Extrait du *Bulletin de Physio-Pathologie Respiratoire*
Volume 9 - 1973

VISCOELASTICITY STUDIES WITH CAPILLARY METHODS

ÉTUDES DE LA VISCO-ÉLASTICITÉ A L'AIDE DE MÉTHODES CAPILLAIRES

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Bull. Physio-path. resp., 1973, 9, 91-101.

ABSTRACT

Simultaneous measurements of viscosity and elastic recoil of sputum can be easily performed with a simple inexpensive capillary method in samples as small as 0.5 ml. However, the intersample variability of viscosity results is still large and multiple sample determinations are mandatory. As this results from the natural physical inhomogeneity of sputum, the same considerations apply to other methodologies presently used. The elastic recoil values on the other hand are quite reproducible and the results vary within a narrow range.

Rheology ; sputum ; viscosity ; elastic recoil ; capillary methods.

Regardless of the methods employed to determine the viscoelasticity of sputum, two major technical problems are still to be resolved. The first problem is that the quantitative results vary considerably regardless of the methodology employed. This variability results from the non-homogeneous character of sputum, the unpredictability of its source and its highly complex physico-chemical composition. The second problem is the lack of agreement among investigators of how to standardize the reading of « apparent » viscosity in this non-Newtonian material where the relation of shear stress and shear rate is constantly changing.

We wish to present here a new method which offers several advantages : 1. — The size of the sputum sample required is very small ; thus, one can always work with fresh material and select for measurement the desired area. 2. — The procedure conforms with rheological theory.

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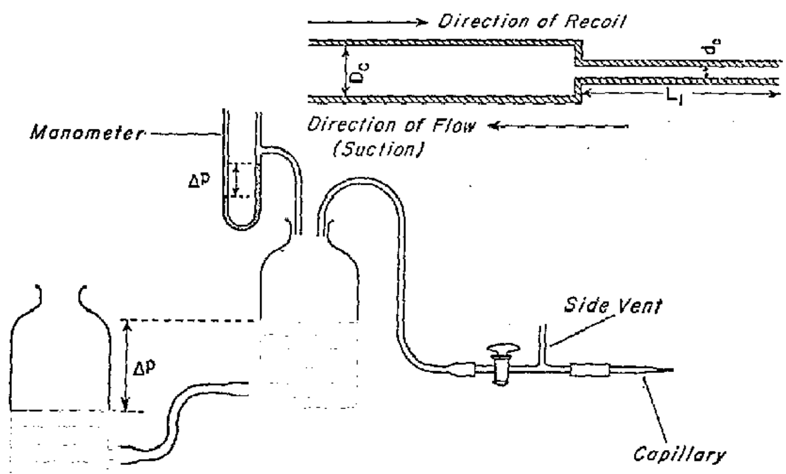

FIG. 1

The critical characteristic of the capillaries, is that the radius of d_c be 10 times smaller than L_1.

Caractéristique des capillaires : le rayon d_c doit être 10 fois plus petit que la longueur L_1.

3. — The viscosity can be determined at any shear rate, even as low as 200th of a reciprocal second. 4. — Viscosity and elastic recoil are measured simultaneously and the shear modulus can be calculated. 5. — The system is inexpensive and easy to operate.

The system as portrayed in Fig. 1 consists essentially of two capillaries in series connected by a valve to a vacuum system consisting of two bottles of water at different levels. This generates a constant suction pressure which is measured by the attached manometer. The two-way valve releases the negative pressure at the end of each measurement. The capillary itself consists of two capillaries of different radii and length. While their actual dimensions may vary, the critical requirements are that the length of the small capillary be about 10 times its radius to insure constant pressure in this area where the greatest degree of shear stress will take place. On the other hand, in the larger capillary, the radius is increased to reduce wall adherence that may interefere with flow. The capillaries are cemented to a glass slide for stability and placed on the stage of a microscope. The flow and recoil of the sputum are observed in the large capillary and measured with a calibrated eyepiece micrometer placed on the ocular of the microscope.

