Tryptophan pyrrolase in haem regulation

The mechanisms of enhancement of rat liver 5-aminolaevulinate synthase activity by starvation and of the glucose effect on induction of the enzyme by 2-allyl-2-isopropylacetamide

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1. Rat liver tryptophan pyrrolase activity is enhanced by a hormonal-type mechanism during the first 2 days of starvation and by a substrate-type mechanism during the subsequent 2 days. 5-Aminolaevulinate synthase activity is also enhanced during the first 2 days of starvation, but returns thereafter to values resembling those observed in the fed rat. Treatments that prevent or reverse the enhancement of tryptophan pyrrolase activity in 24–48 h-starved rats also abolish that of 5-aminolaevulinate synthase activity. Starvation of guinea pigs, which does not enhance the pyrrolase activity, also fails to alter that of the synthase. It is suggested that the decrease in 5-aminolaevulinate synthase activity in 72–96 h-starved rats represents negative-feedback repression of synthesis, possibly involving tryptophan participation, whereas the enhancement observed in 24–48 h-starved animals is caused by positive-feedback induction secondarily to increased utilization of the regulatory-haem pool by the newly synthesized apo-(tryptophan pyrrolase). 2. Glucose, fructose and sucrose abolish the 24 h-starvation-induced increases in rat liver tryptophan pyrrolase and 5-aminolaevulinate synthase activities. Cortisol reverses the glucose effect on 5-aminolaevulinate synthase activity, presumably by enabling pyrrolase to re-utilize the regulatory-haem pool after induction of synthesis of this latter enzyme. 3. The impaired ability of 2-allyl-2-isopropylacetamide to enhance markedly 5-aminolaevulinate synthase activity in 24 h-starved rats treated with glucose is associated with a failure of the porphyrinogen to cause loss of tryptophan pyrrolase haem. Cortisol restores the ability of the porphyrinogen to destroy tryptophan pyrrolase haem and to enhance markedly 5-aminolaevulinate synthase activity, presumably by enhancing tryptophan pyrrolase synthesis and, thereby, its re-utilization of the regulatory-haem pool. It is tentatively suggested that 2-allyl-2-isopropylacetamide destroys the above pool only after it has become bound to (or utilized by) apo-(tryptophan pyrrolase).

Glucose is the component of carbohydrate diets responsible for decreasing the enhanced excretion of porphyrins and their precursors in a variety of experimental (Rose *et al.*, 1961; De Matteis, 1964) and human (Welland *et al.*, 1964; Felsher & Redeker, 1967; Perlroth *et al.*, 1968) hepatic porphyrias, apparently by blocking the induction of the rate-limiting enzyme of the haem-biosynthetic pathway, 5-aminolaevulinate synthase (EC 2.3.1.37)

(Tschudy *et al.*, 1964; Marver *et al.*, 1966b; Bonkowsky *et al.*, 1973). Tschudy (1978) has reviewed the possible mechanisms by which glucose has been proposed to block the induction of various hepatic enzymes, and suggested that none of these mechanisms could explain the glucose effect on 5-aminolaevulinate synthase induction.

It is now well established that 5-aminolaevulinate synthase induction by porphyrinogens such as 2-allyl-2-isopropylacetamide involves a positive-feedback mechanism, the major feature of which is the production of an early loss of liver haem. Although studies of the early loss of cytochrome *P*-450 haem have been instrumental in the development of the

