retreat must sustain heavy predation. Furthermore there is some evidence that the proper feeding reactions of an individual are dependent on its possession of a home range and associated shelter site. Much additional work is called for in the study of territorialism in the amphibians. It would be particularly interesting to determine what specific reactions are involved in a defense by intimidation when there is little or no display apparent.

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On the Conversion of Anthranilic Acid to Indole

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Isotope studies on the utilization of anthranilic acid as a precursor of tryptophan in *Neurospora* have indicated that during the conversion of anthranilic acid to indole the carboxyl group is lost (*1*) and the amino group is retained (*2*). Somewhat different conclusions concerning indole synthesis in *Escherichia coli* have been arrived at by Bergmann *et al.* (*3*) on the basis of inhibition analyses. These workers suggest that in *E. coli* the carboxyl carbon of anthranilic acid is retained during conversion to indole.

The question of whether or not the carboxyl carbon of anthranilic acid is lost in *E. coli* has recently been examined in tracer experiments performed with this organism. The results obtained (*4*) showed conclusively that in *E. coli,* as in *Neurospora,* the carboxyl group of anthranilic acid is removed during the formation of indole. In the synthesis of indole from anthranilic acid, therefore, two carbon atoms must be added to form the pyrrole ring of indole. Since the carboxyl group of anthranilic acid is removed during this process, the pyrrole ring of indole could be formed through either the 1- or the 3-position of the benzene ring of anthranilic acid. The results of an experiment designed to distinguish between these possibilities are presented in this paper (*5*).

Washed cell suspensions of certain tryptophan auxotrophs of *E. coli* readily convert anthranilic acid to indole, and cell-free extracts of such cells also carry out this conversion (*4*). 4-Methylanthranilic acid can be substituted for anthranilic acid, in which case a methylindole is formed. If ring formation in the production of methylindole from 4-methylanthranilic acid occurred through the 1-position of the benzene ring, the product of the reaction should be 6-methylindole (Fig. 1). If, however, the pyrrole ring is formed through the 3-position, 4-methylindole should result. If the ring could be formed through either position, as, for example, if aniline were the product of anthranilic acid decarboxylation, then a mixture of 4- and 6-methylindole might be produced. Thus identification of the methylindole formed from 4-methylanthranilic acid would permit distinguishing between the various possibilities.

Washed cell suspensions of a tryptophan auxotroph of *E. coli* were incubated (with shaking) in the presence of 4-methylanthranilic acid (40 μg/ml), glucose (2mg/ml) and hydroxylamine hydrochloride (200 μg/ml) (*6*). After a 30-min incubation period at 37°C an additional portion of hydroxylamine hydrochloride (200 μg/ml) was added. After 60 min, incubation was stopped and the cells were removed by centrifugation. A few milliliters of alkali were added to the supernatant solution (enough to make it slightly alkaline), which was then extracted with several portions of ether. The ether extracts were combined and concentrated to a few milliliters. Approximately 5 ml of 0.1*N* NaOH were then added to the ether extract and the mixture distilled in vacuum until less than 1 ml of solution remained. At this point the ether-free distillate contained all the methylindole.

The quantity present was determined colorimetrically with Ehrlich's reagent, using 6-methylindole as standard. The distillate assayed 10.2 mg methylindole (assuming that it was 6-methylindole). Picric acid (100 mg) was added to the distillate and the picrate that formed was collected by filtration; 83 percent of the methylindole was recovered as the picrate. The picrate melted (melting points are uncorrected) at 156 to 160°C. The melting point of the picrate of an authentic sample of 6-methylindole was 159 to 161°C. The melting point of a mixture of the two picrates was 158 to 160°C. Reported melting points for the 4- and 6-methylindole picrates are 194 to 195° (*7*) and 157°C (*8*), respectively. The infrared spectra of the isolated material and authentic 6-methylindole picrate were determined by H. Wasserman of Yale University. Comparison of the spectra indicated that the two samples were identical. Since the isolated material is 6-methylindole, ring formation must occur through the 1-position of the benzene ring of 4-methylanthranilic acid (*9*).

