groupements envisagés sont d'ailleurs équivalents et tout groupement mixte série-parallèle peut être assimilé à l'un d'eux, convenablement choisi. Par contre, un groupement quelconque non décomposable en branches montées en série ou en parallèle, présente des propriétés plus complexes qui ne sont étudiées que dans un cas particulier.

Zusammenfassung

Durch Zusammenschaltung einer großen Zahl von Federn und Bremsschienen (Elementen mit reibender Gleitung) entsprechend dem Aufbau der verallgemeinerten *Kelvin-Voigt* und *Maxwell*-Modelle (statt der Kolben die Bremsschienen) lassen sich einige interessante Eigenschaften darstellen. Bei Übergang zu einer unendlichen Zahl von Elementen kann man ein Spektrum von Fließgrenzen und ein Spektrum von Verformungsgrenzen nachbilden. Die zwei betrachteten Gruppierungen sind übrigens äquivalent, und alle anderen gemischten parallel in Serienschaltungen können durch eine von ihnen, passend ausgewählt, dargestellt werden. Im Gegensatz hierzu stellt irgend eine Schaltung, die nicht aus Zweigen von Serien- oder Parallelschaltungen zusammengesetzt ist, komplexere Eigenschaften dar, die nur in einem besonderen Fall untersucht werden.

Bibliographie

1) *Le Rolland* et *Plénard*, Fonderie **130**, 427–438 (1956); Fonderie **131**, 477–495 (1956); Fonderie **134**, 105–112 (1957).

From the Department of Pathology (Haematology), Postgraduate Medical School of London (England)

The Rheological Problem in Chronic Bronchitis

Further Studies on the Constituent Mucoprotein and Nucleoprotein

By J. C. White and P. C. Elmes

With 7 figures in 8 details and 1 table

(Received April 8, 1958)

D 3 Chronic Bronchitis, Excessive Mucoid Secretion and Purulent Infection

In earlier papers (1, 2, 3, 4, 5) we have indicated the relationship of excessive bronchial secretion to the pathology and symptomatology of chronic bronchitis. The main underlying feature is excessive mucous secretion from the glands of the damaged bronchial mucous membrane. Superimposed upon this are intermittent episodes of acute purulent infection.

During acute attacks, the viscidity of the mucus is modified by the presence of extracellular fibrils of deoxyribonucleoprotein derived from polymorph leucocytes. In both phases, the highly-viscid nature of the secretions is the main cause of symptoms. Although inhalations of deoxyribonuclease may diminish the viscous contribution of the nucleoprotein in acute attacks, the persistence of the mucous component is responsible for the chronic nature of the disease (4).

The underlying fibrous structure of both the deoxyribonucleoprotein and of the mucoprotein of the mucus is responsible for the pathological feature of these secretions (3), and defines the rheological problem in this study (5).

Viscosity Studies on Sputum

The sputum in its native, untreated state is not amenable to study by the classical methods of viscometry. To compare the viscidity of the secretions produced by different patients, or in the same individual at different stages of his illness, it is better to use a method which gives a yield-value (5), or to employ a suitable capillary method to obtain an arbitrary estimate of consistency under controlled conditions (7). The deoxyribonucleoprotein and mucoprotein which form the principal macromolecular constituents are present in essentially fibrous form, not in solution, and once the continuity of this structure is destroyed, the viscous properties change profoundly. This is demonstrated as follows (see fig. 1):–

a) Purulent Sputum:

Specimens which were too viscid to flow in an *Ostwald* viscometer were homogenised without dilution, producing a fluid with a relative viscosity of about 10. There was no recovery of viscosity on standing, and microscopic examination showed it to consist of very small fragments of nucleoprotein in suspension. Addition of sodium chloride up to molar concentration dissolved this, with coincident rise in relative viscosity, and exhibition of thixotropy in three instances out of four (fig. 1a).

b) Mucoid sputum

Native mucoid sputum is a gel that will not flow in an *Ostwald* viscometer. Even after homogenisation most specimens are too viscous to flow through an *Ostwald* (No. 2.,

