processes taking place in the respiration system.

The object of this investigation was to modify existing methods of intravital microscopy of the lung [1, 2, 4, 7] in order to carry out experiments on cats under open chest conditions and during artificial ventilation of the lungs. The aims of the investigation were as follows: to halt the cardiorespiratory movements of the lung but preserve physiological conditions at the site of observation; to choose optimal conditions for observing and recording the object, and to construct devices suitable for carrying out experiments on cats.

EXPERIMENTAL METHOD

A Luman 13 luminescence microscope was used for recording and observing, and the OLK-2 system (Fig. 1) was used for investigations by contact microscopy. RF-3 film was used for microphotography. An important and laborious stage was the construction of a number of devices and adaptations for performing experiments on cats, for the microscope itself is not designed for work with large and medium-sized laboratory animals.

A universal table serving simultaneously for surgical preparation of the animal and for manipulations with it under the microscope, was produced by the Institute's workshops (awarded Efficiency Suggestion No. 1, 1981, State Register of Inventions, Institute of General Pathology and Pathological Physiology, Academy of Medical Sciences of the USSR). A halter with connections for the air pipes and a wound retractor, fixed with bolts, were mounted on the table. Complete and reliable fixation of the animal was secured in this way.

Another design innovation was the creation of a strong removable mechanism, made in the experimental workshops of the Institute (awarded Efficiency Suggestion No. 2, 1981, State Register of Inventions, Institute of General and Pathological Physiology, Academy of Medical Sciences of the USSR). This device is an independent lifting mechanism, fixed to the table by means of a strong pedestal (Fig. 2). By turning a wide wheel the rod of the lifting device is moved, and it can be additionally fixed by means of a set screw. Support for the table is provided by a wide metal platform. It is fixed to the rod by means of a collar with flange and lock. The universal stage with the dissected animal is placed on the platform of the lifting device and moved horizontally. In this way the table with the animal can be moved in the necessary directions.

The problem of halting movements of the lung was solved by the use of an original suction chamber, suggested by the All-Union Research Institute of Pulmonology, Ministry of Health of the USSR (G. M. Kudryashev et al.) and modified by P. S. Aref'ev (awarded Effi-

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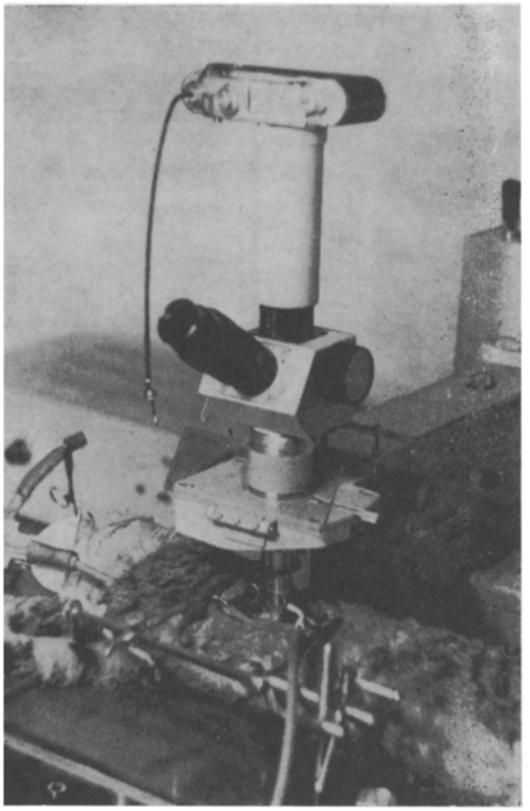

Fig. 1

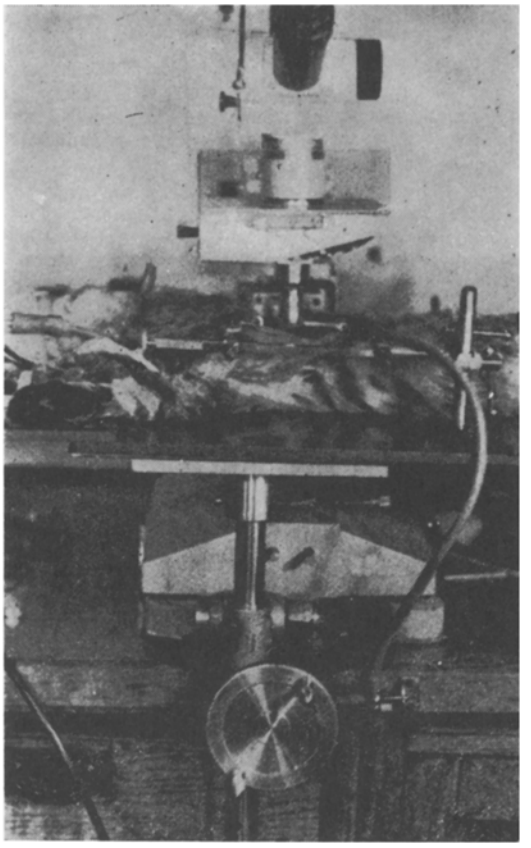

Fig. 2

Fig. 1. The OLK-2 system for contact microscopy, assembled on a Luman 13 microscope.

