possible to ascertain their structures, in spite of the fact that paragonial substance A is a relatively small molecule with only 1 amino-acid. This compound is indeed rapidly transferred from the spermatophore into the hemolymph and the stimulation of oogenesis appears[4] 16–24 h later. Baumann[6,7] has described a paragonial substance PS_2 from *Drosophila funebris*, also active upon oogenesis, and also a low mol.wt glycine containing product. However, in the absence of structure elucidation and of direct comparison, it is not possible to conclude that the 2 products are identical. The biological activity of paragonial substance B is quite original but difficult to analyse. However, the combined action of paragonial substances A and of the antagonistic (versus toxic) paragonial substance B may create a balance ensuring the regulation of ovarian production and oocyte maturation. As has been shown in a number of cases, particularly in insects, the control of reproduction could result from the interaction of 2 antagonistic hormonal factors[8,9]. It is necessary to add that the possibility that the paragonial substance B is a toxic artefact produced by the degradation of paragonial substance A during the manipulations cannot be excluded for the moment. But even, in this case, the fact that an antagonistic property is obtained is also of great interest.

1 Address for reprint requests: Institut de Biocénotique Expérimentale des Agrosystèmes, ERA CNRS 328, Avenue Monge, Parc Grandmont, F-37200 Tours, France.
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Effect of tryptophan on tyrosinase in relation to vitiligo[1]

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

Department of Chemistry, Bose Institute, 93/1 Acharya Prafulla Chandra Road, Calcutta 700009 (India), 25 October 1979

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 of 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 NaOH, 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 7.0) 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

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

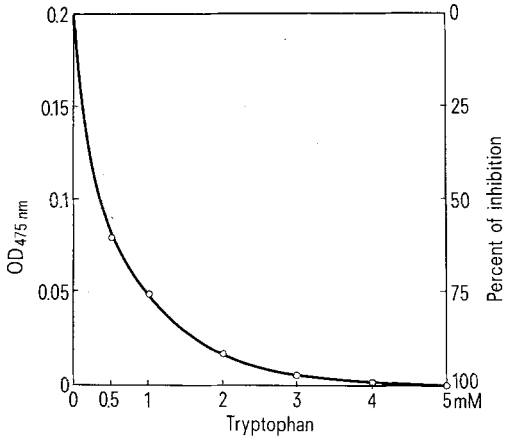
* μM of dopachrome/min/mg of protein ($p < 0.001$); ** μM of kynurenine $\times 10^{-2}$/mg of protein ($p < 0.001$).

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 ($p < 0.001$).

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[4]. 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 the factors involved in the impairment of melanin biosynthesis in vitiligo.



Inhibition of DOPA auto-oxidation by graded amounts of Tryptophan. DOPA (1 mM) was incubated with different concentration of tryptophan (0.5 mM; 1.0 mM; 2.0 mM; 3.0 mM 4.0 mM and 5.0 mM) in a total volume of 1 ml at 37 °C, pH 7.0 for 8 h. OD of the solutions were determined at 475 nm in a Hilger-Watts Spectrophotometer after incubations, which represents DOPA auto-oxidations i.e. DOPA-chrome formation in a given time.

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Hemolymph proteins in an ascitic condition, induced by lethal *1(3)gl* tumorous tissue in *Drosophila hydei*[1]

Ž. Srdić and P. Borner

Laboratoire de Génétique, Université de Genève, CH-1224 Genève, and Zoologisches Institut der Universität Zürich, CH-8057 Zürich (Switzerland), 18 March 1980

Summary. Drosophila hydei larvae homozygous for the gene *1(3)gl* may survive to the age of 3 weeks, become bloated and be incapable of metamorphosis. Wild-type flies after the injection of a fragment of the *1(3)gl* disc, which has been previously culture in vivo for 40–500 days, also become bloated (ascitic reaction). In the hemolymph of both the advanced *1(3)gl* larvae and ascitic adult hosts the accumulation of a specific protein is observed.

The gene *1(3)gl (lethal (3) giant larvae),* a recessive lethal gene in *Drosophila hydei,* is located at the distal end of an autosome which is number 3 according to Berendes[2] and number 4 according to Spencer[3]. Homozygous *1(3)gl* die as advance larvae. Incapable of metamorphosis, they may survive to the age of 3 weeks whereas the normal pupation age is about 7 days. Such larvae are oversized, bloated and rather transparent, and show a characteristic syndrome[4] the most salient features of which are hypertrophy of the brain and degeneration of imaginal discs. The latter disappear during larval life, except for the dorsal mesothoracic and ventral metathoracic discs, which coalesce into a single poorly-organized mass of large dimensions. When fragments of this mass are injected into the abdomens of adult hosts, the tissue behaves as a benign tumor; it proliferates rapidly but does not reduce the lifespan of the host.

Transplanted *1(3)gl* brain tissue by contrast behaves as a malignant tumor, killing the host. These properties of brain and disc tissue, in conjunction with the chromosomal localization of the gene, leave no doubt that *1(3)gl* of *D. hydei* is homologous with *1(2)gl* of *D. melanogaster*[5], although certain features of the phenotype differ between the 2 species.

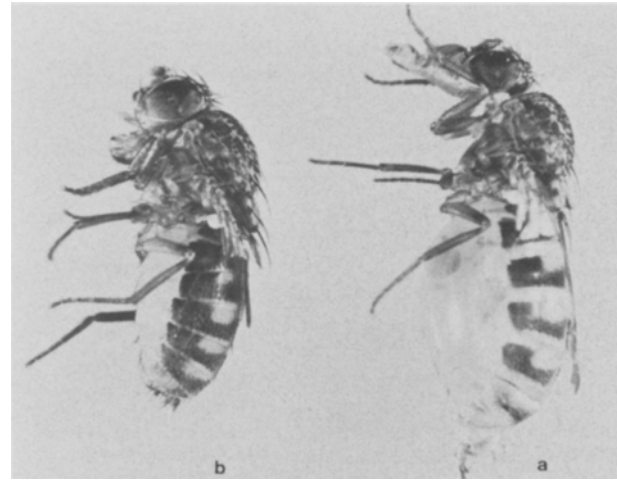

Fig. 1. *Drosophila hydei* females (strain Alicante wild-type) 20 days after the injection of an imaginal disc. (a) Fragment of disc from a *1(3)gl* larva, cultured in vivo for 40 days, (b) disc from a normal larva (control). In both specimens the wings were removed to facilitate photography.