We have been unable to demonstrate the uncoupling of phosphorylation and respiration in liver mitochondria isolated from menadione-toxic rats. Further experiments are in progress to investigate the possibility that menadione is lost during the isolation and preparation of the mitochondrial suspension.

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Complex nature of the proteolytic system of the thyroid gland

Crude preparations of thyroid protease were previously shown to hydrolyse N-acetyl-L-phenylalanyl-L-di-iodotyrosine[1]. Further studies have demonstrated that acetone fractionation of hog thyroid extracts, while providing a substantial purification of the protease[2], can also lead to a separation of the peptidase activity mentioned. The fractionation procedure, which will be described in detail elsewhere, utilised Extract II[2] as starting material. The main protease fraction (PRO/B16) used in the experiments cited below separated at higher acetone concentration and assayed at 620 units/mg§, whereas the fraction (PEP/B16) showing strong peptidase activity (substrate: N-acetyl-L-phenylalanyl-L-tyrosine) was only weakly active towards haemoglobin (1.5 protease units/mg) and separated at lower acetone concentration. The difference in behaviour of the two fractions towards various peptides is illustrated in Table I.

TABLE I

COMPARISON OF THE ACTION OF THYROID PEPTIDASE (PEP/B16) AND PROTEASE (PRO/B16) FRACTIONS ON PEPTIDE SUBSTRATES

Incubation: 1 h at 37° C; pH 3.5 (0.1 *M* ammonium acetate).
Estimation: chromatographically (*n*-butanol-acetic acid-water (80:20:20), ninhydrin sprayed).

Peptide (0.05 ml) (0.01 M)	*Peptidase (0.05 ml)*		*Protease (0.05 ml)*	
	Wt (mg)	*Result*	*Units*	*Result*
(I) N-Ac.-L-Phe.-L-Tyr.*	0.086	strong tyrosine spot (approx. 50% hydrolysis)	265	no hydrolysis
(II) L-Phe.-L-Tyr. Amide	0.17	no hydrolysis	100	no hydrolysis
(III) Cbz.-Gly.-L-Phe.	0.20	very weak phenylalanine spot	104	no hydrolysis
(IV) Phth.-Gly.-Gly.	0.17	no hydrolysis	104	no hydrolysis
(V) Gly.-L-Tyr.	0.17	no hydrolysis	104	no hydrolysis
(VI) L-Lys.-L-Tyr.-L-Leu.	0.20	weak leucine spot	100	no hydrolysis
(VII) L-CySH.-L-Tyr.**	0.27	negligible hydrolysis	108	approx. 75% hydrolysis

* This peptide and its di-iodo derivative behaved similarly.
** 0.04 *M*. N-Ethylmaleimide (0.05 *M*; 0.05 ml) added before chromatography[4].

§ One unit is the amount of enzyme required to liberate 10^{-4} milliequiv. tyrosine in 30 minutes at 37° C (haemoglobin substrate; pH 3.5); compare ANSON[3].

Although peptide I (Table I) and its di-iodo derivative were the most sensitive towards fraction PEP/B16, the same weight of certain cruder preparations of the peptidase (on the basis of their higher contents of protease contaminant), while releasing less tyrosine from peptide I, readily hydrolysed peptide III (Cbz.-Gly.-L-Phe.) under comparable conditions. It is, therefore, considered that there are at least two thyroid peptidases of the carboxypeptidase type, both active at pH 3.5.

In addition to peptide VII the protease fraction hydrolysed the tripeptide, L-CySH.-L-Tyr.-L-*iso*Leu., at the cysteinyl link and, to a lesser extent, L-Tyr.-L-CySH. Fission of peptide VII could also be demonstrated at pH 7.4 (phosphate buffer) to about the same degree as at pH 3.5, if oxygen was excluded during incubation. Crystalline pepsin has been reported to hydrolyse peptides of cysteine and tyrosine[5]. However, such peptidase action in the thyroid may not be inherent in the protease molecule. Thus, although 0.2 mg of each of four fractions obtained by electrophoresis of a sample of protease (550 units/mg), by the procedure reported previously[2], hydrolysed cysteinyl-tyrosine to approximately the same extent, the fractions assayed at 1600, 930, 700 and 130 protease units/mg.

The enzyme fractions PRO/B16 and PEP/B16 were also tested on rat thyroglobulin labelled *in vivo* with ^{131}I. Pooled thyroids from 5–7 rats, each of which had been injected 24–42 hours previously with 30 μc carrier-free ^{131}I, were blended with saline, and the supernatant brought to 45% saturation with ammonium sulphate. The solution of the precipitate, after dialysis, was usually heated for 4 minutes at 80° C to reduce proteolytic activity and precipitated with acetone at —5° C (to remove traces of free iodo-amino acids). Aqueous solutions of this precipitate and the enzyme fractions were incubated at 37° C and pH 3.5 for 2 or 16 hours. Chromatograms (*n*-pentanol–propionic acid–water (20:3:15)) and radioautograms were then prepared. The main qualitative difference between the two enzyme fractions so far observed in such experiments is that the peptidase releases more radioactivity in the form of di-iodotyrosine than of mono-iodotyrosine, whereas the reverse holds for the protease.

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Specific incorporation of adenosine-5'-phosphate-^{32}P into ribonucleic acid in rat liver homogenates*

We wish to report the demonstration that a nucleoside-5'-phosphate can be incorporated specifically into ribonucleic acid (RNA) without randomization or exchange of its phosphorus. Previous experiments with whole animals[1] and with tissue slices and cell suspensions[2], had shown that when either 3' or 5' nucleoside monophosphates, labeled with ^{32}P, are employed as precursors, the ^{32}P is separated from the nucleoside and appears randomly distributed among all four nucleotides obtained from the labeled RNA. These results are explained by the impermeability of animal cells to mononucleotides, which are presumably cleaved at the cell membranes[2].

The development of cell-free systems that are capable of incorporating labeled precursors into RNA[3] has provided the tool for testing whether an intact nucleoside-5'-phosphate could be incorporated as such into RNA.

When adenosine-5'-^{32}phosphate (AM^{32}P, obtained from the liver acid-soluble fraction be partially hepatectomized rats treated with inorganic ^{32}P) was incubated with the "cytoplasmic fraction" obtained by centrifuging the nuclei from a 0.25 *M* sucrose homogenate of rat liver under conditions in which oxidative phosphorylation was maintained, radioactivity was incorporated

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