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Effect of tryptophan on tyrosinase in relation to vitiligo[1]

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Summary. Tryptophan can inhibit DOPA (3,4-dihydroxy phenylalanine) conversion to melanin both by the enzymatic and the nonenzymatic route. Its role has been studied in relation to vitiligo.

Tryptophan is recorded to be an accelerator to tryptophan pyrrolase[3]. Tryptophan pyrrolase has been found to have an antagonistic relationship with tyrosinase during induced depigmentation and repigmentation in *Bufo melanostictus*[4]. It was, therefore, of interest to examine the role of excess tryptophan on tyrosinase in *Bufo melanostictus* which has been used as an experimental animal in the studies of pigment metabolism in relation to vitiligo[5], and also in mammalian system using black mice. In the present communication we report the effect of tryptophan on different aspects of melanin formation.

Materials and methods. L-DOPA and L-tryptophan were purchased from Sigma Chemical Co., USA. Other reagents were of the purest variety possible.

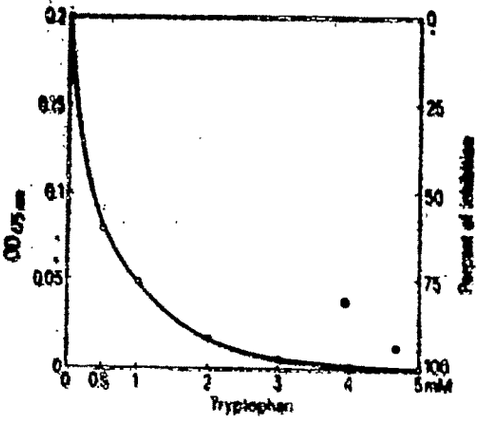
Studies on DOPA auto-oxidation. DOPA auto-oxidation in the formation of melanin was studied in the presence of different concentration of tryptophan.

Studies on enzyme level. Tyrosinase and tryptophan pyrrolase activity of ventral skin and liver of *Bufo melanostictus* (b. wt 40-50 g) and black mice (b. wt 20-25 g) were estimated after 7 days treatment with 1 mg tryptophan per day per animal.

Tryptophan pyrrolase activity was measured according to Knox[6] as slightly modified by Spiegel[7]. The liver and the ventral skin were dissected out. The homogenates of the tissues (12.5%) were prepared with 0.14 M KCl containing 0.0025 M N_2OH, pH 7.0-7.5 and used as an enzyme source. The enzyme activity was measured after incubating with 0.03 M L-tryptophan in 0.2 M phosphate buffer (pH 7.6) solution for 1 h at 37 C. The enzyme activity was expressed in terms of µM of kynurenine/mg of protein.

Tyrosinase activity of the liver and the ventral skin were estimated according to Pomerantz[8] by measuring the rate of formation of dopachrome from L-DOPA at 37 C under the following conditions: L-DOPA (1 µM), sodium phosphate buffer, pH 7.4 (35 µM), enzyme (0.2-0.3 units), total 1 ml. The enzyme activity was expressed as µM of dopachrome formed/min/mg protein.

Results and discussion. It is evident from the experiment that addition of tryptophan can inhibit DOPA auto-oxidation and this inhibition is proportional to the graded amounts of tryptophan (figure). In addition to this observation, tyrosinase activity has been found to be inhibited both in the skin and liver (table 1) of *Bufo melanostictus* along with the rise in tryptophan pyrrolase activity in these tissues, under the influence of tryptophan in vivo. The same effect was also obtained in the case of the mammalian system in vivo (table 2). The present results support our previous observations on the inverse relationship of tyrosinase and tryptophan pyrrolase in *Bufo melanostictus* during experimental pigmentation and depigmentation[5]. It further suggests that increased tryptophan level in the biological system can bring about disturbances in the genesis of melanin. According to Badway[9,10] increase in tryptophan level in body tissues is possible due to the effect of stressful agents like ethanol or catecholamine. Incidentally, stress has been considered to be a factor in the origin of vitiligo[11], so it appears that the inhibition of tyrosinase and DOPA auto-oxidation and activation of tryptophan pyrrolase under the influence of higher concentrations of tryptophan may be factors involved in the impairment of melanin biosynthesis in vitiligo.



