of kidney microsomes. Whether the angiotensinase in liver cell sap is exactly the same as the erythrocyte enzyme remains to be studied. An arylamidase in liver cell sap described by MAHADEVAN and TAPPEL[10] may be related to this angiotensinase activity since both are inhibited by EDTA and thiol reagent. From the present results, we can divide the neutral-active EDTA-inhibited angiotensinases into 2 groups, i.e., sulfhydryl-dependent and -independent. These 2 groups are different not only in their properties but also in their location. The angiotensinase in the liver cell sap belongs to the former group; it is sulfhydryl-dependent and is inhibited by either thiol reagent or EDTA. Microsomal angiotensinase of the kidney which is inhibited by EDTA but not sulfhydryl-dependent belongs to the latter group[11].

Résumé. L'activité de l'angiotensinase neutre contenue dans le suc des cellules hépatiques est inhibée par EDTA ou par PCMS, mais restituée par addition d'ion de calcium ou de composés thioliques. Ces propriétés ressemblent à celles de l'angiotensinase érythrocytaire, mais diffèrent de celles de l'angiotensinase microsomale d'origine rénale laquelle ne nécessite pas la présence de groupes sulfhydryles[11].

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Saliva is Viscoelastic

On reading AFRONSKY's[1] survey of the literature on the properties of saliva one is lead to believe that the viscosity of saliva is directly related to such factors as dry weight of solids, protein or mucin content. Highly viscous saliva has been associated with nervous disorders, pregnancy and diet[2], and numerous correlations exist between saliva viscosity and dental caries[3] and plaque formation[4]. There is even a reported relationship between saliva viscosity and enamel solubility[5]. However, viscosity is defined as the ratio of shear stress to shear rate for a Newtonian fluid, and like many biological fluids saliva is far from Newtonian and it will in some cases exhibit thread formation ('Spinbarkeit')[6]. It is unfortunate, therefore, that almost all rheological measurements on saliva have been carried out with some form of simple capillary viscometer. DEWAR and PARFITT[7] seem to be unique in their attempt to measure elasticity but were faced with severe experimental limitations.

Recently there has been interest in the rheological characterisation of biological materials using the linear viscoelastic model as a convenient starting point[8–10]. This has the great advantage of providing fundamental terms such as viscosity and elasticity which can often be interpreted in terms of molecular structure. Respiratory mucus has been examined in this manner, both by the present author[10] and HWANG and his colleagues in America[8,9] and we now report similar findings for saliva.

Unstimulated samples of Saliva were collected from a healthy male subject and examined without delay with a Weissenberg Rheogoniometer in oscillatory mode and parallel plate geometry.

The equation of state for a linear viscoelastic material undergoing forced harmonic oscillation of small amplitude can be written as[11]:

$$\sigma = 2\,\eta^*\,\dot{\gamma}$$

where $\dot{\gamma}$ is the shear rate, σ the shear stress and η^* the complex dynamic viscosity. The last term can be split up into real and imaginary parts:

$$\eta^* = \eta' - i(G'/\omega)$$

where η' is the dynamic viscosity and G' the dynamic rigidity. ω is the frequency of oscillation in radians sec^{-1}. G' is also known as the storage modulus and is a measure of the energy stored and recovered per cycle[12]. One can also define a loss modulus, $G'' = \eta'\omega$, as a measure of the energy dissipated per cycle. Calculated values of these parameters are shown in Figure 1 for a typical saliva sample. The value of η' is very dependent on frequency, having a value in excess of 10^2 Poise at low frequency, falling to less than 0.5 Poise at high frequency. These values may be compared with those obtained in previous investigations (Table) and they demonstrate the severe limitations of using conventional viscometric tech-

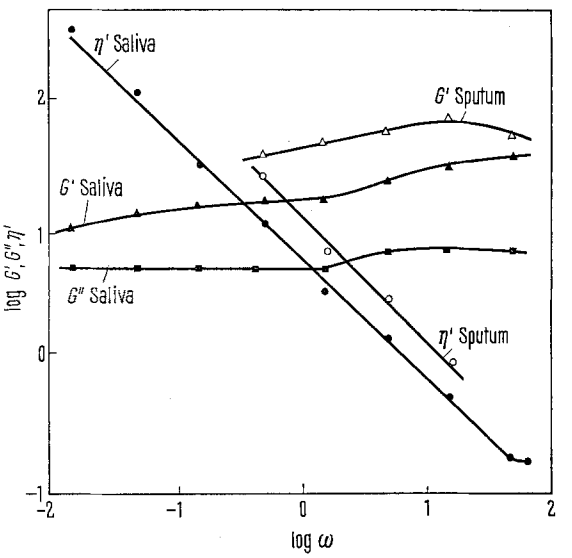

Fig. 1. Dynamic viscoelastic data for saliva and sputum (25 °C). Ordinate: Viscoelastic parameters (log G', G'', η'). Abscissa: Frequency (rad sec^{-1}) (log ω). Sputum data from ref. [10].

