curve (Fig. 1) it is evident that this change occurs when the number of bound ions exceeds *ca.* 6 per molecule of protein (point B).

Fig. 2(b) shows the curve of reduced viscosity $\eta sp./c$ (where c = protein concentration % w/v) against c, obtained by dilution of a stock solution having a concentration of 1.5 % protein and a molar detergent/protein ratio of 200/1. The reduced viscosity remains constant at 0.189 with decreasing protein concentration but below *ca.* 0.9 %, the reduced viscosity decreases and the curve approaches that obtained for native BPA (YANG AND FOSTER[8]). This indicates dissociation of the protein-detergent complex with decreasing protein concentration, a fact which has been confirmed by light scattering and sedimentation velocity experiments.

It appears from these experiments that DTAB combines less strongly with the protein than does the anionic detergent SDS. This is supported by the facts that less of the DTAB is bound at the same equilibrium molar concentration of detergent, and that the binding of DTAB is dependent upon the protein concentration, a situation which is not found in the BPA-SDS system[9] within a protein concentration range of 0.05 to 0.5 % w/v. However, a similarity between the two detergents is that both are able to act as a "molecular wedge", causing the protein to unfold partially and expose new sites for binding. Thus the interaction isotherm (Fig. 1) may be considered as two isotherms, AB the isotherm for the binding of DTAB onto the native protein (P_n), and BC the isotherm for the binding of DTAB onto partially unfolded protein molecules (P_o). The change from P_n to P_o is substantiated by the changes in relative viscosity which occur with increasing concentrations of DTAB (Fig. 2(a)). Further considerations of the reversibility of the process $P_n \rightleftharpoons P_o$, and other physical measurements on the BPA-DTAB system will be given in a later publication.

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The action of fluorodinitrobenzene on ichthylepidin

Ichthylepidin, a structural protein of fish scales first isolated by MÖRNER[1], is notable for its insolubility in the usual solvents for proteins in spite of a low cystine content[2].

In an attempt to determine the N-terminal amino-acid residues of ichthylepidin from scales of the pilchard (*Sardina ocellata* Jenyns) by SANGER's method[3], we have observed that the action of fluorodinitrobenzene (FDNB) in an aqueous-alcoholic solution of sodium bicarbonate brings about the dissolution of more than half the protein, whereas very little is dissolved in the same mixture without the FDNB, or when this is replaced by chlorodinitrobenzene or dinitrophenol. The experimental conditions were as follows:

A sample of scales (10 g) that had been purified by washing with water and Soxhlet extraction for 24 hours with hexane, was demineralised by soaking in a 0.4 *M* solution of trichloracetic acid (500 ml) for 6 hours at 5°. The protein was 44 % by weight of the original scales on an oven-dry basis. Ichthylepidin was then separated from the soluble gelatin by heating the demineralised scales (2 g) in water (100 ml) at 80° for 2 hours. The ichthylepidin was 23 % by weight of the original scales and heating it for another 2 hours in a fresh volume of water dissolved less than 2 % more protein.

To examine the action of FDNB, samples of ichthylepidin (each of 0.10 g of known moisture content) were added to mixtures containing 0.18 g of 1, 2, 4-fluorodinitrobenzene (from Light and Co. Ltd., Colnbrook, England), 0.10 g of sodium bicarbonate ("Analar" from British Drug Houses Ltd.), 2.0 ml of water, and 4.0 ml of ethyl alcohol. Not all of the bicarbonate dissolved under these con-

ditions. The mixtures were shaken in the dark at 30° and after a few hours the ichthylepidin turned pale yellow and soon started to dissolve. Fig. 1, Curve (A), shows the rate of dissolution, expressed as the percentage decrease in weight of the ichthylepidin. This was obtained by filtering off the undissolved ichthylepidin, washing it well with ether, alcohol, and water, and then drying it to constant weight at 105°. No correction was applied for the increase in weight expected from the addition of dinitrophenyl groups. Curve (B) was obtained similarly excepting that FDNB was omitted from the reaction mixture. No dissolution of ichthylepidin occurred in the same time if the FDNB was replaced by an equivalent amount of either chlorodinitrobenzene or dinitrophenol; in fact there was in each case a slight increase in weight owing to absorption. If the mixtures were left without shaking the rate of dissolution was slower, especially at the beginning, and less protein dissolved — the maximum being about 41%. The rate of dissolution was slower still if alcohol was omitted from the mixtures[4], although about the same amount of protein dissolved. The use of ichthylepidin from scales demineralised by means of hydrochloric acid or sulphurous acid did not appear to affect the action of FDNB.

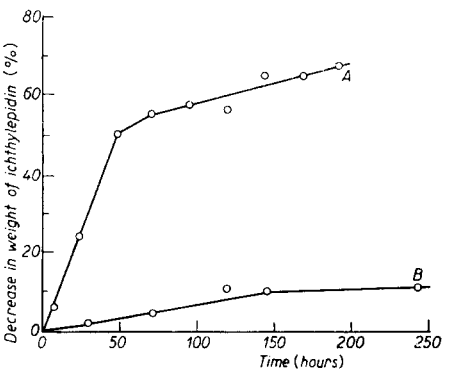

Fig. 1.

The dissolved protein was deep yellow, it could not be precipitated by hydrochloric acid, trichloracetic acid, or ammonium sulphate, and it appeared to be a complex mixture since it could be separated into a number of fractions by chromatography on a talc column[5]. Many of the fractions were of low molecular weight and easily passed through a dialysis membrane.

These results indicate that FDNB is responsible for the dissolution, and probably the degradation, of an insoluble and relatively stable protein. It is known that in bicarbonate solution FDNB can split certain labile peptide bonds in at least two soluble proteins[6,7], but extensive degradation does not appear to have been reported. It should be noted that the period of reaction necessary for maximum dissolution of ichthylepidin was much longer than that usually employed for the dinitrophenylation of soluble proteins, but was comparable to that for insoluble proteins such as collagen[8], and wool[9].

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Specificity of ribonuclease in hydrolyzing cytidine-2′:3′-phosphate

Hydrolysis of ribonucleic acid by crystalline pancreatic ribonuclease[1] has been shown to occur in at least two stages. There is an initial rapid depolymerization of the nucleic acid, accompanied by the formation of cyclic 2′:3′-phosphates of pyrimidine nucleotides, and a subsequent slow hy-