A comparative study of tryptophan metabolism in skins and livers of black and albino rats[1]

C. Chakraborty, A.K. Chakraborty and D.P. Chakraborty[2]

Dept. of Chemistry, Bose Institute, Calcutta 700009 (India), 25 May 1983

Summary. The skin of black rats contains less tryptophan than the skin of albino rats, possibly because the activity of tryptophan pyrrolase is higher. The opposite is found in liver, possibly because tryptophan pyrrolase activity is lower in the livers of black rats.

Several comparative histochemical and biochemical studies on skins and livers of black and albino rats and mice have been made in order to investigate the localization, pattern and concentrations of various enzymes and metabolites and to correlate them with skin pigmentation[3-5]. Albino skins have been shown to possess a significantly higher concentration of tyrosinase than black skins[3,4].The tyrosine-utilizing capacity of albino rat livers is also higher than that in black rats[5]. Investigations in our laboratory show that a vitiliginous patient has an abnormal urinary profile of tryptophan derivatives[6]. The tryptophan metabolites reported from normal human subjects include indole-3-acetic acid (IAA), indican, 5-hydroxyindole-3-acetic acid (5HIAA), 5-hydroxy tryptophan and 5-hydroxytryptamine (5-HT). In the urines of vitiliginous patients, IAA, 5-HIAA and 5-hydroxyanthranilic acid have been detected. Recent investigations by Chakraborty et al[7] further show that vitiliginous patients have abnormal levels of tyrosine, DOPA, tryptophan and tyrosinase and tryptophan pyrrolase in their blood. It has been shown by Kurbanov et al.[8] that with the increase of tryptophan loading, vitiliginous patients have been found to elaborate more kynurenine, 3-hydroxyanthranilic acid and 5-hydroxyindolyl acetic acid through their urine. Recent investigations by Chakraborty et al.[9] show that with tryptophan loading there is inhibition of tyrosinase and DOPA auto-oxidation. Again, it is well known that increased tryptophan loading increases the tryptophan pyrrolase activity[10-12]. All these facts tend to support the theory that tryptophan metabolism is linked to pigment metabolism. In the course of our investigation comparing the levels of various parameters during melanogenesis, and its reversal in experimental animals, we carried out a comparative study of the levels of tryptophan and tryptophan pyrrolase in the skin and liver of black and albino rats. In the present communication we report the results.

Materials and methods. 15 black rats *(Rattus rattus)* and 15 albino rats *(Rattus norvegicus)* with an average body weight of 200 g were kept for few days on the same diet (balanced). Then the rats were sacrificed and the ventral skin and liver tissues were taken. 1 g of skin and 1 g of liver from each rat was used for tryptophan estimation. Each tissue was homogenized with 5 ml of double distilled water (cold) and centrifuged at 12,000 rpm; the supernatants were deproteinized and filtered, and the protein-free filtrate was made neutral. This neutral protein-free filtrate was used for the estimation of free tryptophan. Tryptophan was estimated according to the method of Spies and Chambers[13].

Tryptophan pyrrolase activity was measured according to Knox [14] as slightly modified by Spiegel[15]. Homogenates of the tissues (12.5%) were prepared with 0.14 M KCl containing 0.0025 M NaOH, pH 7.0–7.5, and used as enzyme source. The enzyme activity was measured after incubating with 0.03 M L-tryptophan in 0.2 M phosphate buffer (pH 7.0) solution for 1 h at 37°C. The enzyme activity was expressed in terms of μM of Kynurenine/mg of protein.

Results and discussion. Table 1 shows that the skin tryptophan level is greater in albino rats than in black rats, indicating that the albino skin has less ability to metabolize tryptophan: this is supported by the fact that the albino skin has a lower level of tryptophan pyrrolase activity than that of black rats (table 2). The liver is therefore called upon to carry out this metabolism, resulting in significantly higher tryptophan utilization. The increased levels of tryptophan pyrrolase in the albino liver can account for the higher tryptophan-utilizing capacity of the tissue. The present data add another abnormality in tryptophan metabolism to the list of those found in the skins of albino animals in which defects in pigment metabolism are present.

Table 1. Tryptophan level in the skin and liver of black and albino rats (n = 15)

	Skin* (mean ± SD)	Liver* (mean ± SD)
Black rat	2.68 ± 0.20	2.37 ± 0.21
Albino rat	4.69 ± 0.25	0.34 ± 0.05

* mg/g of dry tissue, $p < 0.001$.

Table 2. Tryptophan pyrrolase activity in the skin and liver of black and albino rats (n = 15)

	Skin** (mean ± SD)	Liver** (mean ± SD)
Black rat	19 ± 4.5	10 ± 4.2
Albino rat	10 ± 4.5	15 ± 5.0

** μM of Kynurenine × 10^{-3}/mg of protein, $p < 0.001$.

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2 To whom correspondence should be addressed.

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