Finally, it would be interesting to know the effect of HCG on GA production by *Fusarium moniliforme*, the original source of GA[13]; if inhibition occurs, conceivably this may prove the ideal system for elucidating the mode of action of HCG at the molecular level[14].

Résumé. La gonadotrophine chorionique peut arrêter la production de l'α-amylase pendant la germination des grains intacts d'orge. Par contre, la synthèse de l'α-amylase controllée par l'acide gibberellique dans les sections de grains d'orge sans embryon n'est pas modifiée. La gonadotrophine chorionique doit donc arrêter la synthèse ou la sécrétion de l'acide gibberellique.

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Hepatic and Renal Neutral Angiotensinases

Although neutral angiotensinase activity has been found in various tissues, it is not clear how many enzymes are involved. The properties of plasma angiotensinases are well documented[1]; they appear to be different from those of erythrocyte angiotensinases[2] but similar to those of kidney angiotensinases[3]. Little is known about liver angiotensinases. The location of kidney angiotensinase in microsomes[3,4] and of liver angiotensinase in the cell sap[5] suggests the possibility of two different enzymes. The purpose of this paper is to further define and compare the properties of the neutral angiotensinases in liver, kidney and erythrocytes.

Materials and methods. Subcellular fractions of rat tissues were prepared according to the method of Ragab et al.[6] for the lirer and to that of Shibko and Tappel[7] for the kidney. Erythrocytes were separated from rat blood taken with disodium ethylenediamine tetracetic acid (EDTA-Na_2) as anti-coagulant. After washing twice with 0.9% saline, erythrocytes were hemolyzed by adding an equal volume of distilled water and centrifuged. The supernatant was diluted to an appropriate concentration with distilled water. Samples of liver cell sap, kidney microsomes or hemolysate, which contained 0.8 mg, 0.1 mg and 2 mg of protein, respectively, were incubated with 0.25–0.5 µg of angiotensin II or its analogues in 4 ml of 0.125 *M tris*-acetic acid buffer (pH 7.5) at 37 °C for 30 min. One part of the samples was dialyzed against EDTA-Na_2 solution (0.22% in 0.9% NaCl) and then against 0.9% NaCl (2.5 m*M*). Dithiothreitol (DTT) (1 m*M*), *p*-chloromercuriphenyl-sulfonic acid (PCMS), di-isopropylfluorophosphate (DFP) (1 drop of a 5% solution in w/v isopropanol), or (5 m*M*) $CaCl_2$ was added to the incubation mixture as activator or inhibitor. After removal of proteins by boiling and centrifugation, the remaining angiotensin was bioassayed in the rat, using [Asn[1], Val[5]]-angiotensin II as standard. Angiotensinase activity was defined as percentage of angiotensin inactivated during incubation.

Results and discussion. The Table shows effects of inhibitors and activators on angiotensinase activity of liver, kidney and erythrocytes on [Asn[1], Val[5]]-angiotensin II. Values are percentages of the activity compared to that of untreated samples.

Angiotensinase activity of these 3 kinds of samples was inhibited by dialysis against an EDTA solution and restored by addition of $CaCl_2$ but was not inhibited by DFP. The activity of liver cell sap and that of hemolysate were also inhibited by PCMS whereas microsomal angiotensinase of the kidney was resistant to this agent. Dithiothreitol prevented this inhibition.

Inhibition and activation of angiotensinase activity at pH 7.5

	PCMS	PCMS + DTT	EDTA	EDTA + Ca	DFP
Liver cell sap	0	94	0	78	111
Kidney microsome	97	102	9	98	99
Hemolysate	0	87	0	90	100

Itskovitz and Miller[2] showed that mercury compounds inhibited most of the angiotensinase activity of hemolysate but not of plasma. Our observation on the erythrocyte enzyme is consistent with their findings. Kokubu et al.[8] reported that a purified erythrocyte angiotensinase which cleaved the 2–3, 4–5 and 5–6 bonds of angiotensin was inhibited by either EDTA or DFP. They also stated that the inhibitory action of EDTA was not reversed by calcium or other divalent cations. This discrepancy from our observations could be explained by the existence of more than one enzyme in the hemolysate[9] or by differences in purity. Liver cell sap and hemolysate inactivated both [Asp[1], Ile[5]]-angiotensin II and [Asn[1], Val[5]]-angiotensin II, but had little effect on [Arg[1], Ile[5]]-angiotensin II.

Thus, the angiotensinase activity of the liver cell sap is similar to that of hemolysate but different from that

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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-

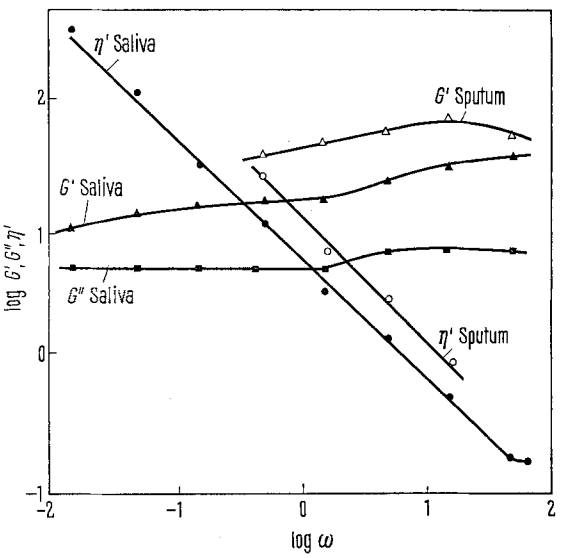

Fig. 1. Dynamic viscoelastic data for saliva and sputum (25 °C). Ordinate: Viscoelastic parameters (log G', G'', η'). Abscissa: Frequency (rad sec^{-1}) (log ω). Sputum data from ref. [10].

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