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concept of feedback regulation of haem biosynthesis (De Matteis, 1975, 1978), there is now considerable evidence (see Badawy, 1982, and references cited therein) suggesting that the haem moiety of this major hepatic haemoprotein does not participate in the above regulatory process, and that the latter may involve a small, rapidly-turning-over and readily-exchangeable pool of haem (the so-called regulatory-haem pool) closely associated with the minor cytosolic haemoprotein, tryptophan pyrrolase (tryptophan 2,3-dioxygenase, EC 1.13.11.11) (see also Badawy, 1979; Badawy & Morgan, 1980; Morgan & Badawy, 1980; Welch & Badawy, 1980; Badawy *et al.*, 1981). In view of these findings and of evidence described below, we propose that the glucose effect on 5-aminolaevulinate synthase induction by 2-allyl-2-isopropylacetamide may involve prevention by the sugar of the destruction of the regulatory-haem pool by this porphyrinogen. Thus Morgan & Badawy (1980) presented evidence strongly suggesting that the failure of 2-allyl-2-isopropylacetamide to enhance markedly 5-aminolaevulinate synthase activity in the adrenalectomized-rat liver is associated with impaired destruction of tryptophan pyrrolase haem, because adrenalectomy prevents the utilization of the regulatory-haem pool by tryptophan pyrrolase by decreasing the amount of this enzyme to a value similar to that in intact fed animals. Morgan & Badawy (1980) also found that cortisol enabled the above porphyrinogen to enhance markedly 5-aminolaevulinate synthase activity in adrenalectomized rats by inducing tryptophan pyrrolase synthesis, thereby increasing the amount of haem bound to (or utilized by) the apoenzyme, thus permitting the porphyrinogen to produce its destruction of haem. Impaired enhancement of 5-aminolaevulinate synthase activity and the absence of loss of tryptophan pyrrolase haem have also been observed (Morgan & Badawy, 1980) in livers of starved intact rats after administration of allopurinol (4-hydroxypyrazolo[3,4-*d*]pyrimidine), which, like adrenalectomy, decreased tryptophan pyrrolase activities and therefore the amount of bound haem to values similar to those observed in intact fed animals. Because similar decreases in tryptophan pyrrolase activities and bound haem are produced in starved intact rats by administration of glucose (Badawy, 1981a), the possibility was considered that, under these latter conditions, 2-allyl-2-isopropylacetamide may also find very little haem to destroy.

In the present paper, we present evidence supporting this hypothesis, and demonstrate the ability of cortisol to render 2-allyl-2-isopropylacetamide capable of destroying tryptophan pyrrolase haem and of causing a marked enhancement of 5-aminolaevulinate synthase activity in livers of glucose-treated starved intact rats. Evidence is also presented for

the first time that un-induced 5-aminolaevulinate synthase activity of 24 h-starved rats is decreased by glucose and sugars forming it, and that the synthase activity is no longer enhanced in rats starved for periods longer than 48 h.

Materials and methods

Locally bred male Wistar rats (140–150 g) were maintained on cube diet 41B (Oxoid, Basingstoke, Hants., U.K.) and water, and most of them were starved for 24 h before death. Some rats were starved for longer periods (of up to 96 h), whereas others were examined in the fed state. A few additional experiments were performed with locally bred adult male Hartley–Dunkin guinea pigs.

The sources of various chemicals and the methods of preparation of solutions for, and the routes of, administration of some of them have previously been described (Badawy & Evans, 1976; Badawy & Morgan, 1980).

Tryptophan pyrrolase activity was determined in liver homogenates (Badawy & Evans, 1975; see also the fuller description by Badawy, 1981b) either in the absence (holoenzyme activity) or in the presence (total enzyme activity) of added ($2\mu\text{M}$) haematin. The apoenzyme activity, obtained by difference, was used to calculate the haem-saturation ratio (holoenzyme activity/apoenzyme activity), which indicates the extent of saturation of pyrrolase with haem (see also Badawy, 1979). 5-Aminolaevulinate synthase activity was determined in liver homogenates (Badawy & Morgan, 1980). Serum glucose concentration was determined by the fluorimetric procedure of Slein (1963). Statistical analysis of results was performed by using Student's *t*-test.

Results and discussion

Effects of 2-allyl-2-isopropylacetamide, cortisol or both on liver 5-aminolaevulinate synthase and tryptophan pyrrolase activities in starved control rats

These effects are shown in Table 1 and serve as control results in relation to those of glucose to be described below. Starvation of rats for 24 h enhanced the activities of liver 5-aminolaevulinate synthase and tryptophan pyrrolase without altering the haem-saturation ratio of the latter enzyme. Also cortisol administration to 24 h-starved rats increased tryptophan pyrrolase activity, but did not alter significantly the haem-saturation ratio of this enzyme or the 5-aminolaevulinate synthase activity. The absence of changes in the haem-saturation ratio of tryptophan pyrrolase by starvation or cortisol confirms previous findings (Morgan & Badawy, 1980). By contrast, 2-allyl-2-isopropylacetamide administration to 24 h-starved rats (Table 1)

Table 1. *Effects of 2-allyl-2-isopropylacetamide, cortisol or both on liver 5-aminolaevulinic synthase and tryptophan pyrrolase activities in 24h-starved control and glucose-treated rats*