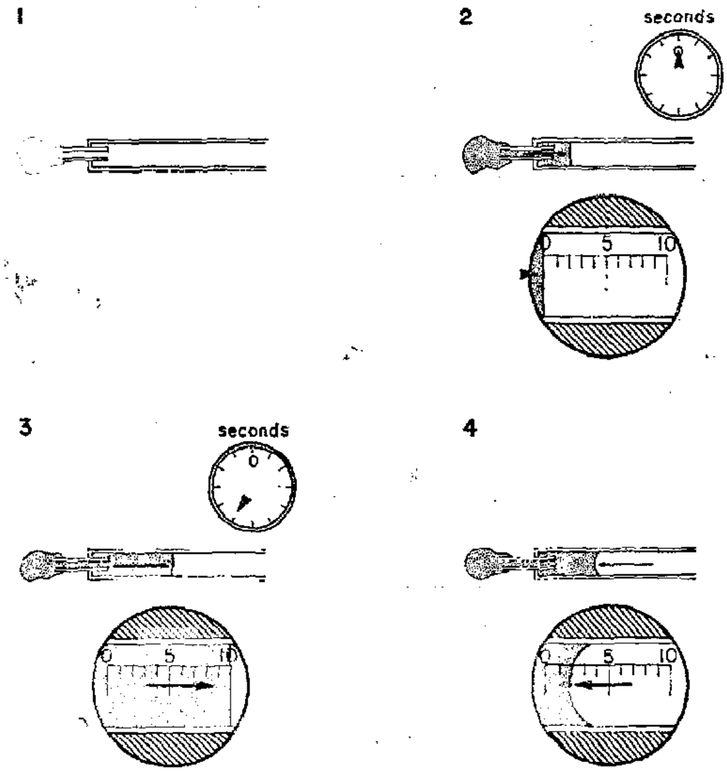

FIG. 2

(1) The sputum blob is in contact with the small capillary. Suction is applied at the other end (see Fig. 1). (2) Sputum enters the large capillary and its front edge is aligned with the beginning of the ocular micrometer. (3) Steady flow over a measured distance and time. (4) Pressure is released and the extent of recoil measured by the micrometer.

(1) La goutte de crachat est en contact avec le petit capillaire. L'aspiration est appliquée à l'autre extrémité (voir Fig. 1). (2) Le crachat pénètre dans le large capillaire et le bord du ménisque est aligné sur le repère 0 de l'oculaire micrométrique. (3) Ecoulement stable sur une distance et un temps donnés. (4) La pression est relâchée et la recouvrance est mesurée à l'aide du micromètre.

The blob of sputum is placed in contact with the small capillary and the negative pressure is applied to the free end of the large capillary (Fig. 2). The sputum is suctioned for a short distance into the large capillary and made to rest there while the zero point of the micrometer is aligned with its front edge. Then, the pressure is again started and the sputum allowed to flow a specified distance measured by the micro-

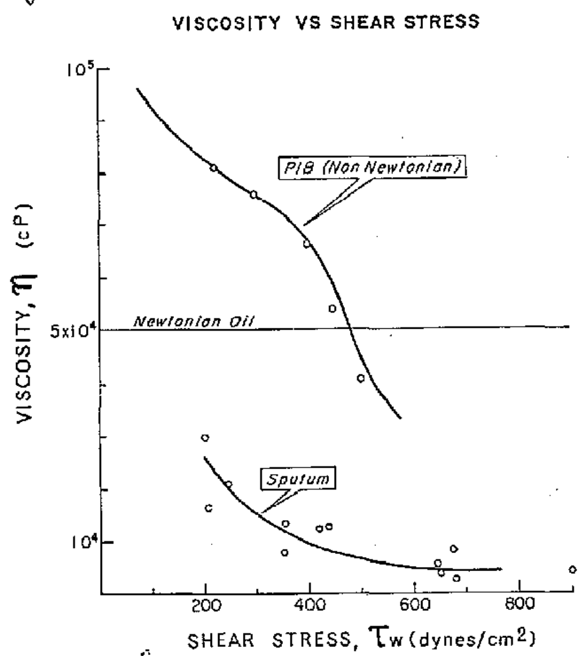

FIG. 3

Viscosity measurements in Newtonian and non-Newtonian materials vs. shear stress. The viscosity of oil is constant. With polyisobutylene (PIB) or sputum « *apparent* » *viscosity varies at different shear stress or shear rate.*

Mesures de la viscosité en fonction de la contrainte de cisaillement pour différentes substances : newtoniennes et non-newtoniennes. La viscosité d'une huile est constante. Pour le polyisobutylène (PIB) ou le crachat, la viscosité « apparente » varie en fonction de la contrainte ou du taux de cisaillement.

meter while the time is recorded. The flow must be steady. When the flow measurements are completed, the side valve is open to the atmosphere to release the pressure. At that moment, the accumulated energy of elongation makes the sputum recoil. Thus, knowing the volume displaced, the pressure, distance and time as well as the dimensions of the capillaries allows to determine the shear rate at the wall and the shear stress, from which the viscosity is calculated. By the same token, the extent of recoil measures the recoverable shear strain from which one can calculate the elastic modulus. These measurements of flow and recoil are repeated several times for each sample employing different pressures, to obtain a graph of the shear stress-shear rate relationships.

