of the side chain and that there are hydroxyl groups on the two carbon atoms adjacent to the indole ring. On the basis of the data presented the intermediate is identified as indole-3-glycerol phosphate.

Neither indole-3-glycerol phosphate nor indole-3-glycerol (prepared by treating indole-3-glycerol phosphate with intestinal phosphatase) support the growth of mutant strains of *E. coli* capable of responding to anthranilic acid or indole. However, indole-3-glycerol (identified on the basis of R_F values in several solvent systems) is apparently accumulated by several tryptophan-requiring mutants of *E. coli*[4,7] suggesting that indole-3-glycerol phosphate is a normal intermediate in indole synthesis. The inability of indole-3-glycerol phosphate to support growth could be ascribed to impermeability while indole-3-glycerol may not be rephosphorylated and, therefore, not utilized as an indole precursor.

In addition to providing evidence for the proposed biosynthetic pathway of indole synthesis, the isolation of indole-3-glycerol phosphate suggests the possibility that tryptophan may be synthesized in some microorganisms by a mechanism other than the coupling of indole and serine. Studies on histidine biosynthesis have demonstrated that in the synthesis of this amino acid an alanine side chain is formed from a glycerol phosphate side chain[8]. If an analogous sequence of reactions were involved in tryptophan synthesis, tryptophan could be formed without indole serving as an obligatory intermediate.

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[1] J. F. NYC, H. K. MITCHELL, E. LEIFER AND W. H. LANGHAM, *J. Biol. Chem.*, 179 (1949) 783.
[2] C. YANOFSKY, *J. Biol. Chem.*, 217 (1955) 345.
[3] C. YANOFSKY, *Biochim. Biophys. Acta*, 16 (1955) 594.
[4] C. YANOFSKY, manuscript in preparation.
[5] S. A. GORDON AND R. P. WEBER, *Plant Physiol.*, 26 (1951) 192.
[6] W. S. BECK, *J. Biol. Chem.*, 212 (1955) 847.
[7] A similar accumulated compound, which may be indole-3-glycerol, has been described by J. S. GOTS, W. Y. KOH AND G. R. HUNT, *J. Gen. Microbiol.*, 11 (1954) 7 and by S. BRENNER, *Proc. Natl. Acad. Sci. U.S.*, 41 (1955) 862.
[8] B. N. AMES AND H. K. MITCHELL, *J. Biol. Chem.*, 212 (1955) 687.

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Incorporation of pyrimidine precursors into ribonucleic acid in a cell-free fraction of rat liver homogenate*, **

The development of cell-free systems for the biosynthesis of nucleic acids was successfully undertaken in this laboratory as soon as it became clear[1] that all of the ribonucleotides exist in animal tissues in the form of the 5′ mono-, di- and triphosphates.

The recent demonstration of a net synthesis of an RNA-type of polynucleotide by a bacterial extract[2,3] and the incorporation of labeled adenosine monophosphate into the RNA of pigeon liver homogenates[4], together with the earlier report that rat liver homogenates using adenine were inactive[5] prompts the present report in which rat liver "cytoplasmic fraction" is shown to label the uridine moiety of RNA when incubated with ^{14}C labeled orotic acid or 5′-UMP. The accompanying report deals with the utilization of the system for the incorporation of 5′-AM^{32}P into RNA[6]. The addition of orotic acid-6-^{14}C to systems that label the uridine moiety of RNA also results in the extensive labeling of all of the acid-soluble uridine nucleotides of the 5′ phosphate series, but does not label added pools of 2′(3′) UMP, 2′(3′) CMP or of 5′-CMP. The latter fact is paralleled by the fact that the cytidine moiety of RNA is unlabeled in these experiments. Previous studies have established the interactions between the uridine phosphates and the adenosine phosphates in these systems[7]. In the present experiment the mitochondria were used to regenerate ATP, and oxidative substrates were added, but the mitochondria can be omitted if glycolytic components or phosphoglyceric acid are added. The addition of 5′ UMP (biosynthetically labeled from orotic-6-^{14}C in separate experiments) to the reaction mixture also results in labeling of the various acid-soluble uridine nucleotides and of the uridine moiety of RNA.

