turnal variation of plasma iron in these subjects.

Summary. A standard dose of ACTH was administered intravenously for 8 hours to normal volunteers. Plasma iron levels were not affected in any way that could not be accounted for by normal diurnal-nocturnal or day to day variation. Altering normal daily periodicity of corticosteroid concentration by administering ACTH at different phases of the cycle did not affect the curves of plasma iron variation.

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Tryptophan Oxidation by Yellow Mouse Skin. (24899)

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There has been for some time a good deal of evidence that tryptophan is the source of intermediates in production of pigments in insects (See 1, 2, for reviews), but in mammals, tryptophan has been suggested as a possible precursor of pigments only recently(3). An interesting example was discovered by Foster(4), while studying pigment formation by skins of a strain of mice with yellow fur due to dominant gene, A^y/a. Foster found that skin powders from these mice showed very little ability to oxidize tyrosine, the usual precursor of melanin, but vigorously oxidized tryptophan with production of yellow pigment. While in insects, tryptophan appears to lead to pigments by way of kynurenine(1,2), the pathway recently suggested for mammals and possibly other animals(3), is quite different, namely *via* a 5-hydroxyindole compound. No experiments have been reported to determine which, if either, of these alternative routes may be followed in skins of A^y/a mice. In addition, it is of interest to determine whether or not kynurenine is formed by these skins, since Knox(5) studied a number of tissues from several species, including rat, rabbit, and guinea pig, and reported conversion of tryptophan to kynurenine only in the liver. The experiments reported here indicate that kynurenine is not utilized in pigment formation by skins of A^y/a mice, and that it is probable that a hydroxyindole is an intermediate.

Materials and methods. Mice, obtained from Jackson Memorial Laboratories were A^y/a females, from various matings with pa/pa individuals, and males of a black inbred strain, C57Bl/6. Grüneberg(6) describes the phenotypes. The mice were maintained on stock ration supplemented with occasional fresh vegetables. Only progeny of yellow phenotype were used. Coat color in the A^y/a mice varied from light yellow to deep orange. At 8-11 days after birth, mice were sacrificed by decapitation. Skins from back and head

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TABLE I. Rates of Oxidation of Tryptophan and 5-Hydroxytryptophan by A^y/a Mouse Skin Powders.

Substrate added	Oxygen uptake* (μl/100 min./ml skin)	mg skin/ml (wet wt)		μl O_2/hr/g skin
25 μM L-tryptophan	55	300		110
	69	261		159
	66	277		143
	58	324		105
			Mean	130
12.5 μM "	34	274		73
	37	324		68.5
			Mean	70.7
5 μM "	23	300		46
25 μM DL-5-hydroxytryptophan	44	324		81.5
12.5 μM "	26	274		61
	36	324		57
			Mean	59
25 μM D-tryptophan	−2	261		−7.7

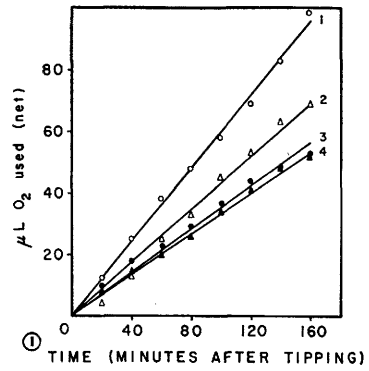
* Total volume 2 ml, conditions as described in text.

