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Determination of tryptophan pyrrolase activity in rat liver homogenates

Stowell & Mørland (1983) suggested that determination of liver tryptophan pyrrolase (tryptophan 2,3-dioxygenase, EC 1.13.11.11) activity in whole homogenates by the methods of Seglen & Jervell (1969) and Badawy & Evans (1975) could be influenced by the following three sources of error: (1) a tryptophan-independent reaction influencing the detection of kynurenine at 365 nm; (2) a haem activation of the enzyme *in vitro* caused by blood left in liver; (3) a post-mortem stimulation of the enzyme (at room temperature). Because only the last can be discounted in relation to the method of Badawy & Evans (1975), which involves rapid homogenization of the liver in an ice-cold solution, we have examined in the present work the possible interference in our method of the so-called tryptophan-independent reaction and the presence of blood in the liver. All experimental details have previously been described (Badawy & Evans, 1973, 1975, 1976; Badawy & Morgan, 1980; Badawy, 1981).

Contrary to the finding by Stowell & Mørland (1983), we could not demonstrate a decrease in the A_{365} when liver homogenates from fed (as well as 24 h-starved) rats were incubated in the absence of added tryptophan (results not shown). It therefore made no statistical difference to the calculation (by linear regression analysis) of tryptophan pyrrolase activity whether or not tryptophan-free blank values were subtracted. This blank-associated decrease in the A_{365} may, as stated by Stowell & Mørland (1983), be related to a dietary factor(s), possibly sucrose (or carbohydrates in general); the carbohydrate content of the food consumed by our rats is 45% (w/w), whereas that of the food of animals used by Stowell & Mørland (1983), though not stated in their paper, is usually 50%.

Stowell & Mørland (1983) reported that holo-(tryptophan pyrrolase) activity and hence the haem saturation of the enzyme were significantly lower in perfused livers, compared with those in livers rinsed with 0.9% NaCl, just blotted on paper or left uncleaned. We could not demonstrate these effects of blood in the present work (results not shown). Moreover, as the results in Table 1 show, perfusion made no difference to pyrrolase activities or saturation with haem under a variety of experi-

mental conditions. All the above perfusion experiments were performed by using 0.14 M-KCl containing 0.0025 M-NaOH as the perfusing fluid. No significant differences in activities or the haem saturation of the enzyme were observed (results not shown) when this fluid was replaced by 0.9% (w/v) NaCl with or without heparin (1000 units/dl).

The possible role of blood in the liver was further examined in experiments in which the ability of allopurinol to prevent the conjugation of apo-(tryptophan pyrrolase) with haem (see Badawy & Evans, 1973) was utilized. It was first important, however, to ascertain whether allopurinol can prevent the conjugation of haem derived from haemoglobin. This was found to be so (results not shown), thus establishing the validity of using this agent in further experiments in relation to the possible role of this source of haem. Furthermore, because in the above experiments both allopurinol and haemoglobin (or haematin) were present simultaneously when liver homogenates were added, it was concluded that prevention by allopurinol of pyrrolase conjugation with haem is a rapid phenomenon and, accordingly, if allopurinol was added to the homogenization solution, then it should have an equal chance of preventing any conjugation (of pyrrolase with haem derived from blood haemoglobin) that may occur upon homogenization of the liver. Thus if such conjugation occurs, as Stowell & Mørland (1983) suggested, then the holoenzyme activity should be expected to be higher when allopurinol is absent, than when it is present, during the homogenization. We found (results not shown) that, although allopurinol (2 mM) present during the homogenization prevented the conjugation of apo-(tryptophan pyrrolase) with added haematin, it failed to cause a decrease in the holoenzyme activity under a variety of experimental conditions involving altered pyrrolase activities and haem saturation, irrespective of whether or not the livers were perfused.

