AN APPARATUS FOR MEASURING THE SURFACE TENSION OF LUNG EXTRACTS FOR SURFACTANT STUDY

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UDC 612.212.014.1.014.462.8-08

The design of an instrument and the technique for measuring the surface tension of lung extracts are described.

The standard method for studying the surfactant of the lungs is by measuring the surface tension (ST) of saline extracts or of endobronchial washings of the lungs in relation to cyclic changes in the surface area of the film formed as a result of adsorption of the surfactant. Wilhelmy's scales [15], of which many modifications exist [1, 2, 4, 6, 7, 9, 10, 12], is most commonly used for this purpose.

Considering the increasing interest in the surfactant problem it was thought worthwhile to give a brief account of a new variant of Wilhelmy's scales.

PRINCIPLE OF THE METHOD

The measurement of ST on Wilhelmy's scales is based on a "tear-off" method – using torsion scales the force required to detach from the surface of the fluid a glass (platinum, quartz) disc partly submerged in it is measured. The test fluid is contained in a flat cuvette fixed with a movable barrier of nonwettable material. By moving the barrier a monomolecular layer of surfactant can be compressed and stretched thereby changing the concentration of its molecules per unit area and, consequently, the surface tension. Other conditions being equal (the rate of movement of the barrier, temperature, etc.), the character of the relationship between ST of the extract and the area of its surface layer gives an idea of the qualitative composition and reserves of the surfactant in the lung studied [4, 7, 8].

DESCRIPTION OF THE INSTRUMENT

The main parts of the instrument (Fig. 1) are: torsion scales (A), the cuvette (B) with the moving barrier, and an apparatus (C) for moving the barrier automatically.

To measure ST, a coverslip (2) measuring $22 \times 22 \times 0.1$ mm is suspended from the lever (1) of the torsion scales (type VT-500). Before each measurement the coverslip is cleaned in hot chromate mixture and washed with distilled water and physiological saline. The Teflon cuvette has internal dimensions of $100 \times 40 \times 15$ mm. If organic glass is used instead of teflon the cuvette must be coated inside with a layer of mineral oil. The walls of the cuvette are lined internally with Teflon tape (3), connected to the moving barrier (4). The bar (5) displacing the barrier makes to-and-fro movements driven by the roller (6) mounted on the shaft of a synchronized reversing electric motor (7) with reducing gear (speed 1 rpm). The direction of rotation is changed automatically by switching over the winding of the stator at times corresponding to the extreme positions of the barrier, by means of a make-and-break contact (8) and two spurs

Departments of Pathological Anatomy and of General and Organic Chemistry, Crimean Medical Institute, Simferopol'. (Presented by Academician of the Academy of Medical Sciences of the USSR A. P. Avtsyn.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 77, No. 2, pp. 120-122, February, 1974. Original article submitted March 26, 1973.

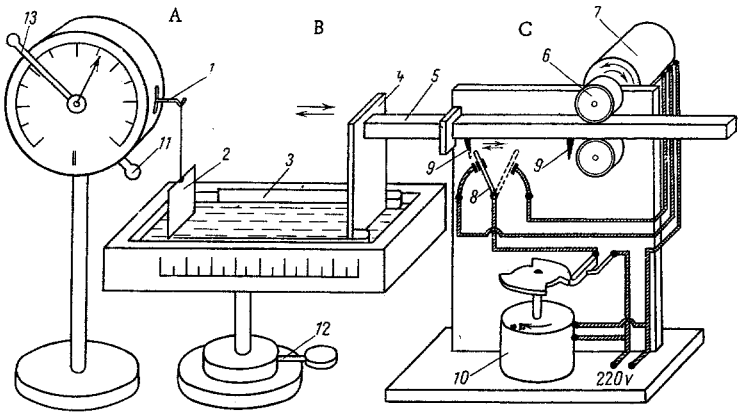

Fig. 1. Scheme of Wilhelmy's scales: A) VT-500 torsion scales; B) cuvette; C) apparatus for moving the barrier; 1) lever of torsion scales; 2) glass; 3) Teflon tape; 4) moving barrier; 5) bar; 6) driving roller; 7) electric motor; 8) make-and-break contact; 9) spurs on bar for switching contact; 10) time relay; 11) screw adjustment of scales; 12) mechanism for adjusting level of cuvette; 13) guide arm of scales.