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032 Tryptophan pyrrolase activity was measured according to 033 Knox[6] as slightly modified by Spiegel[7]. The liver and the 034 ventral skin were dissected out. The homogenates of the 035 tissues (12.5%) were prepared with 0.14 M KCl containing 036 0.0025 M $NaOH$, pH 7.0-7.5 and used as an enzyme 037 source. The enzyme activity was measured after incubating 038 with 0.03 M L-tryptophan in 0.2 M phosphate buffer (pH 039 7.0) solution for 1 h at 37 C. The enzyme activity was 040 expressed in terms of μM of kynurenine/mg of protein.

041 Tyrosinase activity of the liver and the ventral skin were 042 estimated according to Pomerantz[8] by measuring the rate of 043 formation of dopachrome from L-DOPA at 37 C under the 044 following conditions: L-DOPA (1 μM), sodium phosphate 045 buffer, pH 7.4 (35 μM), enzyme (0.2-0.3 units), total 1 ml. 046 The enzyme activity was expressed as μM of dopachrome 047 formed/min/mg protein.

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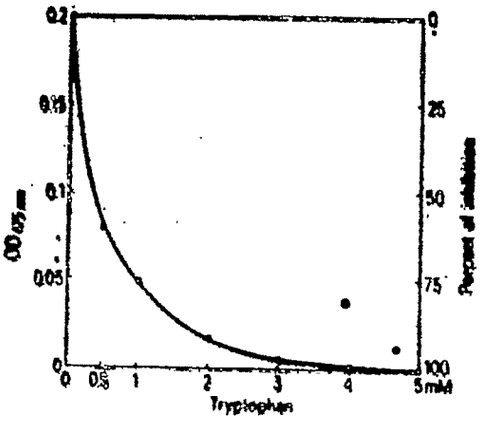

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090 Inhibition of DOPA auto-oxidation by graded amounts of Trypto-
091 phan. DOPA (1 mM) was incubated with different concentra-
092 tion of tryptophan (0.5 mM; 1.0 mM; 2.0 mM; 3.0 mM 4.0
093 mM and 5.0 mM) in a total volume of 1 ml at 37°C, pH 7.0 for
094 8 h. OD of the solutions were determined at 475 nm in a
095 Hilger-Watts Spectrophotometer after incubations, which
096 represents DOPA auto-oxidations i.e. DOPA-chrome forma-
097 tion in a given time.

Table 1. In vivo effect of tryptophan on tyrosinase and tryptophan pyrrolase of ventral skin and liver of *Bufo melanostictus* (n = 30)

Treatment	Tissue	Tyrosinase* (mean ± SD)	Tryptophan pyrrolase** (mean ± SD)
Control	Ventral skin	14.3 ± 2.61	3.02 ± 1.20
	Liver	9.2 ± 1.72	3.1 ± 0.59
Tryptophan (1 mg/day/toad for 7 days)	Ventral skin	7.8 ± 1.15	5.4 ± 2.01
	Liver	5.7 ± 0.86	11.2 ± 1.70

* μM of dopachrome/min/mg of protein ($p < 0.001$); ** μM of kynurenine $\times 10^{-2}$/mg of protein ($p < 0.001$).

Table 2. In vivo effect of tryptophan on tyrosinase and tryptophan pyrrolase of ventral skin and liver of black mice (n = 20)

Treatment	Tissue	Tyrosinase* (mean ± SD)	Tryptophan pyrrolase** (mean ± SD)
Control	Ventral skin	20.6 ± 2.24	4.5 ± 1.11
	Liver	7.1 ± 0.88	2.9 ± 0.58
Tryptophan (1 mg/day/mice for 7 days)	Ventral skin	15.7 ± 1.18	6.9 ± 2.71
	Liver	1.62 ± 0.70	7.3 ± 1.12

* μM of dopachrome/min/mg of protein ($p < 0.001$); ** μM of kynurenine $\times 10^{-3}$/mg of protein ($p < 0.001$)