We can see in Fig. 3 examples of the relationship between viscosity and shear stress in Newtonian and non-Newtonian materials. Oil, which

REPRESENTATIVE FLOW CURVES

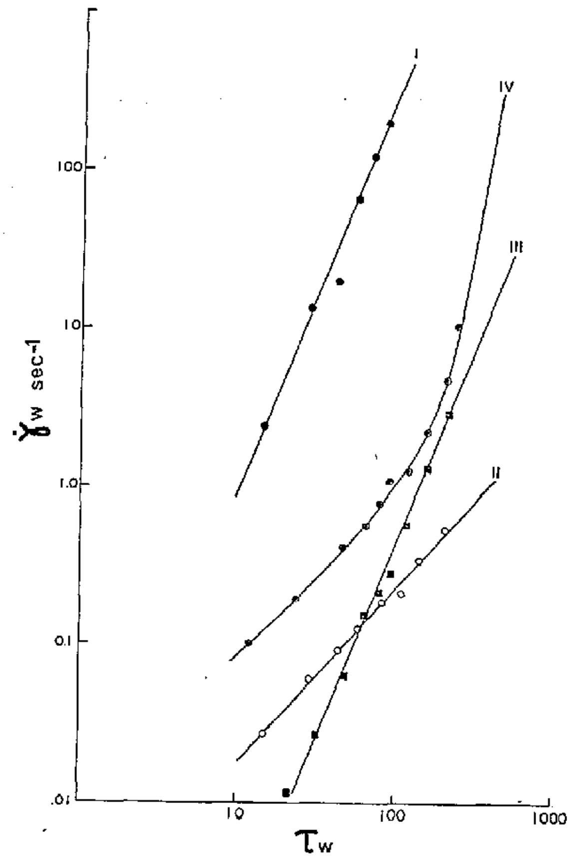

FIG. 4

Representative flow curves. Shear stress (τ_w) versus shear rate ($\dot{\gamma}_w$) of sputum from 4 patients, plotted on log graph. The curves to the right indicate higher viscosity. Sample I is of low viscosity and non-Newtonian. Sample II is highly viscous and Newtonian (constant slope, 45°). Sample III shows high viscosity at low shear stress rapidly decreasing in a non-Newtonian fashion as the stress increases (this behaviour is quite characteristic of purulent sputum). Sample IV shows Newtonian behaviour at low shear stresses but rapidly changes to non-Newtonian at higher shear stresses.

(From DULFANO *et al.*, *Amer. Rev. Resp. Dis.*, 1971, **104**, 88).

Courbes d'écoulement caractéristiques. La contrainte de cisaillement (τ_w) est tracée en fonction du taux de cisaillement ($\dot{\gamma}_w$) (échelle logarithmique). Les courbes de droite témoignent d'une viscosité plus marquée. L'échantillon I est faiblement visqueux et non-newtonien. L'échantillon II est hautement visqueux et newtonien (pente constante : 45°). La viscosité élevée, à faible contrainte de cisaillement, de l'échantillon III, décroît rapidement en fonction de la contrainte, à la manière d'un liquide non-newtonien. (Ce comportement est caractéristique du crachat purulent). L'échantillon IV présente un comportement newtonien à faibles contraintes qui devient rapidement non-newtonien à contraintes plus élevées.

is Newtonian, has a constant viscosity at any shear stress while in non-Newtonian materials such as polyisobutylene (PIB) or sputum, the viscosity varies according to the applied stress. For instance, the « apparent » viscosity of this sample of sputum would be 30,000 centipoises when subjected to a stress of 200 dynes/cm^2 ; however, if the reading is taken at 700 dynes the viscosity would be only 5,000 centipoises. Conversely, the viscosity will be higher as the shear rate decreases. Based on theoretical calculations, we have concluded that in the normal bronchial tree, the shear rate must be quite low, and probably below one reciprocal second. Therefore, for standardization purposes, we have suggested that all measurements of apparent viscosity be expressed at a shear rate of one reciprocal second.