Taken together, the present results therefore strongly suggest that blood present in the liver does not act as a source of haem for tryptophan pyrrolase when the activity of the latter is determined as described by Badawy & Evans (1975). Our findings are at variance with those illustrated in Table 1 of the paper by Stowell & Mørland (1983), which showed that holo-(tryptophan pyrrolase) activity and the haem saturation of the enzyme were higher in unperfused, than in perfused, livers. One possible

Table 1. *Comparison of rat liver tryptophan pyrrolase activities in perfused and unperfused livers*

Rats were either fed or 24 h-starved. Starved rats were either untreated or received a subcutaneous injection of 2-allyl-2-isopropylacetamide (AIA; 400 mg/kg body wt.) or an intraperitoneal injection of cortisol acetate (20 mg/kg body wt.), ethanol (4 g/kg body wt.), haematin hydrochloride (5 mg/kg body wt.) or tryptophan (400 mg/kg body wt.) and were killed at 0.5, 4, 4, 2 and 4 h respectively after these five treatments. Values are means $\pm$ S.E.M. for each group of four rats.

Nutrition	Treatment	Liver perfusion	Tryptophan pyrrolase activity (μ mol of kynurenine formed/h per g wet wt. of liver)		Pyrrolase haem saturation (%)
			Holoenzyme	Total enzyme	
Fed	Nil	—	1.7 $\pm$ 0.15	4.1 $\pm$ 0.31	41 $\pm$ 1
		+	1.8 $\pm$ 0.09	4.5 $\pm$ 0.38	40 $\pm$ 5
Starved	Nil	—	3.0 $\pm$ 0.12	7.3 $\pm$ 0.55	41 $\pm$ 3
		+	3.0 $\pm$ 0.15	7.3 $\pm$ 0.34	41 $\pm$ 3
	AIA	—	1.7 $\pm$ 0.05	7.4 $\pm$ 0.22	23 $\pm$ 0
		+	1.7 $\pm$ 0.11	7.0 $\pm$ 0.14	24 $\pm$ 1
	Cortisol	—	8.0 $\pm$ 0.47	18.4 $\pm$ 0.89	43 $\pm$ 2
		+	8.3 $\pm$ 0.43	18.2 $\pm$ 0.59	46 $\pm$ 1
	Ethanol	—	6.1 $\pm$ 0.18	10.3 $\pm$ 0.48	59 $\pm$ 2
		+	6.1 $\pm$ 0.14	10.0 $\pm$ 0.70	61 $\pm$ 4
	Haematin	—	6.8 $\pm$ 0.47	7.1 $\pm$ 0.57	96 $\pm$ 2
		+	7.0 $\pm$ 0.62	7.2 $\pm$ 0.68	97 $\pm$ 1
	Tryptophan	—	9.5 $\pm$ 0.18	14.2 $\pm$ 0.63	67 $\pm$ 2
		+	9.7 $\pm$ 0.71	13.8 $\pm$ 0.62	70 $\pm$ 3

explanation of these latter findings is that, by freezing the unperfused livers, Stowell & Mørland (1983) might have changed the structure of haemoglobin released from lysed erythrocytes such that haem could have become available for saturation of tryptophan pyrrolase. It is unlikely that freezing could have increased the availability to pyrrolase of the haem of other hepatic haemoproteins, because Stowell & Mørland (1983) found that freezing (and subsequent storage) of perfused livers did not alter pyrrolase activities. In support of the above explanation, we found (results not shown) that haem availability to tryptophan pyrrolase was increased in unperfused (but not perfused) livers were frozen in liquid N₂ even for only 10 min before homogenization, and that addition of allopurinol during homogenization of frozen unperfused livers prevented such an increase. We therefore suggest that only fresh tissue should be used for pyrrolase activity determinations in unperfused livers. The state of oxidation of haemoglobin may also be important, because we found that intravenous administration of a 0.9% NaCl solution of commercial haemoglobin (a significant proportion of which is believed to exist as methaemoglobin, which is the second most commonly used source of haem in pyrrolase activity determinations) increased the haem saturation of rat liver tryptophan pyrrolase and caused a simultaneous decrease in 5-aminolaevulinate synthase (EC 2.3.1.37) activity, whereas neither effect occurred when such a solution was reduced by dithionite before administration (A. A.-B. Badawy & A. N. Welch, unpublished work). The finding in the present work that pyrrolase saturation with haem is

increased by the addition *in vitro* of commercial haemoglobin, but not by the presence of blood in the liver, therefore suggests that blood haemoglobin does not undergo any appreciable oxidation during the 75–80 min period of assay of the enzyme by the procedure of Badawy & Evans (1975) to influence the haem saturation of the enzyme.

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