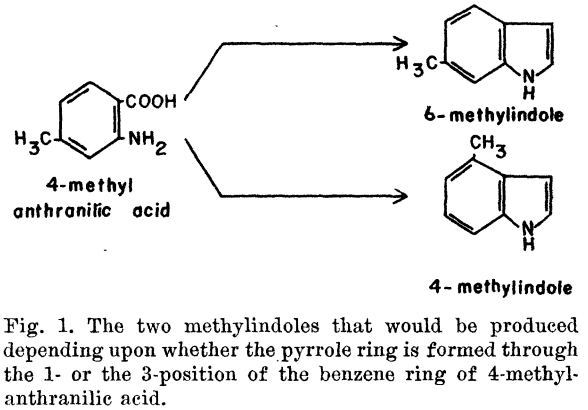

Fig. 1. The two methylindoles that would be produced depending upon whether the pyrrole ring is formed through the 1- or the 3-position of the benzene ring of 4-methylanthranilic acid.

During the conversion of anthranilic acid to indole, therefore, it seems probable that the pyrrole ring is also formed through the 1-position of the benzene ring.

References and Notes

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5. This investigation was supported in part by the Atomic Energy Commission under contract No. AT(30-1) 1017.

6. Hydroxylamine hydrochloride inhibits tryptophan synthetase and thus prevents the further metabolism of the indole derivative.
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Paper Electrophoresis of Steroid Derivatives

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The preparation of pharmaceutical mixtures of estrogens, androgens, and progesterone in various concentrations and ratios has become a common practice. Owing to the lack of specificity of the available chemical and biological tests for the individual steroids in mixtures, there was a need for a technique that could be used in the identification of these compounds. A number of excellent publications have appeared on the use of partition and adsorption chromatography for the separation of the steroids, which may be used in conjunction with the technique described here. These papers have been referred to by Lieberman (*1*) and summarized by Block (*2*).

The steroids are insoluble in aqueous buffers and do not possess any appreciable charge; thus they do not lend themselves readily to a paper electrophoretic separation. Paul and Durrum (*3*) attempted to overcome the solubility problem by using nonaqueous solvents instead of an aqueous buffer. In spite of the low conductivity of such a system, they did find that the steroids moved toward the anode but failed to separate under the conditions employed. Voigt and Beckmann (*4*) esterified the steroids with succinic anhydride and were able to move dehydroandrosterone acetate, desoxycorticosterone, and dehydroisoandrosterone and to separate the last two by paper electrophoresis. Only desoxycorticosterone moved as one band, indicating that more than one product had formed during esterification. These authors also used their technique in a study of the neutral ketonic fraction in urine (*5*). They formed the hydrazones of the ketosteroids with Girard's reagent T (trimethylacethydrazide ammonium chloride) and then esterified this fraction. They do not mention any attempt to separate the hydrazones that would eliminate the esterification process.

In the present study, the hydrazones were prepared by refluxing 2.5 mg of the steroid with 7.0 mg of Girard's reagent in 2 ml of 10-percent acetic acid in methanol for 2 hr in a manner similar to that described by Zaffaroni (*6*). The paper electrophoresis apparatus and the technique of applying the samples to the paper were the same as those that I used in a study of serum lipoproteins (*7*). Several solvent systems were investigated, and the most useful one consisted of a 0.05*M* solution of sodium borate. The hydrazones were detected by viewing the completely dried electropherogram under a quartz-mercury lamp followed by dipping the papers into a solution of the Kraut-Dragondorff reagent (*2*).

The degree of separation obtained for a mixture of progesterone, testosterone, and estrone is shown Fig. 1. The excess Girard reagent has moved off the paper into the cathodic compartment ahead of the larger hydrazone molecules. The dihydrazone of progesterone that possesses two positively charged quaternary groups moves ahead of the monohydrazones that possess only one quaternary group. The monohydrazones of testosterone and estrone move at the same rate in either an acetate buffer at a *p*H of 4.5 or a diethylbarbiturate buffer at a *p*H of 8.6, but the hydrazone of estrone is less mobile than the hydrazone of testo-

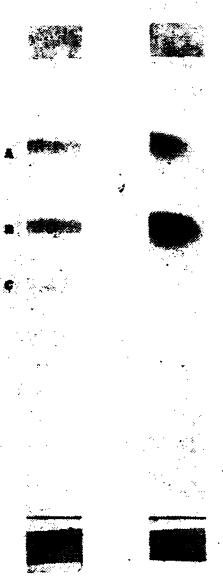

Fig. 1. The electropherogram on the left shows the hydrazones of progesterone (*A*), testosterone (*B*), and estrone (*C*). The electropherogram on the right shows the hydrazones of progesterone and testosterone. The progesterone being the most mobile has moved the greatest distance from the line of application (pencil line on the electropherogram). These electropherograms were run in 0.05*M* sodium borate for 18 hr at a potential of 200 v and a current of 1.5 ma/in. width of paper.