For convenience, the flow curves are presented on log-log graphs where the viscosity can be easily calculated at any selected point of shear stress or shear rate (Fig. 4). We always express it at a shear rate of one reciprocal second. The four examples portrayed here are representative of most samples. In each, the overall slope is obtained by multiple determinations of shear stress and shear rate at different applied pressures. A displacement of the slopes to the right indicates higher viscosity. Sample 1 exemplifies low viscosity and non-Newtonian character throughout. Sample 2 is highly viscous but behaves in a

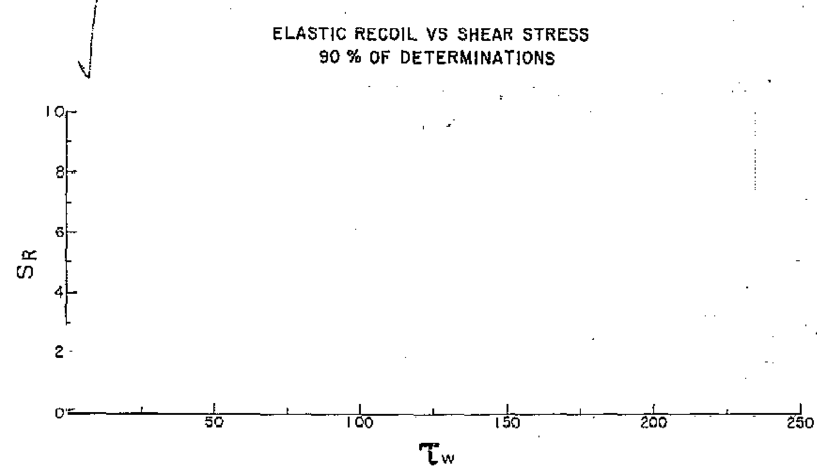

FIG. 5

Elastic recoil vs. shear stress. For 90 % of determinations, the limits of elastic recoil of sputum cover a relatively small band of less than 6 S_R units.

(From DULFANO *et al.*, *Amer. Rev. Resp. Dis.*, 1971, **104**, 88).

Recouvrance élastique en fonction de la contrainte de cisaillement. Pour 90 % des mesures, les limites de la recouvrance élastique S_R du crachat couvrent une bande relativement étroite, inférieure à 6 unités.

Newtonian manner as judged by the constant slope at 45°. Sample 3 shows high viscosity at low shear stress ; however, this viscosity decreases rapidly in a non-Newtonian fashion as the shear stress increases. This behaviour is quite characteristic of purulent sputum. Sample 4 shows Newtonian behaviour at lower pressures ; however, this changes rapidly to non-Newtonian as the pressure increases.

In Fig. 5 we present the elasticity results expressed by the relationship between shear stress and the recoverable shear strain which is the volume that recoils when the pressure is released. You may notice that the variation in elastic recoil among the samples are not very large and cover a rather defined area. At low shear stress, one can see that the elastic recoil changes considerably in relation to the stress but when the stress approximates the level of about 100 dynes, the curves usually tend to level off. Therefore, we standardize our reading of elastic recoil at the level of 100 dynes pressure.

A detailed analysis of sputum elasticity in 50 patients with chronic bronchitis showed an interesting phenomena (Fig. 6). The elastic recoil

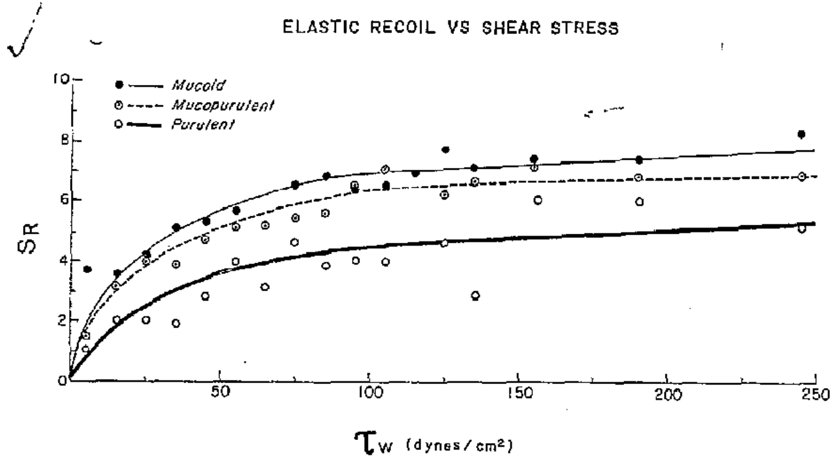

FIG. 6

Average elastic recoil values of sputum plotted for each stress area (all samples) to allow clear correlation with the gross quality of sputum. The mucoid samples show greater elasticity than the purulent ones ; values for the mucopurulent samples are intermediate.

