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.

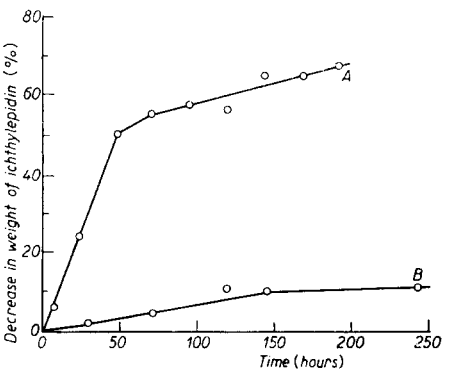

Fig. 1.

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-

drolysis of the cyclic compounds to the free nucleotides or nucleotide groups[2-5]. Ribonuclease also degrades polyribophosphate[6] and apurinic acid[7], and it has been suggested that it is the absence of purines rather than the presence of pyrimidines that determines the specificity of pancreatic ribonuclease[5]. It has been shown, however, that the degradation of apurinic acid by ribonuclease is a nonspecific function of the enzyme, other basic proteins such as lysozyme and cytochrome *c* being equally effective[8]. This, together with the finding that the depolymerizing action of ribonuclease and its action upon the cyclic phosphates do not disappear simultaneously when ribonuclease is hydrolyzed with subtilisin[9], raised the question whether the secondary action of ribonuclease—that is the slow hydrolysis of the cyclic 2′:3′-pyrimidine-phosphates—might not also be a nonspecific property of the enzyme. The data presented here show that this is not the case.

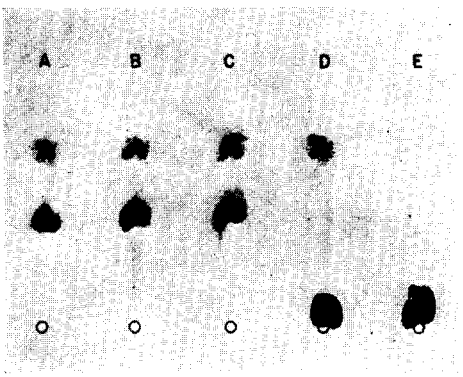

Fig. 1. Ascending chromatogram of cyclic cytidine-2′:3′-phosphate after treatment with (A) distilled water, (B) cytochrome *c*, (C) lysozyme, and (D) ribonuclease; and of (E) cytidilic acid *b*. Spot of origin indicated by circles. Concentration of cyclic phosphate in digestion mixture, 8 mg per ml; concentration of basic protein in digestion mixture, 0.1 mg per ml. Lower spots, cytidilic acid; middle spots, cyclic cytidine-2′:3′-phosphate; upper spots, ammonium cytidine ethyl phosphate, an impurity in the cyclic phosphate.

Mixtures of solutions of cytidine-2′:3′-phosphate with ribonuclease, lysozyme, cytochrome *c*, or water at pH 7.5 were left at 37° C for 24 hours. The cytidine-2′:3′-phosphate had been synthesized from cytidylic acid *b* according to BROWN, MAGRATH AND TODD[10], without removal of the by-product, ammonium cytidine ethyl phosphate. The ribonuclease was a thrice-crystallized preparation[11], and the lysozyme and cytochrome *c* were commercial preparations, the former purchased from the Worthington Biochemical Corporation, the latter from the Sigma Chemical Company. Aliquots were then subjected to ascending paper chromatography according to the procedure of BROWN, DEKKER AND TODD[12], using their *iso*propyl alcohol-ammonia system. As can be seen from Fig. 1—an ultraviolet photograph[13] of a typical chromatogram—only ribonuclease effected conversion of the cyclic phosphate to cytidylic acid. Proteins of similar molecular weight and isoelectric point had no degradative effect on the cyclic phosphate. It appears, therefore, that the hydrolysis of the cyclic 2′:3′-phosphates of pyrimidine nucleotides by ribonuclease is a specific function of the enzyme. LEDOUX has demonstrated[14] that the –SH groups which he finds to be essential for the depolymerizing function of ribonuclease are also essential for its hydrolytic action on cyclic phosphates.

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