Substrate induced alterations in tryptophan pyrrolase activity in two mouse strains[1]

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Summary. Total hepatic L-tryptophan 2,3-dioxygenase activity was studied in 2 mouse strains receiving i.p. injections of L-tryptophan. After a single injection, enzyme activity was increased in albino but not pigmented mice. After 3 injections, enzyme activity was reduced in both strains.

It is now well established that, at least within moderate limits, the quantity of tryptophan presented to the mammalian brain may control the synthesis and turnover of serotonin[2,3]. Despite the obvious importance of this metabolic pathway, however, most of the tryptophan entering a mammal is degraded to kynurenine in the liver. The rate limiting enzyme in this pathway is L-tryptophan 2,3-dioxygenase, or tryptophan pyrrolase. This interesting heme and copper containing enzyme has been the focus of numerous investigations into mechanisms of enzyme regulation, and it has been repeatedly shown that single doses of L-tryptophan, corticosteroids, or agents which increase the plasma concentration of either of these compounds increase the activity of the enzyme[4,5]. Such studies have provided important insights into enzyme regulation, but with increasing interest in chronic modification of serotonergic neuronal function by modifying the tryptophan content of the brain, it has become important to know what effect, if any, repeated elevation of substrate concentration has on this hepatic enzyme. Nearly all of our knowledge of the regulation of this enzyme has come from studies of albino rodents. It seems hazardous to casually extrapolate these findings to pigmented mammals of greater clinical and economic importance, particularly in light of the demonstration of several differences between the pyrrolase in albino rats and mice and that in pigmented mammals[6,7].

Materials and methods. Male mice, 90–180 days of age were used throughout. Albino mice [CD-1 - Crl: COBS CD-1 (ICR)BR] were obtained from Charles Rivers breeding laboratories, Wilmington, MA. Outbred pigmented mice were obtained from our departmental colony. All mice received i.p. injections of sterile L-tryptophan at a dose of 150 mg/kg, or equivalent volumes of 0.9% saline between 10.00 and 12.00 h every other day. 2 h following the 1st, 2nd or 3rd injection, or 24 h following the 3rd injection, fasted mice were decapitated and the total hepatic tryptophan pyrrolase activity was determined by the method of Symanski and Bennet[8]. This assay measures both kynurenine and n-formyl kynurenine, so that any changes in the rate of conversion of n-formyl kynurenine to kynurenine will not obscure changes in the pyrrolase activity. The rate of product formation was standardized to the hepatic protein content determined by the method of Lowry et al.[9] with bovine serum albumin as a standard.

Results and discussion. In albino mice a single 150 mg/kg dose of L-tryptophan caused a substantial increase in pyrrolase activity (fig. 1); similar findings have been reported in albino rats[4,5]. In contrast, an identical dose had no significant effect on the pyrrolase activity in the pigmented strain. Figure 2 shows that the only effect of tryptophan administration in this strain was a transient reduction in pyrrolase activity after the 3rd treatment. In the albino strain the initial increase in pyrrolase activity was followed by a progressive reduction in activity with subsequent treatments, and the pyrrolase activity in tryptophan treated animals remained lower than in controls, 24 h after the 3rd treatment. Although unexpected in view of the extensive literature on substrate induction of this enzyme, the finding of reduced pyrrolase activity after repeated tryptophan administration supports and extends the findings of Badawy et al. These authors found that while acute administration of ethanol increased the pyrrolase activity in albino rats, 1 week, and even more so 2 weeks of ethanol treatment reduced the pyrrolase activity[10,11]. A reduction in the activity of the hepatic pyrrolase has also been reported after chronic administration of morphine, nicotine, and phenobarbitone[12]. Thus, at least in albino rodents, it appears that a reduction in the activity of the hepatic pyrrolase is a frequent consequence of repeated administration of a number

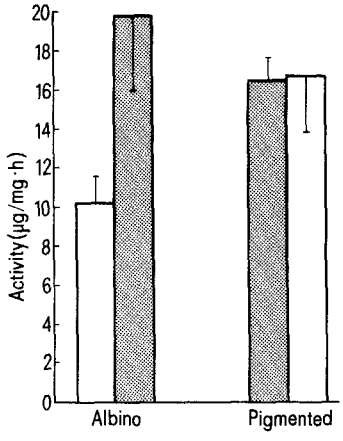

Figure 1. The effect of a single injection of saline (0.9%, open bars) or L-tryptophan (150 mg/kg, shaded bars) on hepatic tryptophan pyrrolase in 2 mouse strains. Enzyme activity is expressed as μg product per mg protein per h. A single i.p. injection of L-tryptophan significantly ($n=12$, $t=5.436$, $p \leq 0.001$) increased the enzyme activity in albino, but not in pigmented ($n=12$, $t=0.266$) mice. Unpaired t-test, direction not predicted.

