Inverse relationship between melanogenesis and endogenous hydroquinone[1]

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Summary. Inhibition or stimulation of melanogenesis have been found to occur as a result of the alteration of hydroquinone levels in the body. Substances which stimulate melanogenesis are found to lower the level of hydroquinone in amphibia, and evidence for the relationship is also given by mammalian experiments.

Hydroquinone, reported to be an inhibitor of tyrosinase[3,4], is formed in the body by quinone reductase present in a number of tissues[5]: DPNH + p-benzoquinone → Hydroquinone + DPN^+. The inhibition of melanogenesis by extracts of mouse melanoma and of normal liver has been attributed to be an enzymatic reduction of quinones formed in the course of DOPA oxidation[6].

In our previous communications[7,9], we reported the reversal of experimental depigmentation by psoralen in *Bufo melanostictus*. Indole compounds have been shown to inhibit or inactivate tyrosinase[10], and tryptophan has recently been considered to cause impairment of melanogenesis by activating tryptophan pyrrolase and depressing the tyrosinase activities[11]. The inhibitory action of ascorbic acid on enzymatic melanogenesis[12,13] has been presumed to be due to the reduction of various quinoid compounds formed in metabolic processes[14]. It was, therefore, of interest to us to examine the role of endogenous hydroquinone under different conditions of experimental depigmentation or its reversal under the influence of the compounds referred to above. The comparison of the hydroquinone levels of black and albino rats has also been carried out to find out the status of hydroquinone in these 2 differently pigmented types.

Materials and methods. Depigmenting and pigmentogenic agents were administered orally to adult male toads *(Bufo melanostictus)*, of 60–70 g of b.wt. Hydroquinone (May & Baker Ltd.), tryptophan (MERCK), ascorbic acid (Sarabhai Chemicals) and other chemicals used for the experiments were all of AR grade. Psoralen (fig.) was isolated in our laboratory from *Psoralea corylifolia* seeds[15].

Hydroquinone was estimated in the skin, liver, blood and urine. For skin and liver, the tissues were homogenized with cold distilled water (5 °C), then centrifuged at 12,000 × g, deproteinized with 20% TCA (trichloroacetic acid), filtered, and the filtrate then made neutral with 1 N Sodium hydroxide. Blood samples were drawn from the hepatic portal vein and citrated, deproteinized with 20% TCA and neutralized with 1 N Sodium hydroxide. The neutral filtrates were kept in the cold (5 °C) before estimation. 6 albino (Wistar strain) rats of 200–250 g b.wt and 6 pigmented Bandicoot rats of the same b.wt were used to compare the hydroquinone levels of the 2 varieties of rats. Hydroquinone was estimated according to the method of Wosilait and Nason[5]. The concentrations of endogenous hydroquinone in all the samples were calculated from the standard curve for hydroquinone which was prepared with known concentrations of hydroquinone following the same method.

Results. During inhibition of tyrosinase activity with ascorbic acid (table 1) and tryptophan (table 2) there is an elevation of hydroquinone levels in skin, liver, blood and urine of *Bufo melanostictus*. On the other hand, on administration of the pigmentogenic drug, psoralen (animals exposed to sunlight) to animals previously treated with ascorbic acid, there is fall in hydroquinone level (table 1). It has further been observed (table 3) that albino rats have a higher concentration of hydroquinone in their skin than black rats.

Discussions. The results suggest that the alteration of the endogenous hydroquinone level in different tissues affects the inhibition or stimulation of melanogenesis in the animals. This is further supported by the presence of a larger concentration of hydroquinone in albino rat skin than in the skin of black rats. Both ascorbic acid and hydroquinone are inhibitors of tyrosinase. The presence of ascorbic acid does not interfere with the increase in the hydroquinone level in the system. This may be due to the difference in the redox potential of the dehydroascorbic acid/ascorbic acid system (+ 0.08 v) (A) and the quinone/hydroquinone system (+ 0.699) (B) at pH 7. When both systems (A) and (B) co-exist in a biological system, as a result of the to higher redox potential of the system (B) the reducing action of the system (A) will predominate, so that excess ascorbic acid will reduce quinone to hydroquinone while dehydro-

Table 1. Hydroquinone level after ascorbic acid and psoralen (animals exposed to sunlight) treatment in toads. Number of animals in each group (n) = 20

	Control (mean ± SD)	Ascorbic acid treatment (mean ± SD)	Blank[b] (mean ± SD)	Psoralen treatment[c] (mean ± SD)
Ventral skin (mg/g of dry tissue)	5.8 ± 2.1	24.28 ± 4.0**	17 ± 1.6	10.6 ± 0.5**
Liver (mg/g of dry tissue)	0.75 ± 0.2	3.97 ± 0.52**	1.7 ± 0.2	1.0 ± 0.2**
Blood (μg/ml of blood)	65 ± 7	186.6 ± 11.6**	98 ± 6	78 ± 8**
Urine (μg/ml of urine)	4.95 ± 1.5	19 ± 3.2**	11 ± 2.1	5.2 ± 1.2**

[a] Treatment carried out for 7 days (25 mg/toad/day); [b] ascorbic treated toads left untreated for 7 days; which were considered as the controls for psoralen treated toads; [c] treatment carried out for 7 days to ascorbic acid treated toads (mg/toad/day). Fisher's t-test was used in those cases where the squares of the 2 SD values (SD_1^2 and SD_2^2) are not too different ($0.6 \leq SD_1^2/SD_2^2 \leq 1.4$). But in those cases where the squares of the SD values are quite different, Welch's test was used. ** Values have high significance at 1% level, indicating that the observed treatment mean is in each case very significantly large as compared to the 'control' mean or 'blank' mean.