In order to show that the RNA contained ^{14}C labeled nucleotides in nucleic acid linkages,

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** Abbreviations as follows: ribonucleic acid, RNA; uridine-5′-monophosphate, 5′-UMP; adenosine triphosphate, ATP; uridine 2′(3′) monophosphates (mixture), 2′(3′) UMP; cytidine-5′-monophosphate, 5′-CMP.

the isolated RNA[8] was subjected to alkaline hydrolysis yielding 2'(3') mononucleotides which were separated chromatographically[9]. Pools of non-radioactive 5' UMP and orotic acid were added to the RNA hydrolysate before chromatography to localize any radioactivity arising from contamination by these substances, but they remained unlabeled. The results (Table I) show that the 2'(3') uridylic acid obtained from the RNA was labeled and it may be concluded that an acid-soluble 5'-nucleotide, or a derivative thereof was incorporated into a diester linkage in a molecule with the properties of RNA. The reaction probably results in the labeling of at least one type of RNA molecule. These conclusions are further supported by the studies with diesterase reported herewith[6]. In this reaction system, the specific activity of the RNA begins to decline after 45 minutes incubation and the proximal precursor is unknown. The studies by OCHOA *et al.*[2,3] offer possible explanations which are being tested in both instances.

TABLE I

RADIOACTIVITY OF NUCLEOTIDES OBTAINED BY ALKALINE HYDROLYSIS OF CYTOPLASMIC RNA

A 20% homogenate of rat liver in 0.25 *M* sucrose was centrifuged 10' at 600 *g* and the pellet was washed once with an equal volume of sucrose which was then combined with the first supernatant fraction to give the "cytoplasmic fraction". Fifteen ml of this preparation were mixed with an equal volume of a reaction supplement consisting of sucrose, 0.25 *M*, fumarate, 0.008 *M*, pyruvate and glutamate, 0.02 *M* each, $MgCl_2$, 0.006 *M*, and inorganic phosphate 0.02 *M*. All acidic compounds were in the form of the potassium salts and the supplement was adjusted to pH 7.2, aft r the orotic acid or 5' UMP were added. All components were at 0° until the incubation at 30° was begun, with air in the gas phase. Supplements of ATP, fructose and ribose-5-phosphate had no beneficial effect and ATP inhibited the incorporation. The reaction was stopped with perchloric acid at 20'.

Added precursor	*Acid-soluble fraction*		*RNA*
	cpm/μM precursor	*cpm/μA 5' UPM**	*cpm/μM 2' (3') UMP***
3 μ*M* Orotic acid-6-^{14}C	$1.5 \cdot 10^6$	342,000	88
25 μ*M* 5' UMP-4-^{14}C	$0.1 \cdot 10^6$	70,000	53

* Dilution of added precursor results from endogenous 5' UMP and equilibrating metabolites.
** The radioactivity of the other 3 nucleotides and of added 5' UMP and orotic acid was negligible.

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1 H. SCHMITZ, R. B. HURLBERT AND V. R. POTTER, *J. Biol. Chem.*, 209 (1954) 41.
2 M. GRUNBERG-MANAGO AND S. OCHOA, *J. Am. Chem. Soc.*, 77 (1955) 3165.
3 M. GRUNBERG-MANAGO, P. J. ORTIZ AND S. OCHOA, *Science*, 122 (1955) 907.
4 E. GOLDWASSER, *J. Am. Chem. Soc.*, 77 (1955) 6083.
5 E. GOLDWASSER, *J. Biol. Chem.*, 202 (1953) 751.
6 C. HEIDELBERGER, E. HARBERS, K. C. LEIBMAN, Y. TAKAGI AND V. R. POTTER, *Biochim. Biophys. Acta*, 20 (1956) 445.
7 E. HERBERT, Y. TAKAGI AND V. R. POTTER, *J. Biol. Chem.*, 213 (1955) 923.
8 R. B. HURLBERT AND V. R. POTTER, *J. Biol. Chem.*, 195 (1955) 257.
9 H. SCHMITZ, V. R. POTTER, R. B. HURLBERT AND D. M. WHITE, *Cancer Research*, 14 (1955) 66.

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Effects of 2:4-dinitrophenol and other agents on the nucleoside triphosphatase activities of L-myosin

Recent work has shown that DNP*[1,2] and PMA[1] increase the ATPase and decrease the ITPase velocity of Ca^{++}-activated L-myosin. We now report observations on other nucleoside-5'-triphosphatase activities of this enzyme.

Thrice-precipitated L-myosin was prepared from rabbit muscle[3]. Activity was measured by incubating the enzyme (0.10 mg/ml) for 5 min at 25° with 0.05 *M* aminotris(hydroxymethyl)-methane chloride, pH 7.5, 2 m*M* substrate, and other additions as noted below. Unless otherwise

* Abbreviations: ATP, CTP, GTP, ITP, UTP, adenosine, cytidine, guanosine, inosine and uridine 5'-triphosphates; DNP, 2:4-dinitrophenol; EDTA, ethylenediaminetetraacetic acid; PMA, phenylmercuric acetate.