chaeta number. The results are also illustrated in Fig. 1. The change of selection system, of course, reduced the difference of mean between the two halves of the population. But a large difference has been retained. There can therefore be no doubt that a considerable difference could be maintained under random mating.

These selection experiments therefore demonstrate that it is in principle possible for ecotypes or biological races to diverge under the divergent selection pressures that might be imposed in heterogeneous habitats. The concept that random mating must "swamp" the genetic differences involved is not sound. Isolation barriers may be involved in much, even most, evolutionary divergence, but they are not a prerequisite of such divergence (*4*).

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Enzymatic O-Methylation of N-Acetylserotonin to Melatonin

Abstract. An enzyme, hydroxyindole-O-methyl transferase, that can transfer the methyl group of S-adenosylmethionine to the hydroxy group of N-acetylserotonin to form the hormone melatonin is described. This enzyme, which is highly localized in the pineal gland, also O-methylates serotonin.

Recently Lerner and co-workers (*1*) isolated a new hormone, melatonin (N-acetyl-5-methoxytryptamine), from the pineal gland and peripheral nerves of man, monkey, and cow. This compound was found to lighten the color of frog melanocytes and block the actions of the melanocyte-stimulating and adrenocorticotropic hormones (*1*). McIsaac and Page have recently shown that serotonin (5-hydroxytryptamine) is converted to N-acetylserotonin in vivo (*2*). We wish to report the isolation of an enzyme that forms melatonin by the O-methylation of N-acetylserotonin.

Table 1. Enzymatic O-methylation of N-acetylserotonin to melatonin. The soluble supernatant fraction obtained from 16 mg of cow pineal gland was incubated at 37°C with 0.1 μmole of N-acetylserotonin, 100 μmoles of phosphate buffer (*p*H 8.0), and 0.1 μmole of S-adenosylmethionine. After 2 hours' incubation melatonin was determined in the incubation mixture (*4*).

System	Melatonin formed (mμmoles)
Complete system	11
S-adenosylmethionine omitted	0

Since melatonin was found to be highly localized in the pineal gland (*1*), this tissue was examined for the presence of an enzyme that could O-methylate hydroxyindoles. Pineal glands from cows (*3*) were homogenized with ice-cold isotonic potassium chloride and centrifuged at 78,000*g*. The resulting soluble supernatant fraction was incubated with N-acetylserotonin and S-adenosylmethionine at *p*H 8.0. After a 2-hour incubation at 37°C the reaction product was extracted from the incubation mixture with chloroform and the organic phase was washed with water to remove residual substrate. The chloroform extract was then evaporated to dryness in a stream of warm air and the residue taken up in 3*N* HCl. A fluorescent metabolite was found to be present in the acid extract with a maximum fluorescent peak in 3*N* HCl at 540 mμ upon activation at 310 mμ; this is characteristic of 5-hydroxy- and 5-methoxyindoles. This metabolite had the same fluorescent spectrum, the same R_f values in butanol, acetic acid, and water (100 : 35 : 70) (0.91) and in N-propanol and 1*N* ammonia (5 : 1) (0.89), and the same color reactions and partition coefficient as authentic melatonin. When S-adenosylmethionine was omitted from the incubation mixture, no melatonin was formed (Table 1). These observations demonstrate the existence of an enzyme (hydroxyindole-O-methyl transferase) that can transfer the methyl group of S-adenosylmethionine to the hydroxy group of N-acetylserotonin. The reaction is shown in Fig. 1.

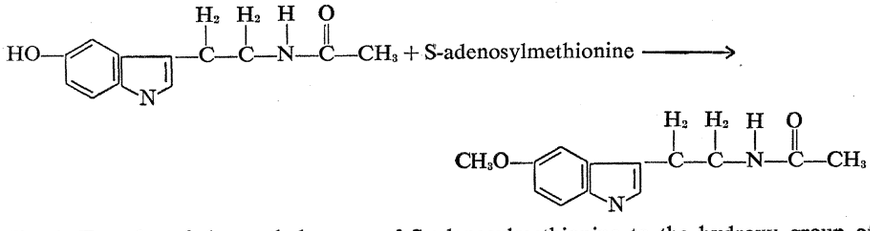

Fig. 1. Transfer of the methyl group of S-adenosylmethionine to the hydroxy group of N-acetylserotonin.

Hydroxyindole-O-methyl transferase has been purified about 20-fold from beef pineal gland by heat treatment, ammonium sulfate fractionation, and adsorption and elution from alumina Cγ gel (*4*). Unlike catechol-O-methyl transferase (*5*), the enzyme has no requirement for Mg^{++}. It could not be detected in liver and kidney of a number of mammalian species, but was found in the pineal gland of the monkey (*4*). The lack of the requirement for Mg^{++} and the unique localization of hydroxyindole-O-methyl transferase indicates that it is different from catechol-O-methyl transferase (*5*) and the other known transferases (*6, 7*).

Incubation of serotonin with hydroxyindole-O-methyl transferase and S-adenosylmethionine resulted in the formation of a product having the characteristics of authentic 5-methoxyserotonin. However, the rate of O-methylation of serotonin was only one-tenth that of N-acetylserotonin (*4*). This finding suggests that acetylation precedes O-methylation in the formation of melatonin as follows:

Serotonin ⟶ N-acetylserotonin ⟶ melatonin

From the results described in this report and elsewhere, it is becoming increasingly apparent that O- and N-methyltransferases (*5–7*) requiring S-adenosylmethionine are playing key roles in the biosynthesis and inactivation of biologically active amines and their derivatives (*8*).

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3. We are greatly indebted to Dr. A. B. Lerner for supplying us with beef pineal glands and melatonin.
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