Further investigations along these lines are at present being made and will form the basis of a subsequent communication.

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Proteolysis of Collagen

It was shown by Linderstrøm-Lang and co-workers[1] that the tryptic hydrolysis of β-lactoglobulin involves the reversible denaturation of the protein as a preliminary stage. A consideration of known facts, together with new evidence obtained in connexion with an investigation of the mechanism of bating of skins in leather manufacture[2], indicates that collagen and procollagen are also denatured by the enzyme prior to hydrolysis. Bating is a process in which skins, after liming and deliming, are treated with a proteolytic enzyme (usually trypsin) in order to produce a soft leather. The process has been shown to involve the removal of degraded collagen (a form of collagen in which the fibrillar structure has disintegrated and which has been named 'progelatin'[2]) produced by the swelling action of lime, and is normally carried out at about 37° C. Temperature control is critical since little or no action takes place below 35° C., and above 40° C. rapid digestion of intact collagen occurs, leading to a loss of leather-making substance. The lower limit is explained by the fact that progelatin must be converted into gelatin before it can be hydrolysed by the enzyme. If, however, the skin is heated to 40–45° C. before adding the enzyme, bating can be carried out at a much lower temperature, for example, at 27° C. in about twice the time required at 37° C., which is in accord with the temperature coefficient of proteolytic enzymes acting on gelatin[3]. The higher temperature required to heat the progelatin in the absence of enzyme than in its presence is indicative of a denaturation stage which can be catalysed by the enzyme. As denaturation is believed to involve rupture of hydrogen bonds between parallel molecular chains, the enzyme functions as a hydrogen bond breaker in a similar manner to chemicals such as alkali, urea, potassium thiocyanate, etc.

Hydrogen bond breaking by chemicals can be followed by the decrease in hydrothermal shrinkage temperature (T_s), but fails with proteolytic enzymes since denaturation is immediately followed by hydrolysis, so that shrinkage does not occur. It is impossible to wash out completely or inactivate the enzyme adsorbed on the collagen, which is remarkably stable so that even plunging the enzyme-treated collagen into boiling water results in appreciable hydrolysis before the enzyme is inactivated[4]. Any chemical treatment that lowers the T_s of collagen results in an increased rate of attack by trypsin at 37–40° C. because the activation energy for enzyme denaturation is reduced. Various native fish collagens are attacked by trypsin at temperatures related to their hydrothermal shrinkage temperatures[5]. Fish collagens are believed to differ from mammalian collagen principally in the degree of hydrogen bonding[6] and so behave similarly to chemically treated mammalian collagen.

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Size and Shape of Cartilage Mucoprotein

The chondroitin sulphate – protein complex of cartilage was extracted from fresh homogenized bovine nasal septa by 30 per cent potassium chloride and precipitated with absolute alcohol after dialysis against running tap water and addition of potassium acetate; all operations were carried out at 2° C. The final product was analogous in chemical composition to the mucoprotein of Shatton and Schubert[1]. Solutions of the mucoprotein in phosphate buffer $M/15$, pH 7·0, were studied by viscometry, sedimentation, light scattering and flow birefringence.

The viscosimetric measurements were carried out at a velocity gradient $G = 0{\cdot}246$ sec.$^{-1}$ in a Couette apparatus; the intrinsic viscosity at 25° C. was $[\eta] = 207$ C.G.S. units. The sedimentation coefficient at infinite dilution and 20° C. was $s_0 = 6{\cdot}85 \times 10^{-13}$ C.G.S. units; the considerable spreading of the sedimenting boundary suggested a wide polydispersity of the mucoprotein. Determinations of light-scattering at $\lambda = 5460$ A. (the specific refractive index increment was $dn/dc = 0{\cdot}1680$ per gm./ml.) gave the following results: $M_w = 1{\cdot}98 \times 10^6$; $I_{45}/I_{135} = 2{\cdot}18$; $R_z = 1180$ A. (R_z being the Z-average radius of gyration); the anisotropy was negligible.

I have carried out the double extrapolation (to zero angle and zero concentration) of the Zimm plot obtained in the diagram $P^{-1}(\theta)$ vs. μ^2R^2 ($\mu = \frac{4\pi}{\lambda'} \sin \frac{\theta}{2}$, λ' being the wave-length of light in the solution, θ the angle between the incident and scattered beams) in order to compare the plot with the curves pertaining to spheres, coils and rods, respectively; it is known[2] that the curves relating to polydisperse systems fall below those of monodisperse systems (the latter are shown in Fig. 1).

As is evident from Fig. 1, the sphere model seems to be quite improbable; furthermore, it would require a hydrated specific volume (as deduced from R_z) of $V' = 4{,}450$ ml./gm., which appears to be much too high. A polydisperse system of rods, such as was suggested by Mathews and Lozaityte[3], though not incompatible with the light-scattering results, seems also highly improbable. The length of the rods (as deduced from R_z) would be about 4000 A.; such a system could scarcely fail to exhibit a strong flow birefringence, like, for example, tobacco mosaic virus

(this latter presents an intrinsic streaming birefringence $[\Delta n] = \lim_{\substack{G \to 0 \\ C \to 0}} \frac{\Delta n}{G\eta_0 C} = 5 \times 10^{-4}$); in the case of cartilage mucoprotein, on the contrary, the flow birefringence was negligible ($[\Delta n] \ll 10^{-6}$), a result which is in agreement with the negative results of Webber and Bayley[4] for an analogous product.