State	Treat- ment	Line no.	Control rats				Glucose-pretreated rats			
			Pyrrolase activity (μmol of kynurenine formed/h per g wet. wt. of liver)		Pyrrolase haem- saturation ratio	Synthase activity (nmol of 5-amino- laevulinate formed/ min per g wet wt. of liver)	Pyrrolase activity (μmol of kynurenine formed/h per g wet wt. of liver)		Pyrrolase haem- saturation ratio	Synthase activity (nmol of 5-amino- laevulinate formed/ min per g wet wt. of liver)
			Holoenzyme	Total enzyme			Holoenzyme	Total enzyme		
Fed	Nil	(1)	1.5 $\pm$ 0.07	3.7 $\pm$ 0.47	0.68 $\pm$ 0.15	0.42 $\pm$ 0.05	1.4 $\pm$ 0.12	1.4 $\pm$ 0.12**	—	0.35 $\pm$ 0.01
Starved	DMF	(2)	3.8 $\pm$ 0.11***	10.7 $\pm$ 0.44***	0.55 $\pm$ 0.04	0.99 $\pm$ 0.11**	1.8 $\pm$ 0.11***	4.8 $\pm$ 0.19***	0.60 $\pm$ 0.06	0.54 $\pm$ 0.04*
	AIA	(3)	2.3 $\pm$ 0.21***	16.5 $\pm$ 1.08**	0.16 $\pm$ 0.005***	2.23 $\pm$ 0.11***	2.6 $\pm$ 0.17	7.5 $\pm$ 0.28***	0.53 $\pm$ 0.03***	0.80 $\pm$ 0.09***
	Cortisol	(4)	7.2 $\pm$ 0.59**	19.2 $\pm$ 0.87***	0.60 $\pm$ 0.05	0.97 $\pm$ 0.05	3.6 $\pm$ 0.07***	10.7 $\pm$ 0.33***	0.51 $\pm$ 0.01	0.88 $\pm$ 0.06
	Cortisol + AIA	(5)	4.6 $\pm$ 0.33*	22.8 $\pm$ 1.96	0.25 $\pm$ 0.004***	2.08 $\pm$ 0.14***	3.2 $\pm$ 0.12*	14.4 $\pm$ 0.56*	0.28 $\pm$ 0.01†	2.15 $\pm$ 0.13

Rats were starved for 24h before death, but were allowed free access to either drinking water or a solution of glucose (10%, w/v). At 5h before death, the animals received an injection of 2-allyl-2-isopropylacetamide (400mg/kg body wt.), cortisol acetate (20mg/kg body wt.), both compounds (in the same doses) or an equal volume (1ml/kg body wt.) of the solvent dimethylformamide. 5-Aminolaevulinic synthase and tryptophan pyrrolase activities and the haem-saturation ratio of the latter enzyme were determined as described in the Materials and methods section. Values are means $\pm$ s.e.m. for each group of four rats. The values obtained in glucose-pretreated rats are compared with those with the corresponding treatment in the lines containing the results with control animals. In these latter animals, the values in line (2) are compared with those in line (1), whereas those in lines (3) and (4) are compared with those in line (2), and those in line (5) are compared with those in line (4). The significance of the differences is indicated as follows: † $P < 0.05$; * $P < 0.01$; ** $P < 0.005$; *** $P < 0.001$. Abbreviations and symbols: DMF, dimethylformamide; AIA, 2-allyl-2-isopropylacetamide; —, not determined.

increased the total tryptophan pyrrolase activity by 54%, but decreased that of the holoenzyme by 39%. This latter decrease, which represents loss of haem, therefore led to a 71% decrease in the pyrrolase haem-saturation ratio, and this was accompanied by a 125% increase in 5-aminolaevulinate synthase activity. The results in Table 1 also show that the ability of the above porphyrinogen to deplete tryptophan pyrrolase haem and to enhance 5-aminolaevulinate synthase activity in 24 h-starved rats was not influenced by the joint administration of cortisol. All these results confirm those previously reported in 24 h- or 48 h-starved rats (Marver *et al.*, 1966a; Padmanaban *et al.*, 1973; Morgan & Badawy, 1980; Badawy, 1981a).

Effects of glucose on liver 5-aminolaevulinate synthase and tryptophan pyrrolase activities in starved rats and in those treated with 2-allyl-2-isopropylacetamide

