In view of WEISS's claims[5] as to the intracellular distribution of protease and peptidase his procedure[5,6] was repeated, using hog thyroids, to ascertain whether the particulate fractions provided better starting materials for the purification of the enzymes. In addition, the microsomal fraction was sedimented from the nuclear and mitochondrial supernatant by centrifuging for 1 hour at 60,000 *g* (mid point of cup). The results of the enzyme assays are given in Table III.

These results confirm essentially the findings of WEISS as regards the distribution of peptidase; in the case of the protease activity, however, this was present to a greater extent in the supernatant from the combined nuclear and mitochondrial fraction. It should be pointed out that such results may bear no relationship to the enzymic distribution *in situ*; the implication may be that during the blending and centrifuging of the cellular material the two enzymes have become selectively adsorbed on the large aggregate surfaces of the particles from which they can then be eluted. No explanation can be offered for the discrepancy between the protease activity value of Fraction II and the combined values of Fractions IIa and IIb (Table III).

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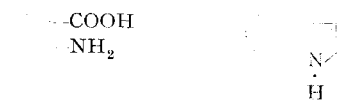
The participation of ribose derivatives in the conversion of anthranilic acid to indole by extracts of *Escherichia coli**

Although the mechanism of tryptophan formation from indole is understood in both *Escherichia coli*[1] and *Neurospora crassa*[2–5], comparatively little is known about the conversion of anthranilic acid to indole in these organisms. The available evidence indicates that the amino nitrogen of anthranilic acid is retained during indole formation (shown with *Neurospora*[6] while the carboxyl carbon is lost (in both *E. coli*[7] and *Neurospora*[8]). Loss of the carboxyl carbon of anthranilic acid necessitates the addition of two carbon atoms to anthranilic acid to form indole. The present experiments with extracts of a mutant strain of *E. coli* demonstrate that these two carbon atoms may be derived from ribose or ribose derivatives.



anthranilic acid indole

Extracts were prepared from a tryptophan auxotroph of the K-12 strain of *E. coli*. The tryptophan requirement of this mutant is also satisfied by anthranilic acid or indole. For the experiments reported here, this strain was grown on a minimal medium[9] supplemented with anthranilic acid. The cells were collected by centrifugation, washed, and subjected to sonic oscillation. The sonic extracts were centrifuged for 20 minutes at 60,000 × *g* in a Spinco ultracentrifuge. The clear supernatant solutions were dialyzed against 0.02 *M* phosphate buffer at pH 7.8 before use.

Dialyzed extracts of the mutant, as can be seen in Table I, readily convert anthranilic acid to indole in the presence of the proper supplements. Both ribose and ribose-5-phosphate (R-5-P) serve as excellent sources of the two carbon atoms needed for indole synthesis from anthranilic acid[10]. However, neither compound is effective in the absence of ATP. The results of a preincubation experiment with potato apyrase[11] (Table II) also indicate that ATP is required for indole synthesis when R-5-P is employed as carbon donor. ATP apparently is not required when 5-phosphoriboxyl-

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pyrophosphate (PRPP) is the source of the two carbons needed to convert anthranilic acid to indole[12] (Table II). These data are interpreted as indicating that PRPP is a more direct precursor of the 2-carbon unit than R-5-P. If PRPP were directly involved in the conversion of anthranilic acid

TABLE I

Supplements	*μM indole formed*
none	0
ATP	0
ribose	0
R-5-P	0.014
ribose + ATP	0.066
R-5-P + ATP	0.195

TABLE II

Supplements	*μM indole formed*	
	preincubated with apyrase	*preincubated without apyrase*
R-5-P	0	0.007
R-5-P + ATP	0	0.056
PRPP	0.029§	0.068
PRPP + ATP	0.037§	0.071

Each tube contained the following in a final volume of 1 ml: 0.5 ml of coli extract, 0.1 ml of 1 *M* tris buffer at pH 7.8, 0.22 μM of anthranilic acid, 50 μM of KCl, 20 μM of hydroxylamine hydrochloride and 1 μM of $MgSO_4$. ATP (1.3 μM), ribose (30 μM), and R-5-P (2.6 μM) were added as indicated in the table. Incubation was at 37° C for 30 minutes. The hydroxylamine was added to prevent the conversion of any of the indole formed to tryptophan.

The supplements indicated in the table were preincubated at 37° with or without potato apyrase. Each tube also contained 40 μM of tris buffer at pH 7.8 and 1 μM of $MgSO_4$. After 15 minutes the tubes were chilled and a mixture containing 0.22 μM of anthranilic acid, 2 μM of hydroxylamine hydrochloride, 40 μM of KCl, 60 μM of tris buffer and 0.3 ml of coli extract was added to each tube. The tubes were reincubated at 37° for 15 minutes. The final volume in each tube was 0.85 ml. R-5-P (0.25 μM), ATP (0.6 μM) and PRPP (0.25 μM) were added as indicated in the table.

§ The presence of apyrase appears to prevent the rephosphorylation of a product of the action of *E. coli* extracts on PRPP. This product may be R-5-P.

to indole it would be reasonable to assume that a reaction analogous to that reported for nucleotide synthesis[13] occurs in indole formation. This reaction would consist of the transfer of the R-5-P moiety of PRPP to the amino group of anthranilic acid to form a ribotide. It is also conceivable, however, that a ribotide is not formed during indole synthesis but that a 2-carbon unit derived from an active form or derivative of ribose is transferred to the amino group of anthranilic acid. Whether or not a two carbon unit is formed from PRPP prior to condensation with anthranilic acid is the subject of present studies. Consistent with both possibilities is the finding[7] that in *E. coli* extracts, C_1 of ribose-1-^{14}C is converted to the carbon atom of indole adjacent to the pyrrole nitrogen. The possible involvement of reactions of the hexose monophosphate shunt mechanism in the formation of the two carbon atoms in question will be discussed in a separate publication[7].

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