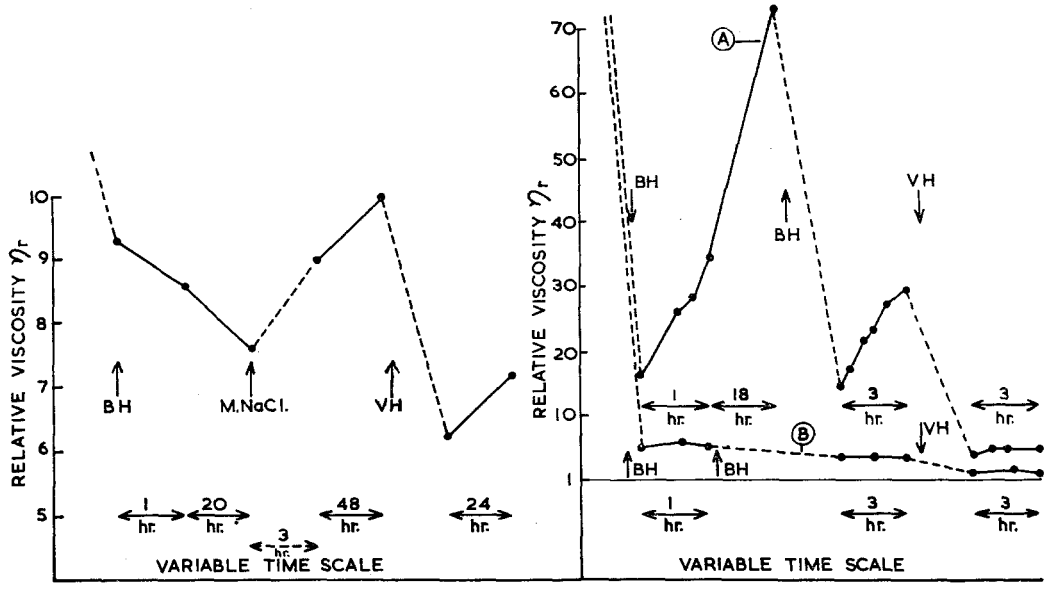


Fig. 1a. *Purulent sputum.* *BH* = brief homogenisation. *VH* = vigorous homogenisation. M. Na Cl. = salt added to molar conc. ←→ = periods of standing at 5°C

Fig. 1b. *Mucoid sputum.* *A* = homogenised in equal vol. of water. *B* = homogenised in M. Na Cl.

B. S. I.) viscometer, but homogenisation for a short time in an equal volume of water produces a liquid of relative viscosity about 20. On standing at 25°C., this shows a rapid and marked increase (fig. 1, b, line *A*); further brief homogenisation again reduces the viscosity, but recovery still occurs over several hours. A more vigorous and prolonged homogenisation at this stage reduces the viscosity again, but there is no recovery.

Hydration and swelling of the mucoprotein structure in water appears to play a large part in these reversible effects. In M. NaCl solution, the recovery of viscosity on standing is very much less (fig. 1, b. line *B*), whilst an intermediate result is obtained with 0.14 M. NaCl.

Orientation of the Secretions

Both mucoid and purulent sputum exhibit weak birefringence in the native, unfixed state. Whereas the mucoid sputum has weakly positive birefringence with respect to the fibre-direction in the specimen, that of the purulent sputum is stronger and negative in sign (6). The deoxyribonucleoprotein fibrils form coarse bundles, much more readily-apparent on phase-contrast examination than the highly-hydrated fibrous mucoprotein. Owing to the uncertainty in thickness, no measurements of absolute birefringence have been obtained.

The orientation of these constituents in the fresh sputum has also been demonstrated by the ultra-violet dichroism of samples simply laid out on a quartz slide, with the apparent fibre-direction corresponding to the length of the slide but without any deliberate attempt to draw out or orient the specimen, and covered by a quartz cover-slip.

The specimens were examined with Mr. *D. Hill*, using a simple assembly constructed by him. The slide was rotated on a turntable in front of a film of stretched Congo red-stained polyvinyl alcohol (8), transmitting polarised light from a *Flood-Hilger* Type FL. 12 hydrogen-arc and monochromator movable behind a fixed output-slit. An area of 2 × 2 mm. of the specimen was illuminated via a quartz-window. The transmitted light entered an E.M.I. quartz-window photomultiplier, type 6255, connected with a direct-couple amplifier and ink-writer for recording and measurement. A shutter was included in the light path in oder to record the base line as required.