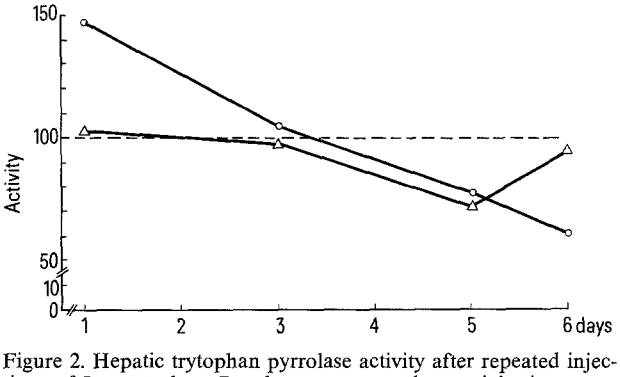

Figure 2. Hepatic trytophan pyrrolase activity after repeated injections of L-tryptophan. Results are expressed as activity in tryptophan-treated animals as a percent of that in saline-treated animals. Albino (circles) and pigmented (triangles) mice were injected i.p. with saline (0.9%) or L-tryptophan (150 mg/kg) on days, 1, 3 and 5, and pyrrolase activity (μg product/mg protein - h) was assayed on days 1, 3, 5 and 6. Tryptophan-treated animals were significantly ($p \leq 0.05$, unpaired t-test, direction not predicted) different from saline treated animals on day 1 (albino), day 5 (albino and pigmented), and day 6 (albino). Each data point represents 12 animals.

of compounds which affect the CNS. In this regard, it is noteworthy that the only effect of tryptophan administration in the pigmented mouse strain was a reduction in pyrrolase activity following repeated treatments. Although the dearth of similar studies on other pigmented mammals makes generalizations hazardous, it may well be that reduction in pyrrolase activity is more common than is induction. Investigations on this possibility may elucidate additional mechanisms of enzyme regulation, as well as have therapeutic ramifications.

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Catalase in free-living and parasitic platyhelminths[1]

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Summary. Catalase was found in all of the free-living species of platyhelminths studied, but could not be detected in any of the parasitic species (trematodes or cestodes).

Catalase (EC 1.11.1.6) is ubiquitously distributed in aerobic organisms and is generally present in fairly large amounts. Only in strict anaerobes is catalase normally absent[2]. Some parasitic protozoa such as *Entamoeba histolytica*, some species of *Trichomonas* and certain stages of *Leishmania mexicana, L. donovani* and *Trypanosoma brucei* lack catalase[3–5], whilst in some microorganisms catalase is an inducible enzyme. The principle function of catalase is to protect cells against the damaging effects of peroxides. There have been reports that catalase activity may be low or absent in a number of parasitic helminths[4,6,7]. It was, therefore, of interest to see if this lack of catalase was a peculiarity of the phyla concerned or whether it could in any way be related to the parasitic mode of life.

A comparison of the catalase activity in a number of free-living and parasitic platyhelminths is shown in the table.

The distribution of catalase in free-living and parasitic platyhelminths

Species	Activity* (units/mgprotein × 10^3)
Free living	
Polycelis nigra	98 ± 20
Procerodes littoralis	6 ± 0.8
Leptoplana tremellaris	20 ± 3
Thysanozoon sp.	120 ± 30
Parasitic	
Anoplocephala magna	< 1
Moniezia expansa	< 1
Hymenolepsis diminuta	< 1
Hymenolepis nana	< 1
Schistocephalus solidus (plerocercoid)	< 1
Ligula intestinalis (plerocercoid)	< 1
Bothriocephalus scorpii	< 1
Schistosoma magrebowiei	< 1
Fasciola hepatica	< 1

Values are mean ± SEM, n = 6.

Catalase was assayed as described previously[7], the activity (units/mg protein) was calculated from the formula $K = {}^1/_t (\ln E_o/E_t) \times$ dilution factor, where E_o is the initial extinction at 240 nm and E_t the extinction after 10 sec. Catalase was present in all of the free-living species, but could not be detected in any of the parasitic ones. Catalase has also been shown to be absent from the adult and larval stages of *Taenia pisiformis*[4]. The lack of catalase would, therefore, seem to be correlated with the parasitic mode of life. Several parasitic helminths including *Fasciola hepatica* and *Moniezia expansa* have been reported to produce hydrogen peroxide when particulate fractions are incubated with substrate under aerobic conditions[8,9]. The origin of this hydrogen peroxide is unknown, but it is probably formed by the branched cytochrome chains[10]. The production of hydrogen peroxide could be a direct reflection of the lack of catalase. However, there is no evidence that helminths produce significant amounts of hydrogen peroxide in vivo[7], and it is possible that peroxidases may functionally replace catalase in parasitic platyhelminths. The absence of catalase from parasitic platyhelminths would appear to be a potential site for chemotherapy.

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