These effects are also shown in Table 1. Glucose administration in drinking water abolished or strongly decreased the 24 h-starvation-induced increases in 5-aminolaevulinate synthase and tryptophan pyrrolase activities and caused a 79% inhibition of the net enhancement of activity of the former enzyme by 2-allyl-2-isopropylacetamide. In glucose-treated rats, this porphyrinogen caused 44–56% increases in tryptophan pyrrolase activity [typical of the hormonal-induction mechanism (see also Badawy & Evans, 1973)] and therefore did not alter significantly the pyrrolase haem-saturation ratio. These results therefore demonstrate for the first time the failure of 2-allyl-2-isopropylacetamide to cause a significant loss of tryptophan pyrrolase haem in glucose-pretreated rats, and that this was associated with an impaired enhancement of 5-aminolaevulinate synthase activity (of only 48%, compared with 125% in starved controls). Both findings resemble in relative terms those previously obtained after administration of this porphyrinogen to starved adrenalectomized rats or to starved intact animals given allopurinol (Morgan & Badawy, 1980). The ability of glucose to prevent the starvation-induced increase in 5-aminolaevulinate synthase activity is also reported here for the first time, whereas the prevention of the increase in tryptophan pyrrolase activity confirms a previous finding (Badawy, 1981a). Similarly, the glucose blockade of 5-aminolaevulinate synthase induction by the above porphyrinogen has previously been reported (Tschudy *et al.*, 1964; Marver *et al.*, 1966b; Bonkowsky *et al.*, 1973).

Reversal by cortisol of the effects of glucose on liver 5-aminolaevulinate synthase and tryptophan pyrrolase activities in starved rats and in those treated with 2-allyl-2-isopropylacetamide

Cortisol administration to glucose-pretreated star-

ved rats (Table 1) abolished the sugar-induced decreases in 5-aminolaevulinate synthase and tryptophan pyrrolase activities and enabled 2-allyl-2-isopropylacetamide to cause an enhancement of the synthase activity in glucose-pretreated animals to an extent similar to that achieved in controls. Under these conditions, cortisol also enabled the above porphyrinogen to cause a significant decrease in the tryptophan pyrrolase haem-saturation ratio, thus suggesting that loss of haem has occurred. Such loss may not, however, be evident in absolute terms from the small decrease in the holo-(tryptophan pyrrolase) activity (from 3.6 to 3.2 units), but, had there been no loss of haem, and therefore no decrease in the haem-saturation ratio, then the total tryptophan pyrrolase activity value observed in Table 1 (14.4 units) should have been accompanied by a holoenzyme activity value of about 4.8 units, because 2-allyl-2-isopropylacetamide is known (Badawy & Evans, 1973) to cause a hormonal-type induction of tryptophan pyrrolase activity. It may therefore be concluded that cortisol has indeed enabled this porphyrinogen to cause loss of tryptophan pyrrolase haem in glucose-pretreated rats.

The finding (Table 1) that cortisol reversed the glucose-induced decrease in 5-aminolaevulinate synthase activity in control (non-porphyrinogen-treated) rats is of additional interest, because it could explain the variable clinical response of patients with acute hepatic porphyrias to therapy with carbohydrates (Tschudy, 1978, and references cited therein) in view of the possibility (which has not been examined as far as we could ascertain) that glucocorticoid abnormalities in such patients may influence the glucose repression of 5-aminolaevulinate synthase synthesis and, hence, the therapeutic efficacy of carbohydrates.

Mechanism of the glucose repression of 5-aminolaevulinate synthase induction by 2-allyl-2-isopropylacetamide

The findings in Table 1 therefore strongly suggest that glucose blocks 5-aminolaevulinate synthase induction by 2-allyl-2-isopropylacetamide by a mechanism resembling that exerted by adrenalectomy and by allopurinol administration to intact rats (see Morgan & Badawy, 1980), namely that of preventing the above porphyrinogen from destroying the regulatory-haem pool by rendering apo-(tryptophan pyrrolase) unable to utilize it, and that, by restoring this latter utilization by inducing tryptophan pyrrolase synthesis, cortisol enables the above porphyrinogen to exert its usual effects on tryptophan pyrrolase haem and 5-aminolaevulinate synthase activity in livers of adrenalectomized and glucose-treated intact rats. Because glucocorticoids do not enhance haem biosynthesis, it may be suggested that prevention of the utilization by tryptophan pyrro-

lase of the regulatory-haem pool by the above three treatments leaves this pool in a form (possibly free) that is unavailable for destruction by the above porphyrinogen and may therefore become capable of repressing 5-aminolaevulinate synthase synthesis. This is a mere and tentative suggestion, but it implies that 2-allyl-2-isopropylacetamide can destroy the regulatory-haem pool only if the latter is bound to apo-(tryptophan pyrrolase). This latter implication is certainly supported by the inability of this porphyrinogen to enhance markedly 5-aminolaevulinate synthase activity in adrenalectomized and in allopurinol- or glucose-treated intact rats, in which the regulatory-haem pool is not being utilized by tryptophan pyrrolase. 2-Allyl-2-isopropylacetamide destroys the haem moiety of cytochrome *P*-450 at the site of activation (Ortiz de Montellano *et al.*, 1979), and is also known (De Matteis, 1973) to destroy cytosolic haem. The above suggestion can best be examined by monitoring the fate of this un-utilized haem pool under the above experimental conditions and in control rats in relation to haem destruction in both microsomal fraction and the cytosol.