In frankly purulent sputum, rich in deoxyribonucleoprotein fibrils, a fairly strong negative dichroism with respect to fibre-length is present with a maximum at a wavelength in the region of 260 mμ. (fig. 2). This appears attributable to the orientation of the purine and pyrimidine rings of the nucleoprotein in a direction across the long-

axis of the fibrils (2, 9). At longer wavelengths, the negative dichroism is replaced by a smaller positive dichroism, particulary in the region 280–310 mμ. Although form-dichroism would be expected to rise towards the U.V.-wavelengths, the fourth-power scattering factor of *Rayleigh* (10) only holds for bodies far below the wavelenght in size, and the fibre-diameters concerned here appear to range from 100–400 Å up to 0.2–0.4 μ (3). A contribution to the positive intrinsic dichroism from the aromatic amino-acids of the polypeptide chains may (11) therefore be expected from the proteins of the fibrils.

Basic dyes are readily taken up by the deoxyribonucleoprotein fibrils of purulent sputum and the dye-molecules are sufficiently oriented to exhibit dichroism in the region of their near ultra-violet and visible absorption bands. This is well-shown with toluidine blue (C. I. 925) and brilliant green (C.I. 662) (figs. 3, 4). In both instances, positive form dichroism is replaced by negative intrinsic dichroism increasing rapidly in the region of the absorption maxima

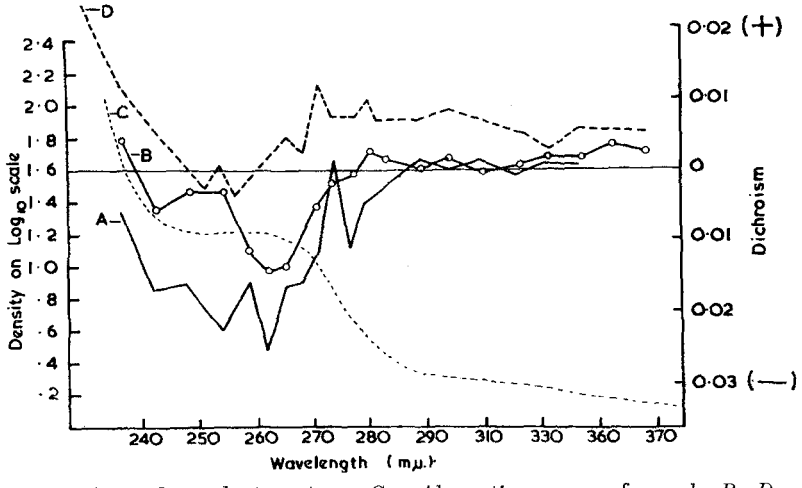


Fig. 2. *A* & *B* = Dichroism of purulent sputum. *C* = Absorption curve of sample *B*. *D* = Dichroism of a mucoid sputum sample

A sample of sputum richer in mucus than in nucleoprotein adds weight to this view (fig. 2). The negative dichroism is small in the region of 260 mμ, and although a positive form-dichroism persists above this wavelength, a maximal positive dichroism from 280–300 mμ again suggests a contribution from the protein-chains.

of the dyes. Absorption curves for the pure dye-perchlorates, from data prepared by Dr. *G. H. Beaven*, are plotted in the figures. The wavelength scale becomes less precise in the visible spectral region, owing to the dis-