The hypothesis of a polydisperse system of coils seems to be the most satisfactory. My light-scattering results also indicate the absence, or at most a very small extent, of branching in the mucoprotein; indeed, Benoit[2] showed that branching increases the general upward curvature of curve 2, whereas my curve 4 exhibits a strong downward curvature. The possibility of a system of non-Gaussian coils, which could also explain such curvature[5], seems to be ruled out by the high value of the ratio L/r (L being the length of the filament and r the root-mean-square end-to-end distance of the coil).

On the assumption of such a polydisperse system of coils, I can, by the method of Sadron and Benoit[6], calculate the approximate value of M_n (strictly speaking, its upper limit); I have found M_n = 480,000; therefore, $M_w/M_n \geqslant 4$, a result which confirms the polydispersity observed in the sedimentation.

In order to check the mutual consistency of my experimental results as well as my conclusions, I applied the following equation proposed by Flory[7]:

$$\Phi^{1/3} P^{-1} = \frac{N s_0 [\eta]^{1/3} \eta_0}{M^{2/3} (1 - \bar{v}\rho)},$$ where $\Phi^{1/3} P^{-1}$ should be a universal constant independent of solvent, temperature and nature of polymer. Introducing in the above equation my values of $s_0, M_n, [\eta]$ and assuming $\bar{v}$ = 0·60 ml./gm., I find $\Phi^{1/3} P^{-1} = 2 \cdot 1 \times 10^6$; the good agreement between this value and the theoretical one seems to confirm the consistency of the experimental results and the validity of the proposed model.

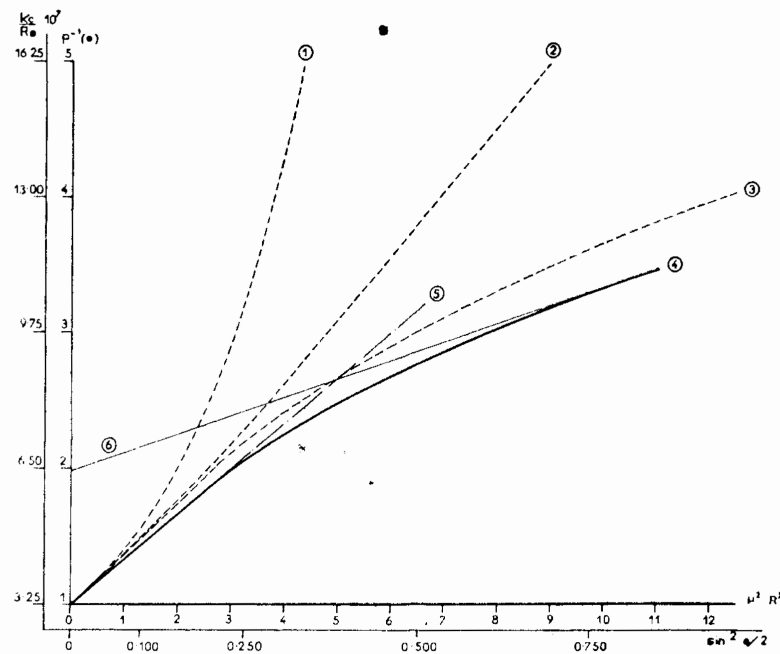

Fig. 1. Curves 1, 2 and 3 relate to monodisperse systems of spheres, coils and rods, respectively. Curve 4 is the Zimm plot extrapolation for $c = 0$, with its tangent at the origin (5) and the asymptote (6)

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Ion Permeability of the Plasmalemma of the Plant Cell

IT has recently been suggested by several workers[1] that the plasmalemma of the plant cell does not constitute a barrier to the diffusion of ions, and that there is a Donnan distribution of ions between the cytoplasm of the plant cell and the extracellular medium. As a corollary, it has been suggested[1c] that the electric resistance and capacity of plant tissue are to be attributed wholly to the properties of the tonoplast and bulk cytoplasm. However, it has been suggested that recent measurements of resistance and capacity on *Nitella* give the properties of the plasmalemma[2]; and a.c. measurements on *Chlorella*[3], which has no significant vacuole, appear to require a similar explanation. Results which I have obtained with cells of *Nitella* indicate that the plasmalemma of the *Nitella* cell acts as a barrier to the inward diffusion of calcium ions, and is responsible for the d.c. resistance of the cell bathed in a solution of potassium, sodium or calcium chloride. Thus if the cytoplasm contributes to the apparent free space for ionic diffusion, it cannot be in the simple way proposed for higher plant tissue[1]. Although one cannot argue from single cells of an alga to pieces of angiosperm tissue, it may be noted that there is evidence from *Nitella* that labelled ions do cross the plasmalemma much more rapidly than the tonoplast[4]; this situation is comparable with that in angiosperm tissue[1c].

Microelectrode experiments on *Nitella*[5] showed that the electric potential difference between cell sap and extracellular medium is located at the plasmalemma, the value being −160 mV. (interior negative) with the cells bathed in 0·1 mM potassium chloride solution. The location of the p.d. and its value were qualitatively in agreement either with the hypothesis of a Donnan distribution across a freely permeable plasmalemma, or with hypotheses involving a semi-permeable membrane. Further experiments by the same method (Walker, N. A., and Cooper, J., unpublished work) have, however, found a p.d. of −160 mV. across the plasmalemma of similar cells bathed in