Table 2. Hydroquinone levels after tryptophan treatment in toads (n = 20)

	Control (mean ± SD)	Tryptophan treatment[d] (mean ± SD)
Ventral skin (mg/g dry tissue)	14.2 ± 0.6	16.2 ± 0.25**
Liver (mg/g of dry tissue)	1.66 ± 0.52	2.93 ± 0.60**
Blood (μg/ml of blood)	104 ± 8	123 ± 12**
Urine (μg/ml of urine)	4.8 ± 1.5	14.5 ± 3.1**

[d] Treatment (1 mg/toad/day) for 7 days; **Values have high significance at 1% level.

Table 3. Hydroquinone levels of black and albino rats (n = 6)

Tissues	Black rat (mean ± SD)	Albino rat (mean ± SD)
Skin (mg/g of dry tissue)	1 ± 0.2**	4.5 ± 1.5**
Liver (mg/g of dry tissue)	0.597 ± 0.2 (NS)	0.627 ± 0.2 (NS)

** Mean values have high significance at 1% level; NS, not significant.

ascorbic acid will be unaffected by hydroquinone. Thus the higher hydroquinone level in tissues in ascorbic acid-treated subjects could be explained. Previous reports[12,13] on the lowering of tyrosinase activity by the action of ascorbic acid support these results.

Amelanosis due to tryptophan may be attributed to its selective toxicity[10] to melanotic cells while that due to ascorbic acid is attributed to its reducing action. On the other hand, the induced melanosis due to psoralen (plus sunlight or UV) has not been clearly explained, but the overall effect of administration of this pigmentogenic agent is to lower the hydroquinone level. This suggests that activation of melanogenesis requires an overall lowering of the tissue inhibitor concentration, and points out that the interference of the agents causing acceleration or retardation of melanogenic process. The suggestions put forward by Chakraborty et al.[9] as well as by Nordlund and Lerner[16] support this idea. It was suggested by Chakraborty et al.[9] that psoralen could interfere with inhibitors of tyrosinase to augment melanogenesis, while Nordlund and Lerner[16] suggest that psoralen (plus UV) may damage a postulated inhibitory agent that is responsible for killing pigment cells, producing vitiligo. Recent isolation of an inhibitor by Vijayan et al.[17] from vitiliginous skin is in conformity with the experimental results and the suggestions presented here.

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Noninvolvement of a rat intestinal folate binding protein in physiological folate absorption

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Summary. Folic acid and its derivatives are observed to be instantaneously bound to rat intestinal mucosal cell curface binding protein. Various parameters were measured such as the optimal pH for binding (6.5), the saturation kinetics observed for folate binding and the avid affinity with which polyglutamyl folates are preferentially bound to the binder. A comparison of the observed results with the conditions reported as favorable for intestinal folate absorption in the rat precludes the involvement of this folate-binding protein in the physiological absorption of folates.

Specific folate binding proteins (FABP) have been reported in serum, milk, leukocytes and a variety of tissues such as liver, kidney and intestinal mucosa[1-6]. FABP have been purified from cows' milk[7], human milk[8], umbilical cord serum[9], human choroid plexus[10] and intestinal mucosa[6]. The physiological functions of FABP have not been well established though it is implicated in folate absorption and transport[11,12]. Here we report observations on a folate binder in the intestinal mucosal cell surface and assess its role as a carrier in the physiological absorption of the vitamin.

Materials and methods. Folic acid, tetrahydrofolic acid, 5-formyltetrahydrofolic acid and 5-methyltetrahydrofolic acid were all obtained from Sigma Chemical Co., USA. (2[14]-C) folic acid with specific activity of 54.3 mCi/mmole was obtained from Amersham, U.K. Dihydrofolic acid was synthesized as described earlier[13]. Yeast folates which consist of almost 80–90% polyglutamyl forms, as characterized in our laboratory earlier[14], served as a source of polyglutamyl folates.

Male albino rats of the Wistar strain fed a complete laboratory stock diet and weighing 150–170 g were used throughout the study. 24-h fasted rats were killed, and the upper two-thirds of the small intestine removed and washed with ice-cold 0.9% saline. The intestine was slit open and the mucosal cells were gently scraped off with a fine scalpel and suspended in Ringer solution.

Mucosal cells equivalent to 40 mg protein were suspended in 5 ml Krebs-Ringer phosphate medium, pH 6.5, containing 0.4 μCi (2-^{14}C) folic acid and incubated at 37 °C with constant shaking. Aliquots of 0.1 ml each were removed at various time intervals during incubation, and filtered under suction through 0.22 m μ pore size millipore filters. The filters with trapped cells were washed thoroughly with 30 ml of 154 mM cold saline, dried and their radioactivity determined. The experiment was repeated at various pH values and at various other ^{14}C-folate concentrations.

In studies measuring competition by other folate compounds for the folate binding sites, various concentrations of the particular folate derivative under study were incubated with cells for 5 min at 37 °C prior to the addition of radioactive folic acid. Incubation with shaking was continued for another 5 min prior to trapping and washing the cells on the millipore filters. The relative amounts of various folate derivatives required for a 50% displacement of ^{14}C-folic acid binding were recorded. All radioactivity measurements were made in a Beckman Liquid Scintillation Counter LS 100 using Bray's solution.

Results. As can be seen in figure 1 the binding of ^{14}C-folic acid