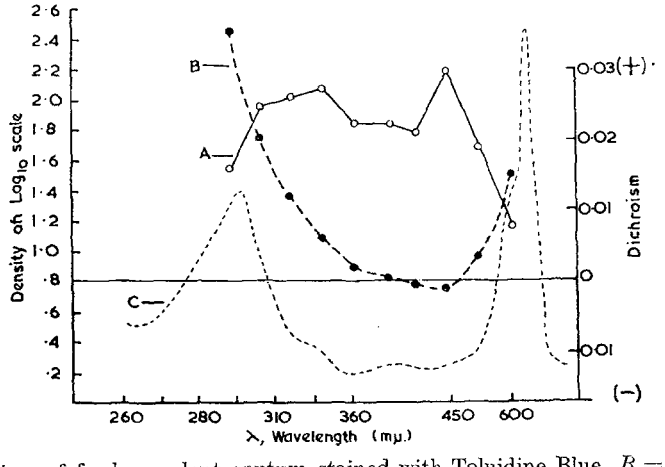


Fig. 3. *A* = Dichroism of fresh purulent sputum stained with Toluidine Blue. *B* = Absorption curve of specimen. *C* = Absorption curve of Toluidine Blue perchlorate

persion of the monochromator being very low here; however the dichroism is clearly related to the visible absorption maxima of the dyes, whilst in the ultraviolet the nucleoprotein absorption also appears to contribute to the dichroism.

sodium chloride crystals (2). After solution of the host-salt in methanol, the negatively-birefringent fibrils take up toluidine blue and exhibit a similar dispersion of the birefringence to the sputum-film above (fig. 5). An orientation of the dye-molecules on the

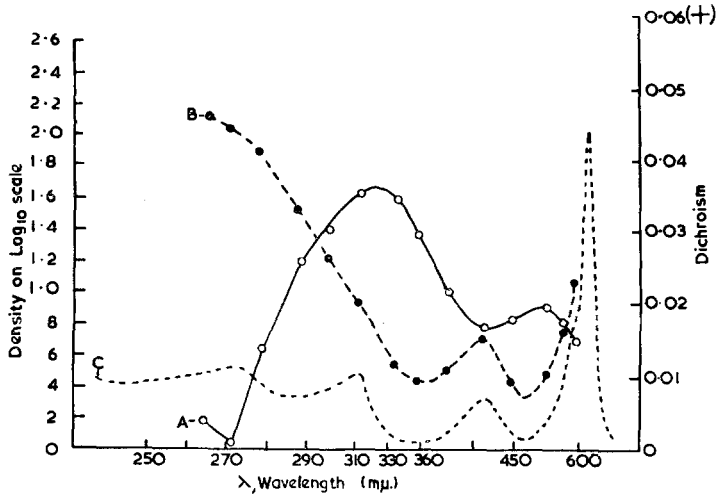


Fig. 4. *A* = Dichroism of Carnoy-fixed purulent sputum stained with Brilliant Green. *B* = Absorption curve of the specimen. *C* = Absorption curve of Brilliant Green perchlorate

The Birefringence of Sputum and of Derived Fibres

In the following experiments, the samples were all wet-fixed in *Carnoy* fluid. Some samples were then treated with fuming sulphuric acid for 5 minutes to increase dye-uptake (12) from 0.005% toluidine blue solution in water. In the case of crystal-fibrils of deoxyribonucleoprotein, staining in 0.5% toluidine blue followed fixation directly. Retardations were measured by the *Brace Kohler* method, using a 0.04 λ plate (13).

a) Purulent Sputum

The birefringence of a film of lightly-spread purulent secretion is negative in sign and there is little dispersion although the retardation decreases towards longer wavelengths, presumably due to form dispersion (fig. 5). The film takes up toluidine blue strongly, and orientation of the dye molecules is very apparent from anomalous dispersion of the birefringence, which is positive at 520 mμ. changing at about 630 mμ to negative sign of birefringence in the red (fig. 5).

Pure deoxyribonucleoprotein can be extracted from such sputum, and microfibrils prepared as oriented inclusions within pre-existent ordered structure of the nucleoprotein fibrils may be inferred in both types of preparation.