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niques for complex fluids. The gradient of the log η' versus log ω relation is -1.0 indicating that the product $\eta'\omega = (G'')$ will be effectively constant over the same frequency range. G' gradually increases with frequency and has a value between 10 and 50 dyne cm^{-2}. Also shown in Figure 1 are similar results obtained in earlier investigations on sputum[10].

The behaviour of saliva over the frequency range 1.6×10^{-2} to 60 rad sec^{-1} is typical of an uncross-linked polymer in what is known as its plateau region of viscoelasticity[12]. No attempt has been made to represent the data in the form of a retardation spectrum but it is evident from the plateau that a whole range of retardation times will exist. On a molecular basis, saliva can be tentatively described as being made up from a transient network of polymer chains that can be formed either by the adherence of individual molecules at widely separated points or by some entanglement mechanism. A highly branched structure with side chains of differing lengths would give rise to a wide distribution of retardation times. This picture compares favourably with established biochemical structure. Salivary mucins are rod shaped glycoproteins with a molecular weight around 10^6[6, 15, 16]. Their carbohydrate moiety is a typical sialomucin constructed of numerous short prosthetic side chains linked to a polypeptide skeleton by means of hexosamine and having sialic acid in the terminal position[15, 17].

It is the viscoelastic, polymeric nature of saliva that gives it its exceptional lubricant properties. At low shear rates viscous and elastic contributions will give saliva a considerable structure of a gel-like nature. This has obvious implications in the role that saliva plays in dental health and the removal of particles of debris from crevices in the teeth. Possible correlation between saliva elasticity and dental caries would be worth investigating. The suggestion by MIZUMA[5], that a reduction in saliva consistency by artificial means would act as a caries preventative, is also worthy of further thought.

Saliva from the same subject varied in consistency from day to day, no doubt due to factors such as the method of collection, time of day, and dietary intake. The pH remained almost constant but there was a noticeable difference in dry weight content. There appears to be a far more satisfactory correlation between elasticity and dry weight than with dynamic viscosity (Figure 2). This is perhaps as to be expected, for an increase in polymer content will lead to a greater gel structure and gel strength but have little effect on the viscosity of the surrounding fluids. Elasticity is, therefore, of far greater importance than the usually quoted 'viscosity' when considering the rheological properties of saliva and their relation to organic content and dental health. DENTON et al.[9] have recently arrived at similar conclusions for respiratory mucus and its role in cilia transport.

The similarity between the results for saliva and sputum suggests to us that it may be possible to employ saliva as a readily available and easily characterizable model for assessing the action of mucolytic agents in vitro. In this way we should be able to have a greater understanding of the mode of action of such drugs and be able to quantify the popular statement in promotional literature concerning 'reduction in mucus viscosity' in rheologically meaningful terms such as viscosity and elasticity. Even today there is no coherent picture as to what the ideal mucolytic agent should achieve and findings such as those of LUCUS and DOUGLAS[18], who showed that the thicker

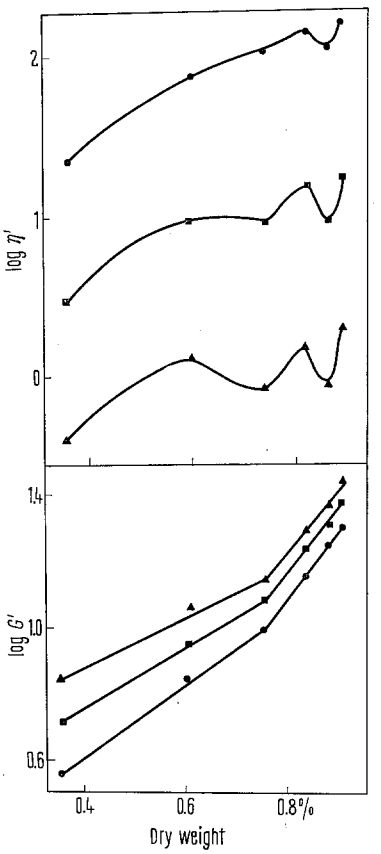

Fig. 2. Correlation of viscoelastic parameters with dry weight content of saliva. Ordinate: Top; log dynamic viscosity (log η'). Bottom; log dynamic rigidity (log G'). Abscissa: % dry weight. Selected frequencies (rad sec^{-1}). ▲, 2π; ■, $2\pi \times 10^{-1}$; ●, $2\pi \times 10^{-2}$.