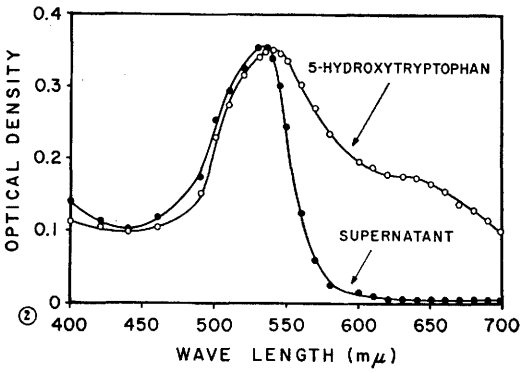
were rapidly removed, weighed on torsion balance, and frozen between blocks of dry ice. Skins from 2-6 mice were pooled. Skin powders were prepared by grinding in mortar as described by Foster(4). They were either used at once or after storage at −20°C for 1-5 days. Measurements of oxygen uptake were made in Warburg vessels(7) in an atmosphere of oxygen at 37.3°C. Routinely, 1 or 1.5 ml of skin powder suspension made up in sodium phosphate buffer, pH 6.8, or 6.0, 0.2 molar, was placed in main compartment, with 0.5 ml of substrate in buffer or buffer alone in the side arm, and 0.2 ml of 20% KOH in center well. Phosphate buffer was used to bring contents to a total of 2 or 3 ml. All substrates were purchased from California Fn. for Biochemical Research. Substrate concentrations in sidearm varied from 10 to 50 μM/ml of L-tryptophan, DL-5-hydroxytryptophan, and 5-hydroxytryptamine; L-kynurenine and D-tryptophan were 25 and 50 μM/ml. After incubation, skin particles were removed by centrifugation and 1 ml of supernatant solution was deproteinized with 1 ml of 0.3 molar zinc sulfate, or total reaction mixture was added to 2 ml 0.3 M zinc sulfate. This precipitant permitted assay of kynurenine formed by liver homogenate from tryptophan as readily as by Knox's method(5), and requires less dilution. Measurements of optical density were made in Beckmann model DU spectrophotometer with cells of 1 cm light path. These measurements were made, with appropriate blanks, both on supernatants after treatment with zinc sulfate and on an aliquot of supernatant assayed for 5-hydroxyindole by the nitrosonaphthol method of Udenfriend *et al.*(8), run at 40°C.† Spectrum of the chromophore produced was compared with that of authentic 5-hydroxytryptophan or 5-hydroxytryptamine added to a skin suspension blank just before centrifugation. Chromophores were extracted into equal volumes of n-butanol, and spectra were again recorded.

Results. Initial experiments showed that skins from 10-day-old mice oxidized L- but not D-tryptophan (Table I). This is in agreement with Foster's observation that L-tryptophan was oxidized more rapidly than DL-tryptophan(4). Rates of oxidation depend on concentration of L-tryptophan over the range studied (Table I). The resultant supernatants directly after incubation were red, but on standing at room temperature changed to yellow.

The following experiments were carried out to determine whether or not kynurenine might be an intermediate. First, after incubation

† Tryptophan contributes to absorption when more than 4 or 5 μM are present in the assay, but does not change the peak or shape of curves. Since its final concentration is not known no correction has been made for it in Fig. 2.





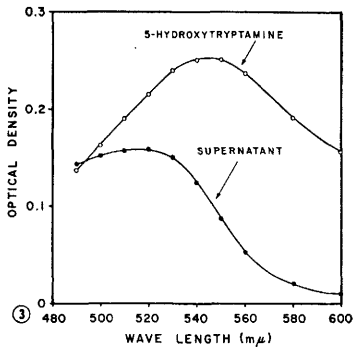


FIG. 1. Oxygen uptake by skin suspensions in presence of : Curve 1, 25 μM L-tryptophan; curve 2, 25 μM DL-5-hydroxytryptophan; curve 3, 12.5 μM L-tryptophan; curve 4, 12.5 μM DL-5-hydroxytryptophan.

FIG. 2. Absorption spectra of chromophores produced with nitrosonaphthol by supernatant after incubation with 25 μM L-tryptophan and by authentic 5-hydroxytryptophan (0.2 μM).

FIG. 3. Absorption spectra of chromophores of supernatant and of 5-hydroxytryptamine after extraction into n-butanol.

with tryptophan and precipitation with zinc sulfate, absorption spectra of supernatant solutions were examined. No peak was observed at 365 mμ, corresponding to absorption maximum for kynurenine. Secondly, L-kynurenine was incubated with skins for 2 and 3 hours to see whether oxygen uptake would result, as pigment formation would be expected to require further oxidation. No net oxygen uptake over controls was observed, nor was any additional color produced in the supernatant. Thirdly, disappearance of kynurenine was studied. Kynurenine was incubated with skins for 2 and 3 hours. Optical density at 365 mμ of diluted aliquots of supernatants was then compared with that from vessels containing buffer alone plus an equivalent amount of kynurenine. These optical densities were the same, within a 5% error of measurement.