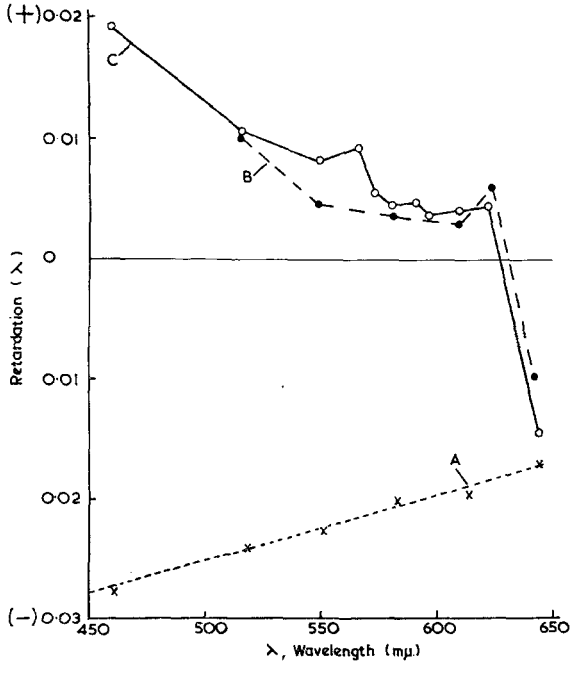


Fig. 5. *A* = Birefringence of fresh purulent sputum. *B* & *C* = Comparison of Toluidine Blue-staining of purulent sputum (fixed, acid-treated, *B*) and crystal-fibre of deoxyribonucleoprotein (*C*)

b) Mucoid sputum

Wet-fixed films of mucoid sputum possess positive birefringence with little dispersion (fig. 6). Dyes such as pyronin and toluidine blue are taken up strongly, and exhibit marked dispersion of birefringence (fig. 6).

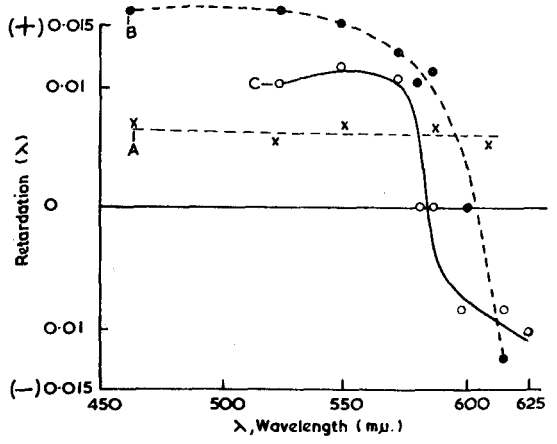


Abb. 6. *A* = Mucoid sputum, Carnoy-fixed. *B* = Ditto, acid-treated and 0.005% Toluidine Blue. *C* = Mucoprotein crystal-fibre. Toluidine Blue after fixation and acid treatment

Both whole mucoid sputum, and partially-dispersed mucoprotein extracted from it by 0.15 M. sodium chloride solution, readily form oriented inclusions in host-crystals of sodium chloride (3). The mucoprotein takes up toluidine blue and exhibits a positive birefringence at 520 mμ, changing at about 580 mμ to a negative birefringence in the red (fig. 6). Here again, the analogy from such evidence suggests pre-existent orientation of the mucoprotein molecules in the mucoid sputum.

Electron-microscopy

Direct electron-microscopic observation of freeze-dried preparations of purulent and mucoid bronchial secretions reveal a fibrous structure, which bears direct comparison with the corresponding micro-fibrils produced artificially by orientation within sodium chloride crystals (3, 5). The latter micro-fibrils possess diameters as small as 90 Å in the case of deoxyribonucleoprotein, but apparently no smaller than 400 Å for the mucoprotein (3).

Interaction of Deoxyribonucleoprotein Micro-fibrils within their Host-Crystal

In this instance, evidence has been obtained from electron-diffraction that the deoxyribonucleoprotein micro-fibrils associate with micro-crystallites of the host-salt. Well-marked diffraction patterns of the host-salt are obtained with evidence of preferred orientation, but a lattice-distortion in which the d_{III} spacing is enlarged, perhaps due to the assumption of a tetragonal lattice (4). Evidence for a similar interaction has not as yet been obtained for the mucoprotein.