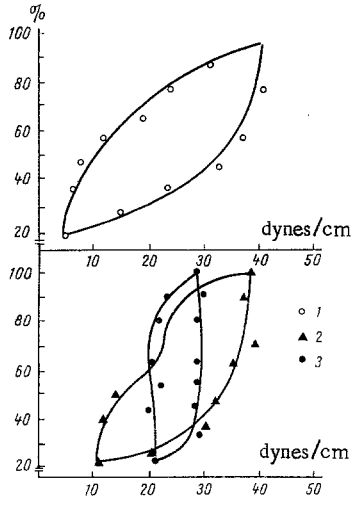

Fig. 2. Curves of surface tension (abscissa) versus surface area of film (ordinate) of lung extracts from a healthy rabbit (1), from a newborn infant dying from a non-pulmonary disease (2), and from a newborn infant dying from respiratory failure – hyaline membrane disease (3). Explanation in text.

(9) on the bar. The speed of movement of the barrier depends on the diameter of the roller (6) and is approximately 1 mm/sec. Every 10 mm (corresponding to a change in area of 10%) the mechanical time relay (10) switches off the electric motor (7) for 6 sec, sufficient to allow measurement of the ST. The whole "compression-stretching" cycle of the film, i.e., a change in its area from 100 to 20% and vice versa, takes 4 min.

ORDER OF THE MEASUREMENTS

About 50 ml of the extract is poured into the cuvette and allowed to stand for 30 min for the film to form. The measuring pointer of the scales is set at zero and the coverslip balanced by turning the screw adjustment (11). Next, using the level-adjusting mechanism (12) the cuvette is raised until the lower edge of the coverslip comes into contact with the surface of the extract and a meniscus is formed. Considering the good wetting properties of the glass, the angle of contact was taken as 0. The lever of the holding device is released and the partly submerged coverslip returned to the equilibrium position by turning the guide (13). Since equilibrium is always reached at the same level of immersion of the slide, the effect of the force of repulsion can be disregarded. The value of ST can therefore be read in milligrams of the scale. To convert into dynes/cm the instrument is first calibrated for distilled water, for which ST is taken to be 72 dynes/cm.

To obtain stable results the measurements of ST are begun after a period of 1-2 h of cyclic changes in the film area and are continued until the ST values in two consecutive cycles coincide. The results of the measurements are plotted on a graph with ST in dynes/cm along the abscissa and the relative area, in %, along the ordinate. The measurements are carried out at room temperature (18-22°C).

A fragment of lung is first weighed, cut into slices 150-200 μ thick, treated with physiological saline (to give a concentration of 6-10%), stirred for 10 min, and filtered through six layers of gauze. Endobronchial washings of the lungs are prepared by filling and emptying the lungs with physiological saline up to 10 times through the trachea or the main bronchus.

This method was used to investigate extracts of the lungs of rabbits and also of newborn infants dying from various causes (birth trauma, asphyxia). Extracts of the lungs of healthy rabbits and newborn infants dying from nonpulmonary diseases gave a wide range of changes of ST, from 5-10 to 30-40 dynes/cm, characteristic of the surfactant [5, 7, 14]. The relationship between ST and the area of the film plotted on the graph appeared as a typical hysteresis loop because the curves corresponding to compression and stretching of the film did not coincide (Fig. 2). Extracts of the lungs from newborn infants dying from asphyxia due to hyaline membrane disease showed very low surface activity: the minimal ST was increased to 20 dynes/cm, indicating absence or inactivation of the surfactant. The results are in agreement with data in the literature concerning the role of surfactant deficiency in the pathogenesis of some forms of respiratory failure in the newborn [4, 8, 11].

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