Since 5-hydroxytryptamine, another metabolite of tryptophan, may give rise to pigments when incubated with various tissues(9, 10,3), its precursor, 5-hydroxytryptophan, was incubated with skin suspensions. It was found that with 2 concentrations of DL-5-hydroxytryptophan oxygen was taken up nearly as rapidly as with equimolar concentrations of L-tryptophan (Fig. 2, Table I). 5-hydroxytryptophan gave rise in all cases to solutions which were yellow in color both initially and on standing. 5-hydroxytryptamine was also oxidized, yielding paler solutions.

The nitrosonaphthol test for 5-hydroxyindoles was performed on supernatants after incubation with tryptophan, and a distinctive chromophore resulted, the yield being greater after incubation at pH 6.0 than pH 6.8. However, it was not violet in color, as is that formed by 5-hydroxyindoles, but red. The absorption spectrum was compared with that of the chromophore due to 5-hydroxyindoles added to skin blanks before centrifugation. A slight but definite shift in peak absorption was observed and a marked difference in optical density at wave lengths above 550 mμ (Fig. 3). The chromophore due to 5-hydroxyindole was not changed above 500 mμ by presence of excess tryptophan, nor was it altered by presence or absence of skin suspension prior to precipitation. Differences between chromophores produced by the supernatant after incubation and by 5-hydroxyindoles persisted when chromophores were extracted into n-butanol (Fig. 3).

Discussion. These results indicate that kynurenine does not appear to be an intermediate in pigment formation by skins of A^y/a mice. The fact that DL-5-hydroxytryptophan is oxidized nearly as rapidly as equimolar L-tryptophan means that L-5-hydroxytryptophan is probably oxidized more rapidly than L-tryptophan. This suggests that the hydroxylated form may be an intermediate in formation of the colored substance. In the absence of strong nitric acid, the nitrosonaphthol reagent is said to be quite specific for 5-hydroxyindoles when the violet chromophore results, and a number of compounds have been examined(8). Since supernatants after incubation with tryptophan yield a substance forming a similar but not identical chromophore, it is possible that a related hydroxyindole or a di-hydroxyindole is formed. It is unlikely that the difference in the chromophore is due to an impurity, since 5-hydroxytryptophan was added to skin suspensions before taking the spectra, and the chromophore produced was identical with that from 5-hydroxytryptophan alone. In addition, it was found that the chromophore of 5-hydroxytryptophan was not altered by excess tryptophan, and finally, an impurity might be expected to add, rather than subtract, optical density. Further experiments will be required to determine whether the colored substance formed may be due to action of an amine oxidase on a hydroxytryptamine, as has been described(9,10), or whether a di-hydroxyindole may lead to a quinone, as has been suggested(3).

Summary. 1. Skin powders from 10-day-old mice with a dominant gene for yellow hair (A^y/a) oxidize L-tryptophan, DL-5-hydroxytryptophan, and 5-hydroxytryptamine, but not D-tryptophan. Yellow solutions are formed. 2. L-kynurenine does not appear to be an intermediate, since it is not oxidized, nor does it disappear at an appreciable rate when incubated with skin powders from these mice. It does not appear when tryptophan is incubated with skin powders. 3. When tryptophan is oxidized by these skin powders, a substance appears which gives a reaction with nitrosonaphthol in the absence of strong nitric acid. The chromophore is distinctive, and differs from that formed by 5-hydroxyindoles.

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Differential Action of Styramate and Meprobamate on Spinal Reflexes. (24900)

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Styramate, (Sinaxar®) 2 phenyl-2 hydroxyethyl carbamate, a drug with central depressing action has been recently recommended by us as skeletal muscle relaxant for human use. Its pharmacological, toxicological and anticonvulsive properties have been described(1) and preliminary data on its skeletal muscle relaxant activity have been presented(2). In general its properties are similar to those of mephenesin except that it is 3 to 6 times longer acting. The influence of styramate on spinal polysynaptic transmission is not asso-