(From DULFANO *et al.*, *Amer. Rev. Resp. Dis.*, 1971, **104**, 88).

Le tracé des valeurs moyennes de la recouvrance élastique du crachat en fonction de chaque niveau de contrainte (ensemble des échantillons) montre clairement l'existence d'une corrélation avec la qualité du crachat. Les échantillons mucoïdes sont caractérisés par une élasticité plus marquée que les échantillons purulents ; des valeurs intermédiaires caractérisent les échantillons mucopurulents.

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in mucoid samples is invariably higher than that of purulent samples, while the mucopurulent tend to fall in between. This measurement of elastic recoil also allows to determine the value of its opposite, namely the elastic modulus. If one keeps in mind that the elastic modulus is proportional to the concentration of a material divided by the molecular weight, the significance of this determination is obvious since from a simple physical determination of elasticity, one can surmise the essential chemical make-up of a given material. In the case of sputum, where the concentration of solids is very low, if one would assume a concentration of only about 1 %, the values obtained for the elastic modulus indicate that the elastic behaviour depends on a substratum of very large molecular

VARIABILITY IN MEASUREMENTS OF SPUTUM VISCOELASTICITY

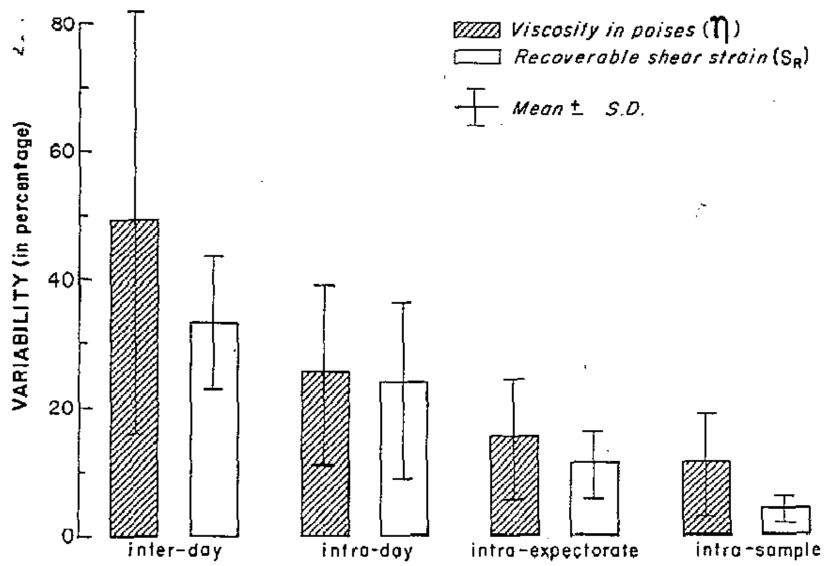

FIG. 7

The « normal » variability in viscoelasticity measurements. The viscosity variations are expressed as percentage difference from the average viscosity, in poises, at shear rate of 1 sec^{-1} ; and the elastic recoil at 100 dynes/cm^2. The inside bars indicate mean values and one standard deviation.

Variabilité « normale » des mesures de la visco-élasticité, les variations de la viscosité sont exprimées sous la forme d'une différence en pourcentage, calculée à partir de la viscosité moyenne exprimée en poises et mesurée à un taux de cisaillement de 1 sec^{-1} ; la mesure de la recouvrance élastique est effectuée à 100 dynes/cm^2. Les barres indiquent les valeurs moyennes ± un écart type.

weight in the order of 5 to 12 millions. This kind of knowledge will be of great help in the future to indicate the kind of chemical structures responsible for the rheological properties of sputum and pave the way for a more intelligent therapeutic approach.