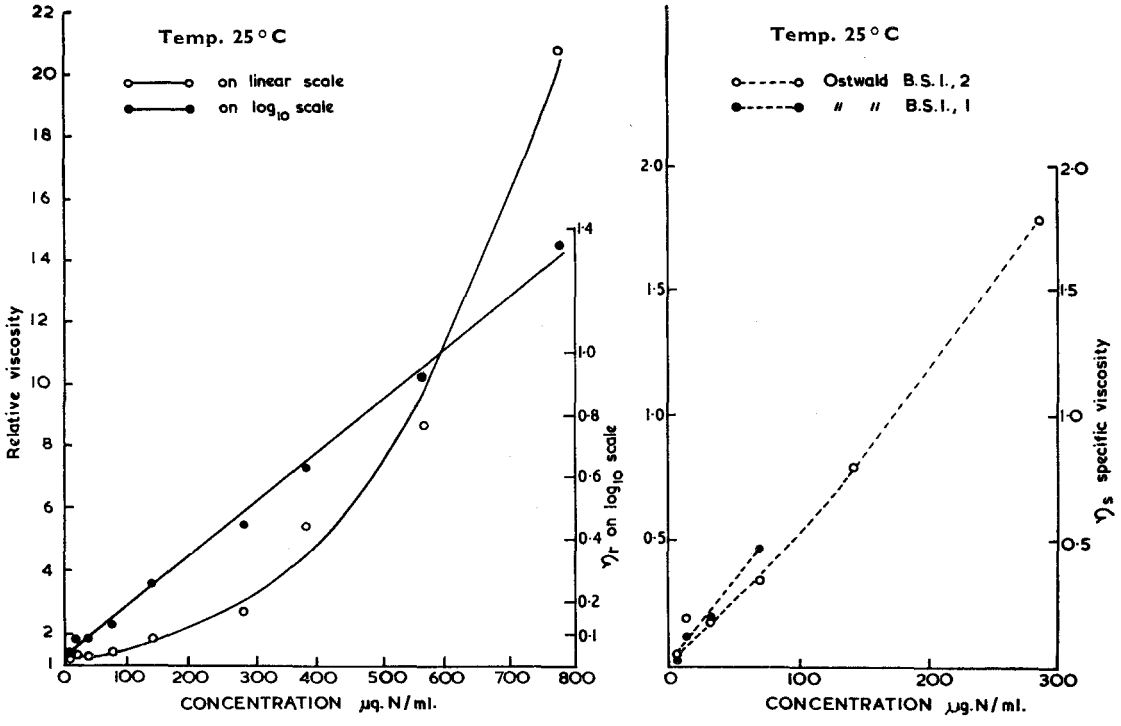
The Mucoprotein of Mucoid Sputum

A mucoprotein has been extracted by the procedure of *Curtain* and *Pye* (15), which appears to be a principal constituent of the mucoid secretion. Hydrolysis and chromatography reveal the constituent sugars as hexosamine, galactose, mannose and fucose (3). The average composition of 4 samples has been determined by Miss *W. Tucker*:

Table 1

	g.% of dry-weight
Nitrogen	8.1
Total reducing Sugar.	25
Hexosamine	6.3
Fucose	6.5
Sialic acid	4.9
Bound Sulphate as S.	< 0.2

The structural relationship of the mucoprotein to the parent mucus has not been fully determined, and its preparation involves disruption of the mucus and precipitation and separation of the mucoprotein via the barium complex. Both the mucus and the protein form fibrous inclusions in sodium chloride crystals, with a micro-fibrillar structure in the electron-microscope; the smallest fibrils are of the order of 400 Å diameter (3). Solutions of the mucoprotein are viscous and opalescent, and at a comparable nitrogen concentration to the whole mucus will not flow in the *Ostwald* viscometers (B.S.I., 0–2). More dilute solutions give an exponential curve of relative viscosity against concentration, similar to deoxyribonucleic acid (16). The plot of the logarithm of the relative viscosity against concentration on a linear scale gives a fairly good straight-line relationship over the range of nitrogen concentration of 10–800 μg. N/ml. (fig. 7). At low concentrations, anomalous surface-tension effects are encountered in the *Ostwald* viscometers (17). The mucoprotein solutions exhibit non-Newtonian behaviour on varying the working volume in an *Ostwald* viscometer with a cylindrical limb.



a b

Fig. 7a. Relative Viscosity of Mucoprotein in *Ostwald* Viscometer (B. S. I., 1)
Fig. 7b. Specific Viscosity of Mucoprotein as function of Concentration

Discussion

Of the two principal constituents of bronchitic secretions, both appear to exist in the material as an oriented fibrous gel, but with differing properties. In both cases disruption destroys the rheological structure. For viscometric examination, simple methods giving a yield value are probably the most applicable in practise, such as the syringe with perforated plunger (4, 5), although torsional methods have been used (18).