Effects of glucose and other sugars on 5-aminolaevulinate synthase and tryptophan pyrrolase activities in 24 h-starved rats

As far as we could ascertain, the ability of glucose to decrease 5-aminolaevulinate synthase activity in starved control rats (Table 1) has not previously been reported. Similarly, 5-aminolaevulinate synthase activity of starved rats has not been examined after administration of glucose-forming sugars, although one such sugar, fructose, has been reported

(Bonkowsky *et al.*, 1973) to block 5-aminolaevulinate synthase induction by 2-allyl-2-isopropylacetamide. The results in Table 2 show that the 24 h-starvation-induced increase in 5-aminolaevulinate synthase activity was abolished by fructose and sucrose, as well as by glucose, whether the three sugars were provided in drinking water during the entire 24 h-starvation period or administered by stomach tube (in equimolar doses) 3 h before death. These findings therefore demonstrate the ability of glucose-forming sugars (as well as glucose itself) to decrease the un-induced activity of 5-aminolaevulinate synthase in livers of starved rats. The results in Table 3 show that, under the above conditions, all three sugars abolished the 24 h-starvation-induced enhancement of tryptophan pyrrolase activity.

Glucose prevents the enhancement of tryptophan pyrrolase activity by starvation for 24 h by blocking the increased release of corticosterone into the circulation (Badawy, 1981a), whereas it inhibits the basal tryptophan pyrrolase activity in livers of fed rats by increasing the hepatic concentrations of the allosteric inhibitors NADPH and NADH (Badawy & Evans, 1976). That these two reduced dinucleotides are not involved in the glucose effect in starved rats is also suggested by the findings (results not shown) that the sugar-induced decreases in tryptophan pyrrolase activities in these latter animals are not reversed by the addition *in vitro* of NAD(P)⁺ or by administration *in vivo* of phenazine methosulphate (10 mg/kg body wt.) or fructose (400 mg/kg body wt.) 2 h previously by the intraperitoneal route. All these treatments *in vitro* and *in vivo* reverse the inhibition of tryptophan pyrrolase activity by glucose in livers of fed rats (Badawy &

Table 2. *Effects of glucose, fructose and sucrose on liver 5-aminolaevulinate synthase activity in 24 h-starved rats*
Rats were starved for 24 h before being killed. Sugars were administered orally either in drinking water during the entire 24 h-starvation period or by stomach tube 3 h before death. When administered in drinking water, the concentrations of glucose, fructose and sucrose were 10, 10 and 20% (w/v) respectively, whereas the doses given by stomach tube were 15, 15 and 30 g/kg body wt. respectively. Control rats received either drinking water or 30 ml of water/kg body wt. by stomach tube. 5-Aminolaevulinate synthase activity was determined as described in the Materials and methods section. Values (including those in fed animals) are means $\pm$ S.E.M. for each group of four rats. The activity of the enzyme in control (water-treated) starved rats is compared with that given for fed animals, whereas those observed in starved rats treated with sugars are compared with the corresponding control (water) values in starved animals. The significance of differences is indicated as follows: †††*P* < 0.02; ***P* < 0.005; ****P* < 0.001.

Nutrition	Treatment	Method	Synthase activity (nmol of 5-aminolaevulinate formed/min per g wet wt. of liver)	
			Drinking water	Stomach tube
Fed	Nil		0.49 $\pm$ 0.03	0.49 $\pm$ 0.03
Starved	Water		0.90 $\pm$ 0.08***	1.21 $\pm$ 0.12**
	Glucose		0.34 $\pm$ 0.05**	0.29 $\pm$ 0.01***
	Fructose		0.44 $\pm$ 0.04**	0.50 $\pm$ 0.03**
	Sucrose		0.59 $\pm$ 0.03†††	0.34 $\pm$ 0.03***

Table 3. *Effects of glucose, fructose and sucrose on liver tryptophan pyrrolase activity in 24 h-starved rats*
Experimental details and comparisons of the results are as described in Table 2. Tryptophan pyrrolase activities were determined as described in the Materials and methods section. Values are means $\pm$ S.E.M. for each group of four rats. The significance of differences is indicated as follows: ††† P < 0.02; ** P < 0.005; *** P < 0.001.