Fig. 2. Lifting mechanism with universal stage and animal dissected for the investigation.

ciency Suggestion No. 3, 1981, Institute of General Pathology and Pathological Physiology, Academy of Medical Sciences of the USSR). In the course of the design research chambers made to the patterns of Wagner and Krasnikov and also many chambers modified by the writers themselves, were tested. Disadvantages of the former were that because of the large channel and also the frequency distribution of suction holes around the region under observation, a ring of deformed lung tissue was formed and the circulation disturbed. The first chamber, modified by ourselves, enabled the objective to be manipulated within the area of the central hole, so that a given region of lung tissue could be selectively observed within the area of this hole. However, this region of the lung was found to be liable to move, so that it was difficult to observe and photograph the object.

The chambers used in this investigation are free from the above defects. Since there are only four holes, each about 1.5 mm in diameter, the region of the lung can be fixed sufficiently firmly and supplied with blood through vessels passing between the holes. A combination of definite sizes of the central hole and of the area of the fixed region of lung tissue means that the latter is no longer liable to move. The chamber is fixed to the objective by a set screw. In this way the necessary advancement and withdrawal of the frontal lens of the objective can be secured. The chamber is connected by a hose to an electric suction pump, which creates the necessary vacuum for fixing the region of the lung.

Pentobarbital (50-100 mg/kg) was injected intraperitoneally into cats, tracheotomy was performed, followed by thoracotomy in the 6th left intercostal space, after which the animal was artificially ventilated (400-600 ml/min). The table with the animal was then placed on the lifting platform under the microscope. The electric suction pump was switched on and, when the vacuum was formed, the table was raised by the lifting device until the lung made contact with the end surface of the chamber. The cock connecting the pump with the chamber was opened and the lung drawn in by suction.

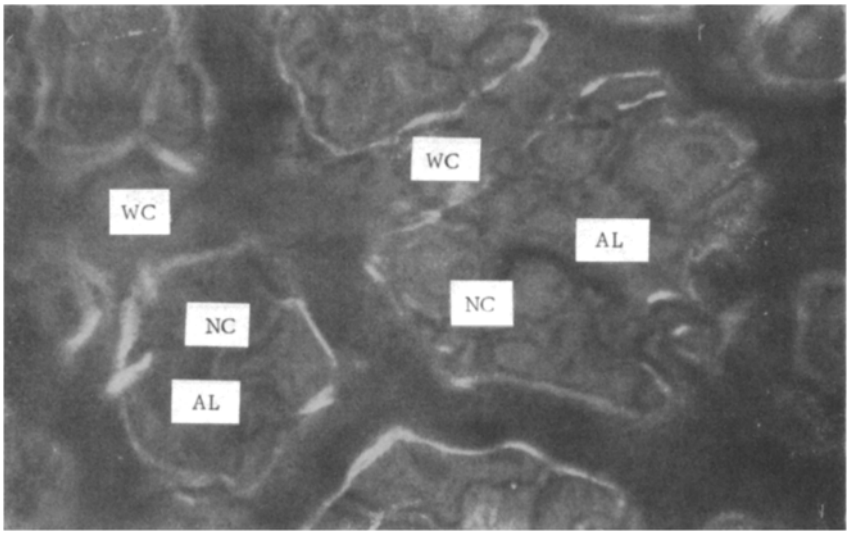

Fig. 3. Microcirculation of cat lung. WC) Wide capillaries, NC) narrow capillaries, AL) alveolus. 100 ×.

EXPERIMENTAL RESULTS

The structure of the microcirculation of the lung tissue is shown in Fig. 3. The surface of each alveolus is crossed by narrow capillaries, which unite with each other and form a single lace-like network. In the narrow capillaries blood cells flow very rapidly one after the other. If the slightest obstruction is present (the holdup of a large leukocyte) the erythrocytes instantly change their course and run into the nearest branches of the capillary on their path. Wide capillaries run between the alveoli in the interstices of the lung [6]. They form a frame around each alveolus and unite with each other into a single reticular framework. The net of narrow capillaries is closely connected with the framework of wide capillaries. Blood cells pass easily and quickly from one capillary network into another. Blood cells in wide capillaries follow one another in a general flow. The blood flow in the capillaries takes place rapidly, in continually changing directions, suggesting the high functional mobility of the lung capillaries [3]. It is these distinguishing features of the capillary circulation which account for the high oxygenation of the blood and gas exchange in the lungs.

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