The deoxyribonucleoprotein forms extracellular fibrils which can be disrupted mechanically, by deoxyribonuclease or by solutions of sodium chloride at concentrations which extract the nucleoprotein. At high salt concentrations the nucleoprotein dissociates (19), and the high viscosity is due to rheological properties of the free nucleic acid.

In the intact fibrils, the nucleoprotein appears to be in a gelatinous state, such as has been suggested for the existence of deoxyribonucleoprotein in the chromosomes (20). This state is preserved at normal physiological salt concentrations (21). The recent work of *Ambrose* (22) on spontaneous fibre formation at critical concentrations of nucleoprotein, but not of nucleic acid, is also interesting in this respect.

The mucoprotein gel is different from nucleoprotein in its behaviour towards salt solutions, and in particular shows considerable ability to take up water. This aspect of sorption might be profitable to study further, as would be the effects of mucolytic enzymes on the intact mucus and on the isolated mucoprotein.

We gratefully acknowledge a grant from the Peel Trust, enabling Miss *W. Tucker*, B. Sc. to assist in part of this work. The Medical Research Council has given financial assistance throughout.

Summary

In chronic bronchitis the two principal constituents of the bronchial secretions are mucoprotein and deoxyribonucleoprotein. Both exist as oriented fibrous structures in the secretions, and are responsible for the viscidity. The special properties of each are responsible for their distinctive rheological behaviour.

Zusammenfassung

Bei der chronischen Bronchitis sind die zwei wesentlichen Bestandteile der Bronchialsekretion Mucoprotein und Desoxyribonucleoprotein. Beide existieren als orientierte Faserstrukturen in den Sekreten und sind verantwortlich für die Klebrigkeit und Dickflüssigkeit Die speziellen Eigenschaften einer jeder Komponente sind ausschlaggebend für das ausgeprägte rheologische Verhalten.

References

1) *Armstrong, J. B.*, and *J. C. White*, Lancet **1950**, 11, 739.

2) *White, J. C.*, and *P. C. Elmes*, Nature **169**, 151 (1952).

3) *White, J. C., P. C. Elmes* and *A. Walsh*, J. Path. Bact. **67**, 105 (1954).

4) *Elmes, P. C.*, and *J. C. White*, Thorax **8**, 295 (1953).

5) *Elmes, P. C.*, and *J. C. White*, Proceedings of the 2nd Int. Congress of Rheology, Oxford 1953. Ed. *V.G.W. Harrison*, p. 382 (London 1954).

6) *Elmes, P. C.* and *J. C. White*, Discussion, Ref. (5).

7) *Scott Blair, G. W.*, and *F. A. Glover*, Lab. Practise **5**, 125 (1956).

8) *Barer, R.*, J. Sci. Instrum. **26**, 325 (1949).

9) *Wilkins, M. H. F., R. G. Gosling*, and *W. E. Seeds*, Nature **167**, 759 (1951).

10) *Lord Rayleigh*, Phil. Mag. **41**, 274 (1871). [See Physical Optics by *R. W. Wood*. 3rd Ed., p. 425 (New York 1954) and Cell Growth and Cellfunction by *T. O. Caspersson*, p. 27 (New York 1950)].

11) *Perutz, M. F., M. Jope* and *R. Barer*, Faraday Soc. Disc. No. 9, 423 (1950).

12) *Kramer, H.*, and *G. M. Windrum*, J. Histochem. Cytochem. **2**, 196 (1954).

13) *Swan, M. M.*, and *J. M. Mitchison*, J. Exp. Biol. **27**, 226, (1950).