		Tryptophan pyrrolase activity (μ mol of kynurenine formed/h per g wet wt. of liver)			
		Drinking water		Stomach tube	
Nutrition	Treatment	Holoenzyme	Total enzyme	Holoenzyme	Total enzyme
Fed	Nil	1.7 $\pm$ 0.05	3.8 $\pm$ 0.13	1.7 $\pm$ 0.05	3.8 $\pm$ 0.13
Starved	Water	4.8 $\pm$ 0.05***	9.6 $\pm$ 0.85***	3.5 $\pm$ 0.04***	8.2 $\pm$ 0.96**
	Glucose	1.7 $\pm$ 0.07***	3.5 $\pm$ 0.30***	2.1 $\pm$ 0.16***	4.9 $\pm$ 0.14†††
	Fructose	1.5 $\pm$ 0.07***	3.1 $\pm$ 0.15***	1.6 $\pm$ 0.05***	2.2 $\pm$ 0.14***
	Sucrose	1.8 $\pm$ 0.04***	3.0 $\pm$ 0.14***	1.5 $\pm$ 0.09***	2.0 $\pm$ 0.08***

Evans, 1976). Similarly, these treatments *in vitro* and *in vivo* also failed to reverse the glucose-induced decrease in 5-aminolaevulinate synthase activity of 24h-starved rats (results not shown). The mechanism by which glucose abolishes the enhancement of 5-aminolaevulinate synthase activity in 24h-starved rats is discussed below in relation to starvation.

Effects of starvation on rat liver 5-aminolaevulinate synthase and tryptophan pyrrolase activities

Very little work has been done on the effects of starvation on rat liver 5-aminolaevulinate synthase activity. Thus the enzyme activity is at least doubled by starvation for 24h (Tables 1 and 2) or 48h (Morgan & Badawy, 1980), and is increased by 39–77% (depending on the assay method) by starvation for 40h (Bock *et al.*, 1971). The results in Table 4 show that starvation of rats for 24h or 48h enhanced 5-aminolaevulinate synthase activity by 163 and 107% respectively, but that the activity of the enzyme returned to values not significantly different from that observed in livers of fed animals at 72–96h after the start of starvation.

The results in Table 4 also show that starvation of rats for 24h or 48h caused a hormonal-type induction of tryptophan pyrrolase activity characterized by proportionate increases in both the holoenzyme and total enzyme activities, and therefore did not alter the haem-saturation ratio of the enzyme (see Badawy, 1979, 1981a). By contrast, starvation for 72h or 96h did not cause a further increase in the total tryptophan pyrrolase activity above that observed at 48h, but elicited a further enhancement of that of the holoenzyme. This therefore led to an increase in the haem-saturation ratio of the tryptophan pyrrolase, of 100% and 124% at 72h and 96h respectively. These latter changes are characteristic of the substrate (tryptophan) or the cofactor (haem) mechanisms of tryptophan pyrrolase activation (see also Badawy &

Evans, 1975; Badawy, 1979), and together with the decrease in 5-aminolaevulinate synthase activity may be caused by tryptophan (see Badawy *et al.*, 1981), whose availability to the liver could be increased secondarily to its likely release from tissue proteins during the above prolonged periods of starvation. The possibility that the above changes observed at 72–96h of starvation could have resulted from an increased availability of haem secondarily to a possible acceleration of turnover of hepatic haemoproteins, in particular cytochrome P-450, cannot be ruled out entirely, but is unlikely, because tryptophan pyrrolase does not utilize microsomal haem (Badawy & Morgan, 1980; A. A.-B. Badawy, unpublished work comparing whole-liver homogenates and cytosolic preparations). It may also be suggested that the decrease in 5-aminolaevulinate synthase activity from the enhanced values at 24–48h of starvation to those observed at subsequent time intervals represents negative-feedback repression of synthase synthesis. These results also suggest that, in studies on the regulation of 5-aminolaevulinate synthase in the rat, the animals must not be starved for periods longer than 48h.

In experiments not shown here, it was found that glucose administration in drinking water (10%, w/v) was capable of preventing the enhancement of tryptophan pyrrolase activity during the 4-day starvation period and the increase in that of 5-aminolaevulinate synthase during the first 2 days of starvation.

