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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into the RNA. Following isolation, the RNA was hydrolyzed with diesterase (prepared according to HURST AND BUTLER[4] from dialyzed* snake venom), which cleaves the internucleotide phosphodiester so as to give the 5'-mononucleotides[5], the radioactivity was concentrated almost entirely in the 5'-AMP obtained from the hydrolysate (Table I). Thus the incubated nucleotide was incorporated into RNA without loss or randomization of its phosphorus, and shows clearly that 5'-nucleotides are the actual intermediates in RNA biosynthesis.

TABLE I

RECOVERED RADIOACTIVITY FROM RNA

	Diesterase hydrolysis (5')	*Alkaline hydrolysis (2' + 3')*
Adenylic acid	92	5.4
Cytidylic acid	0.6	87.4
Uridylic acid	0.5	1.2
Guanylic acid	0.4	1.2
Inorganic phosphorus	6.1	4.8

Rat liver homogenized in 0.25 *M* sucrose, and the supernatant from 600 × *g* centrifugation was used. A 0.5 g equivalent of tissue was incubated in air at 30° C for 40 minutes in a total volume of 5.0 ml including the following concentrations of: 0.01 *M* glutamate, 0.01 *M* pyruvate, 0.004 *M* fumarate, 0.01 *M* phosphate buffer (pH 7.2), 0.01 *M* magnesium chloride, 0.03 *M* fructose, 0.25 *M* sucrose and 1 micromole of AM^{32}P (specific activity, 608,000 c.p.m./μM)[4]. Four flasks were used and pooled. Acid-soluble fractions[6] and RNA[7] were isolated, enzymic and alkaline hydrolyses were carried out[2], and the nucleotides isolated by gradient elution chromatography on Dowex-1[7]. The uridylic acid was separated from contaminating inorganic phosphate by adsorption on charcoal, washing with water, and elution with 10% pyridine.

When the same sample of RNA was hydrolyzed with alkali, which cleaves the internucleotide phospho diester to give a mixture of the mononucleotide-2' and 3'-phosphates, the radioactivity was localized almost entirely in the cytidylic acid, and very little was found in the other three nucleotides (Table I). These results suggest that in the particular RNA that was labeled under these conditions, adenosine is adjacent to cytidine, with the radioactive phosphate attached to the 5'-carbon of the adenosine and the 3'-carbon of the cytidine.

GOLDWASSER[8] has very recently reported the specific incorporation of ^{14}C-adenine-labeled AMP into the adenylic acid, obtained by alkaline hydrolysis of RNA, in a pigeon liver homogenate. However, in a similar experiment using ^{32}P-labeled AMP, he found the label to be distributed among all four nucleotides resulting from alkaline hydrolysis, with the radioactivity in adenylic acid below the average and in uridylic acid, above the average. Our results are also complementary to the elegant work of GRUNBERG-MANAGO, ORTIZ, AND OCHOA[9], who have obtained RNA-like polynucleotides by incubation of nucleoside-5'-diphosphates with a polynucleotide phosphorylase obtained from *Azotobacter vinelandii*. In our case, ADP may be the actual intermediate, with the ^{32}P of the AMP actually incorporated into the RNA since the system produced ADP and ATP and the original inorganic phosphorus was unlabeled.

Similar experiments with other nucleotides are being continued and a more detailed report will be published elsewhere.

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