14) *White, J. C., P. C. Elmes, V. Balashov, R. D. Preston*, and *G. W. Ripley*, Nature **180**, 696 (1957).

15) *Curtain, C. C.* and *J. Pye*, Austr. J. Exp. Biol. med. Sci. **33**, 315 (1955).

16) *Kay, E. R. M., N. S. Simmons*, and *A. L. Dounce*, J. Amer. Chem. Soc. **74**, 1724 (1952).

17) *Bull, H. B.*, J. Biol. Chem. **133**, 39 (1940).

18) *Palmer, K. N. V.*, Lancet **1**, 611 (1957); *J. Forbes* and *L. Wise*, ibid. **11**, 767 (1957).

19) *Stern, K. G.*, and *S. Davis*, Fed. Proc. **5**, 156 (1946). *J. A. V. Butler, P. F. Davison*, and *D. W. F. James*, Proc. Biochem. Soc. **54**, 21 (1953); *C. F. Crampton, R. Lipschitz*, and *E. Chargaff*, J. Biol. Chem. **206**, 499 (1954).

20) *Stern, K. G., G. Goldstein, J. Wagman*, and *J. Schryver*, Fed. Proc. **6**, 296 (1947).

21) *Mirsky, A. E.*, and *A. W. Pollister*, J. Gen. Physiol. **30**, 117 (1946).

22) *Ambrose, E. J.*, Proc. Roy. Soc. B. **148**, 57 (1958).

Aus dem Laboratorium für Polarisationsmikroskopie, Bremen

Versuche zu einem „shear-rate blockage" Effekt am Protoplasma zentrifugierter Gewebe

Von Hans H. Pfeiffer

Mit 3 Abbildungen

(Eingegangen am 8. April 1958)

Biorheologische Untersuchungen sind nach Zielsetzung und angewandter Technik überaus mannigfaltig, wie schon ein Querschnitt durch die Referate der ersten internationalen Konferenz in Lund (3) vor sieben Jahren besser als eine eingehende Erörterung zeigen kann. So habe ich mir in der folgenden Mitteilung einen Beitrag zur Analyse eines Phänomens zur Aufgabe gestellt, für welches das übliche Strömungsverfahren ebenso wie der Versuch des Fadenziehens nicht anwendbar sind. Bei dem Ziel, die des öfteren in Strömungsversuchen mit Kapillaren (8a) durch Demonstration des capillary birefringent effect (8c; 10) oder durch Messung des Rheodichroismus (8e; f) nachgewiesene Nicht-*Newton*sche Natur des Protoplasmas auch für das Protoplasma von Zellen im Gewebeverbande und mit dicht gepackten granularen Komponenten nachzuweisen, habe ich Schnitte pflanzlicher Stärkescheiden (Phloeoterma der Achsen besonders von *Phaseolus multiflorus*) nach Zentrifugieren untersucht. Zur Annäherung an eine niedrigere Größenordnung ging ich später dazu über, Explantate *in vitro* wie vorher schon [über die Eignung zu den Versuchen s. (8d)] von Nieren- und Leberfragmenten des Wasserfrosches *(Rana esculenta)* mit Zellen, in denen Mitochondrien stark angereichert sind, zu zentrifugieren. Beide Objekte wären wegen des zellulären Zusammenhanges der Gewebe für die bisher angewandten Versuchstechniken unbrauchbar. Für sie kam nur die Messung der zentrifugalen Verlagerung der Zellkomponenten in Betracht, wenn der Viskositätsgrad des Protoplasmas in Abhängigkeit von der abgestuften Rotationskraft als Scherkraft erhalten werden sollte.

Die anzustellende *Technik* (1) braucht hier nicht genauer behandelt zu werden. Anfängliche Versuche mit einer Art Zentrifugenmikroskop erwiesen sich schon bei dem botanischen Objekt aus optischen Gründen wenig brauchbar. So wurden die frischen Gewebe mit einer kleinen, motorisch angetriebenen Zentrifuge abgestuften Rotationsgeschwindigkeiten ausgesetzt und dann sofort zu lokaler Festlegung der Zellkomponenten bis 10 min mittels 10% Trichloressigsäure