Finally, we have to be careful of the quantitative variability of these rheological measurements. If viscosity and elasticity are measured repeatedly in a given patient, the differences among samples may be quite high (Fig. 7). Different areas within a one ml sample may yield values for viscosity that vary by as much as 18 %. The variability increases if two samples of the same expectorate are evaluated separately and becomes larger as samples are collected throughout one or several days. For instance, the percentage difference in viscosity values over a three-day period taking three samples per day may amount to 80 %. Measurements of elastic recoil are much less variable. These results are significant as a warning that relatively small changes may be entirely fortuitous. Our present thinking indicates that these variations do not depend on instrumentation or instrinsic changes in the physico-chemical composition of the glycoproteins but rather reflect the presence of additives, like cells and debris which in varying quantities are mixed with the expectorate.

APPENDIX

Regular capillary equations for shear stress, shear rate and recoverable shear strain are modified for the double tube system as shown below. Shear stress (τ_w) measures the extent of the applied force, which at the wall of the capillary, is defined as :

$$\tau_w = \frac{r\ P_t}{2\ L_1} \qquad (1)$$

where P_t = pressure in cm H_2O (from manometer) ; r = radius of small capillary (0.0595 cm) ; L_1 = length of small capillary (0.5 cm).

Shear rate at the wall of the tube ($\dot{\gamma}_w$) is inversely proportional to the time necessary for the moving edge to cover the distance λ :

$$\dot{\gamma}_w = \frac{4\ R^2\ \lambda}{r^3 t} \qquad (2)$$

where λ = distance of flow (0.1 cm) ; R = radius of large capillary (0.0780 cm) ; t = time (in seconds).

Recoverable shear strain, S_R, is proportional to the distance of recoil of the edge upon release of the pressure. Since the edge changes to a meniscus upon recoil, and the distance measured (X_0) is to the utmost concavity of this meniscus, the S_R, which measures total volume of recoiling fluid, must be multiplied by a meniscus correction constant, K, which is inversely proportional to the applied stress (usually about 0.9 on stresses up to 150 dynes/cm² and never is less than 0.7). Thus,

$$S_R = \frac{4 X_0 K}{r} \cdot \left(\frac{R}{r} \right)^2$$

where X_0 = recoil distance in cm ; K = meniscus constant.

For any given sample, (1), (2) and (3) are measured several times at different suction pressures to obtain graphs of τ_w vs. $\dot{\gamma}_w$ and τ_w vs. S_R. Viscosity at any shear stress of rate is defined as :

$$\eta = \frac{\tau_w}{\dot{\gamma}_w} \quad (4)$$

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RÉSUMÉ

Des mesures simultanées de la viscosité et de la recouvrance élastique du crachat peuvent être facilement effectuées à l'aide d'une simple méthode capillaire, peu coûteuse, ne nécessitant que des échantillons de faible volume (0,5 ml). Cependant, la variabilité des mesures de la viscosité selon les échantillons rend indispensable la multiplicité des mesures. Cette remarque, probablement relative à l'hétérogénéité du crachat, s'applique également aux autres méthodes de mesure actuellement utilisées.

En ce qui concerne les valeurs de recouvrance élastique, la reproductibilité des résultats est satisfaisante, les valeurs ne varient que dans des limites étroites.

ZUSAMMENFASSUNG

Simultane Messungen der Viskosität und der elastischen Nachverkürzung des Sputums lassen sich mit Hilfe einer einfachen Kapillarmethode in Materialproben von 0,5 ml durchführen. Die Streuung zwischen den Ergebnissen von Mehrfachstimmungen der Viskosität ist groß, so daß Mehrfachuntersuchungen zu empfehlen sind. Sie Streuung beruht auf der von Hause aus vorliegenden Inhomogenität des Sputums, die obigen Überlegungen gelten also auch für andere Untersuchungsmethoden. Die Werte für die elastische Nachverkürzung andererseits sind befriedigend reproduzierbar, sie schwanken innerhalb eines engen Bereiches.

RESUMEN

Medidas simultáneas de la viscosidad y del retroceso elástico pueden efectuarse facilmente mediante un método capilar sencillo, económico, y que tan solo necesita muestras de pequeño volumen (0,5 ml). Sin embargo, la variabilidad de las *medidas de la viscosidad,* según las muestras, lo que hace indispensable multiplicar las medidas. Este detalle, relativo probablemente a la heterogeneidad del esputo se aplica tambien a los otros métodos de medida que se utilizan actualmente.

Con respecto a los valores de retroceso elástico, la reproductibilidad de los resultados es satisfactoria, los valores varian dentro de limites estrechos.