The viscosity of saliva

Method	Viscosity (Poise)	Shear rate (sec^{-1})	Reference
Ostwald U-tube viscometer	1.4×10^{-2}	Variable and unknown	1
Hess viscometer	$3.5\text{–}18 \times 10^{-2}$	Variable and unknown	1
Epprecht viscometer	$1\text{–}3 \times 10^{-2}$	Not stated	13
Ferranti-Shirley viscometer	1.0×10^{-1}	750	14
Rheogoniometer (in oscillation)	3.8×10^{2}	5.4×10^{-3} (maximum)	This work
	2.0×10^{-1}	2.1 (maximum)	

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and denser the strands of mucus the more efficient the upward movement, require rationalization.

Zusammenfassung. Untersuchungen mit dem Weissenberg-Rheogoniometer zeigen, dass Speichel viskoelastisch ist und dass dessen dynamische Viskosität sich über einen Frequenzbereich von 2.5×10^{-3} bis 10 Hz und von 400 bis 0.1 Poise bewegt.

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The Development of 3′,5′-cyclic Nucleotide Phosphodiesterase in White and Brown Adipose Tissue of the Rat

It is now well established that lipolysis in adipose tissue is induced by cyclic adenosine monophosphate[1]. This compound is formed from ATP by a cyclase and is broken down to AMP by phosphodiesterase. The presence of both enzymes has been demonstrated in adult white adipose tissue[1]. No data are available for brown adipose tissue and nothing is known about the development of either enzyme in both white and brown adipose tissue.

It has been shown previously that the activity of hormone sensitive lipase in rat adipose tissue increases with age[2] and this might be related to changes in the activities of adenyl cyclase or phosphodiesterase. Hence phosphodiesterase activity was determined during postnatal development of the rat.

Adipose tissue was homogenized in an all glass homogenizer with 0.25 *M* sucrose, in 10 volumes for interscapular and 2 volumes for gluteal white tissue. Phosphodiesterase was determined according to[3] in 0.1 ml of the fat free supernatant obtained by centrifuging the homogenate in the cold at $10{,}000 \times g$ for 20 min. The supernatant was incubated with 25 μmoles *Tris* HCl, 2.5 μmoles $MgCl_2$, 10 μmoles ammonium sulfat and 0.75 μmoles cyclic AMP for 20 min at 37 °C. After boiling in water and cooling, snake phospholipase (*Crotalus adamanteus* venom) was added and incubation was continued for another 20 min. The reaction was stopped with 10% trichloracetic acid.

It is evident from the Table that in both types of adipose tissue phosphodiesterase activity is highest in the youngest age groups. Per unit wet weight activity is always higher in brown adipose tissue than in white adipose tissue. Per unit protein content, however, activity is higher in brown than in white tissue only in the suckling period.

Phosphodiesterase activity of white and brown adipose tissue

Age	μmoles *P* released/ mg protein		μmoles *P* released/ g wet weight	
	B.F.	W.F.	B.F.	W.F.
Fetus	0.49 ± 0.015	–	10.8 ± 0.32	–
3 days	0.50 ± 0.010	0.31 ± 0.02	11.0 ± 0.31	2.2 ± 0.16
10 days	0.52 ± 0.02	0.11 ± 0.006	11.2 ± 0.40	0.8 ± 0.06
20 days	0.19 ± 0.011	0.09 ± 0.010	3.5 ± 0.15	0.8 ± 0.05
30 days	0.21 ± 0.009	0.15 ± 0.01	2.0 ± 0.11	0.8 ± 0.04
Adult	0.11 ± 0.006	0.105 ± 0.007	1.9 ± 0.10	0.8 ± 0.031

4 to 6 determinations for each group ± S.E.

It is tempting to relate these developmental changes in phosphodiesterase activity to changes in the activity of hormone sensitive lipase[2]. The postnatal rise in the activity of the latter enzyme might be due to a decrease in the rate of breakdown of cyclic AMP during that period of development. Another fact that is in agreement with such a mechanism is the higher rate of spontaneous lipolysis in white than in brown adipose tissue in the neonatal period[4]. In white adipose tissue, however, this rate decreases with age, a change not to be expected if phosphodiesterase were the decisive factor for lipolysis. Undoubtedly many factors regulate the rate of lipolysis in adipose tissue and probably control of adenyl cyclase activity is more important than the regulation of phosphodiesterase. In brown adipose tissue at least, adenyl cyclase activity does not seem to change during postnatal development[5], though its hormone sensitivity does, and hence it is possible, though not proven, that the postnatal decline in phosphodiesterase activity in both types of brown adipose tissue is related to the high fat diet consumed by the rat during that period[6] and the assumed lesser need for lipolysis.

Zusammenfassung. Nachweis einer Abnahme der Phosphodiesterase im braunen und weissen Fettgewebe mit dem Alter erklärt die erhöhte Hormonempfindlichkeit der Depotfettgewebe bei älteren Ratten.

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