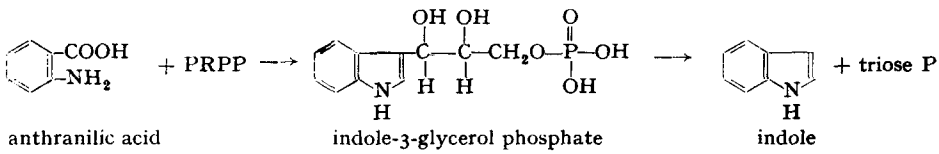
Indole-3-glycerol phosphate, an intermediate in the biosynthesis of indole*

Previous studies on the conversion of anthranilic acid to indole have shown that the carboxyl carbon of anthranilic acid is lost during this conversion[1,2] and that two carbon atoms are added to complete the pyrrole ring of indole[2]. The results of isotope experiments indicated that the two added carbon atoms, the eventual C-2 and C-3 of indole, are derived respectively from C-1 and C-2 of ribose or a ribose derivative[2]. 5-phosphoribosyl-1-pyrophosphate (PRPP) has been implicated as the actual donor of the two carbon atoms on the basis of studies with extracts of *E. coli*[3]. These studies have further suggested that the ribose-5-phosphate moiety of PRPP may add directly to the amino group of anthranilic acid in the biosynthesis of indole. The isolation of indole-3-glycerol phosphate, described below, supports this view. On the basis of the data presented here the following scheme of indole synthesis from anthranilic acid is proposed:



anthranilic acid — indole-3-glycerol phosphate — indole

In previous studies it was shown that the conversion of anthranilic acid to indole is catalyzed by cell-free preparations of *E. coli*[3]. Ammonium sulfate fractionation of such preparations has provided two separate protein fractions, arbitrarily designated A and B, which catalyze different reactions in the transformation of anthranilic acid to indole[4]. Fraction A, in the presence of PRPP and Mg^{++} ions, converts anthranilic acid to a phosphorylated compound which appears to be an intermediate in indole synthesis. This intermediate is converted to indole by Fraction B. The intermediate formed by Fraction A has been isolated by the following steps: adsorption and elution from Darco G-60, gradient elution from a Dowex-1 chloride column, adsorption and elution from Darco G-60 and precipitation as the barium salt by the addition of Ba^{++} ions, alcohol and acetone to an aqueous solution of the intermediate. During purification, the intermediate was assayed colorimetrically with a modified Salkowski reagent for indole acetic acid[5] or by enzymic conversion to indole with Fraction B. C, H, N and P analyses of the isolated intermediate gave the following results:

	C	H	N	P
Found**	29.13	3.51	3.16	6.8
Calculated for $C_{11}H_{12}O_6NPBa.2H_2O$	28.81	3.52	3.05	6.76

The absorption spectrum of the intermediate is very similar, but not identical with that of indole. The compound yields 0.9–0.95 moles of indole per mole of P on treatment with enzyme Fraction B. When heated with dilute alkali the intermediate is cleaved to form indole (isolated as the picrate and identified by melting point and mixed melting point determinations); in dilute acid solution at room temperature the intermediate is rapidly destroyed. Isotope experiments carried out with carboxyl-labeled anthranilic acid*** and with PRPP*** labeled with ^{32}P in the 5-position indicate that the intermediate formed by Fraction A does not contain the carboxyl carbon of anthranilic acid, but does contain the P from the 5-position of PRPP. When treated with enzyme Fraction B the intermediate yields, in addition to indole, a compound which gives a 2,4-dinitrophenylosazone with the same absorption spectrum as the dinitrophenylosazone formed by the triose phosphates[6] (the bis-dinitrophenylosazone of methylglyoxal). The phosphate group of the intermediate is not hydrolyzed by 1 *N* alkali at room temperature; however, following treatment with enzyme Fraction B the organic phosphate present in the reaction mixture becomes alkali labile. These findings suggest that the intermediate is cleaved by Fraction B to yield indole and triose phosphate. When treated with 0.1 *N* sodium metaperiodate for 20 minutes at room temperature the intermediate is quantitatively converted to indole-3-aldehyde (identified by melting point and mixed melting point determinations and by the characteristic absorption spectrum of this compound). This indicates that the phosphate group of the intermediate is on the terminal carbon

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** The C, H and N analyses were performed by the Huffman Microanalytical Laboratories, Wheatridge, Colorado.

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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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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.