Mechanism of the early starvation-induced enhancement of rat liver 5-aminolaevulinate synthase activity

That the enhancement of 5-aminolaevulinate synthase activity in 24h-starved rats is not caused by the associated hypoglycaemia is suggested by the results in Table 5, which show that this enhance-

Table 4. *Effects of starvation on the activities of rat liver 5-aminolaevulinate synthase and tryptophan pyrrolase*
Rats were starved for up to 96 h before death, but were allowed free access to drinking water. 5-Aminolaevulinate synthase and tryptophan pyrrolase activities and the haem-saturation ratio of this latter enzyme were determined as described in the Materials and methods section. Values are means $\pm$ S.E.M. for each group of four rats. The values in starved rats are compared with those in fed animals, and the significance of the differences is indicated as follows: * $P < 0.01$; ** $P < 0.005$; *** $P < 0.001$.

Starvation (days)	Tryptophan pyrrolase activity (μ mol of kynurenine formed/h per g wet wt. of liver)		Tryptophan pyrrolase haem-saturation ratio	Synthesis of 5-aminolaevulinate (nmol formed/ min per g wet wt. of liver)
	Holoenzyme	Total enzyme		
0	2.4 $\pm$ 0.13	5.2 $\pm$ 0.12	0.86 $\pm$ 0.04	0.43 $\pm$ 0.04
1	5.2 $\pm$ 0.39***	11.7 $\pm$ 0.28***	0.80 $\pm$ 0.03	1.13 $\pm$ 0.17*
2	5.5 $\pm$ 0.59**	11.9 $\pm$ 1.42**	0.86 $\pm$ 0.05	0.89 $\pm$ 0.09**
3	8.1 $\pm$ 0.59***	12.8 $\pm$ 0.48***	1.72 $\pm$ 0.12***	0.50 $\pm$ 0.05
4	8.1 $\pm$ 0.89***	12.3 $\pm$ 1.23***	1.93 $\pm$ 0.10***	0.53 $\pm$ 0.05

Table 5. *Effects of allopurinol on the 24 h-starvation-induced changes in rat liver 5-aminolaevulinate synthase and tryptophan pyrrolase activities and in serum glucose concentration*

Fed and 24 h-starved rats received, 2 h before death, an intraperitoneal injection of either allopurinol (20 mg/kg body wt.) or an equal volume (4 ml/kg body wt.) of 0.9% NaCl. Liver 5-aminolaevulinate synthase and tryptophan pyrrolase activities and serum glucose concentration were determined as described in the Materials and methods section. Serum glucose concentration is expressed in mg/dl, whereas the activities of 5-aminolaevulinate synthase and tryptophan pyrrolase are expressed as in Table 1. Values are means $\pm$ S.E.M. for each group of six (serum glucose determinations) or of four rats (all other determinations). The values in columns (2) and (3) are compared with those in column (1), whereas those in column (4) are compared with those in column (3). The significance of differences is indicated as follows: ** $P < 0.005$; *** $P < 0.001$.

Determination	Nutritional state	0.9% NaCl		Allopurinol	
		Fed rats (1)	Starved rats (2)	Fed rats (3)	Starved rats (4)
Serum glucose		130.00 $\pm$ 2.00	76.00 $\pm$ 2.00***	141.00 $\pm$ 9.00	75.00 $\pm$ 6.00***
Synthase activity		0.58 $\pm$ 0.03	1.62 $\pm$ 0.08***	0.59 $\pm$ 0.07	0.97 $\pm$ 0.03**
Pyrrolase activity:					
Holoenzyme		3.40 $\pm$ 0.10	7.00 $\pm$ 0.76**	3.20 $\pm$ 0.10	3.70 $\pm$ 0.23
Total enzyme		7.60 $\pm$ 0.20	15.70 $\pm$ 0.83***	3.40 $\pm$ 0.00***	6.10 $\pm$ 0.49**

ment was decreased by allopurinol in the absence of any normalization of serum glucose concentration. Allopurinol administration to starved rats also reversed the enhancement of tryptophan pyrrolase activity, a finding previously reported in 48 h-starved animals (Morgan & Badawy, 1980; Badawy *et al.*, 1981). The results in Table 5 also show that, in fed rats, allopurinol exerted no significant effects on serum glucose concentration or on 5-aminolaevulinate synthase or holo-(tryptophan pyrrolase) activities, but that it decreased that of the total tryptophan pyrrolase by 55%. This latter effect is caused by prevention by allopurinol of the conjugation of the apoenzyme with haem added *in vitro*, and, since this does not involve endogenous hepatic haem *in vivo*, it is not surprising that allopurinol did

not influence 5-aminolaevulinate synthase activity in these fed rats.

These results therefore exclude the possible involvement of decreased serum glucose concentration in the enhancement of 5-aminolaevulinate synthase activity in 24–48 h-starved rats. It is also unlikely that this enhancement involves an increased turnover of hepatic haemoproteins, because, although such increased turnover (of cytochrome P-450) has been demonstrated in starved rats given endotoxin or haemin, it is associated with a decrease, rather than an increase, in 5-aminolaevulinate synthase activity (Bissell & Hammaker, 1976a,b). Moreover, we have obtained evidence (A. A.-B. Badawy & C. J. Morgan, unpublished work) suggesting that the endotoxin-induced decrease in

5-aminolaevulinate synthase activity, which is associated with an increased saturation of tryptophan pyrrolase with haem (see Bissell & Hammaker, 1977), is not caused by increased turnover of hepatic haemoproteins, but is elicited by tryptophan itself secondarily to the lipolytic effect of the lipopolysaccharide.

This leaves us with the only apparent alternative possibility proposed by Morgan & Badawy (1980), namely that enhancement of 5-aminolaevulinate synthase activity in 24–48 h-starved rats is caused by an increase in the utilization of the regulatory-haem pool (which is presumably present normally in the liver of the fed rat un-utilized by tryptophan pyrrolase and therefore capable of playing its normal repressive role) by the newly synthesized apo-(tryptophan pyrrolase), thus removing this pool from its normal role. This possibility is supported by a number of findings. (1) The enhancement of 5-aminolaevulinate synthase activity by starvation is always prevented or reversed by treatments that cause parallel changes in tryptophan pyrrolase activity and, hence, the amount of haem bound to the apoenzyme. Such treatments include adrenalectomy, allopurinol administration to 48 h-starved intact rats (Morgan & Badawy, 1980), benserazide administration to 48 h-starved intact rats (Badawy *et al.*, 1981) and administration to 24 h-starved rats of allopurinol, glucose, fructose or sucrose (the present work). (2) The ability of cortisol to restore tryptophan pyrrolase activities of glucose-pretreated 24 h-starved rats to normal (i.e. to the observed values in starvation) and to reverse the glucose-induced decrease in 5-aminolaevulinate synthase activity in such rats (Table 1). (3) The failure of the β -adrenoreceptor-blocking agent propranolol to reverse the starvation-induced enhancement of tryptophan pyrrolase activity (Badawy, 1981a) and the associated increase in that of 5-aminolaevulinate synthase (A. A.-B. Badawy & C. J. Morgan, unpublished work). (4) The absence of any increases in tryptophan pyrrolase or 5-aminolaevulinate synthase activities by starvation of guinea pigs. Thus in guinea pigs starved for 0, 1 and 2 days, tryptophan pyrrolase activity (only the holoenzyme, because the apoenzyme is permanently absent; in μmol of kynurenine formed/h per g wet wt. of liver, average for four animals per group) was 1.9, 2.1 and 2.1 respectively, whereas the corresponding values for 5-aminolaevulinate synthase activity (in nmol of 5-aminolaevulinate formed/min per g wet wt. of liver) were 0.39, 0.40 and 0.41 respectively. As stated above, guinea-pig liver tryptophan pyrrolase exists only as the holoenzyme and the activity cannot be induced by cortisol (Badawy & Evans, 1974). This latter finding may explain why tryptophan pyrrolase activity is not enhanced in the guinea pig by starvation. Also, the absence of apo-(trypto-

phan pyrrolase) from guinea-pig liver suggests that less haem may be utilized by the enzyme of this species, compared with that of the rat, and that, accordingly, there may be an excess of the regulatory-haem pool in the former species to explain the usually low activity of guinea-pig liver 5-aminolaevulinate synthase (Irving & Elliott, 1969).

The above findings are therefore compatible with the hypothesis that 5-aminolaevulinate synthase activity is enhanced in 24–48 h-starved rats by a positive-feedback mechanism involving an increased utilization of the regulatory-haem pool by the newly synthesized apo-(tryptophan pyrrolase), and that this further suggests that 5-aminolaevulinate synthase synthesis is normally maintained in the liver of fed rats by the inability of the basal tryptophan pyrrolase to utilize the above pool.

Concluding remarks

The present findings have provided explanations of the possible mechanisms by which starvation of rats enhances liver 5-aminolaevulinate synthase activity and of how glucose and other treatments block this enhancement and the induction of the enzyme in experimental porphyria. As well as providing further support to the role of tryptophan pyrrolase in haem regulation, the results have also illustrated the complexity of the relationship between 5-aminolaevulinate synthase activity and the haem saturation of, and haem utilization by, this minor haemoprotein, the use of which in further studies may lead to a better understanding of the regulation of haem biosynthesis.

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