Biochem. J. (1976) **158**, 79–88
Printed in Great Britain

Animal Liver Tryptophan Pyrrolases

ABSENCE OF APOENZYME AND OF HORMONAL INDUCTION MECHANISM FROM SPECIES SENSITIVE TO TRYPTOPHAN TOXICITY

BY ABDULLA A.-B. BADAWY and MYRDDIN EVANS
University Hospital of Wales, Addiction Unit Research Laboratory, Whitchurch Hospital, Cardiff CF4 7XB, Wales, U.K.

(*Received* 1 *March* 1976)

1. Liver tryptophan pyrrolase exists as holoenzyme and apoenzyme in rat, mouse, pig, turkey, chicken and possibly man. 2. The apoenzyme is absent from cat, frog, gerbil, guinea pig, hamster, ox, sheep and rabbit. 3. The hormonal mechanism of induction of the pyrrolase is absent from species lacking the apoenzyme. 4. The concentrations of tryptophan in livers and sera of these species are lower than in species possessing the apoenzyme. 5. Species lacking the apoenzyme or the hormonal induction mechanism have a deficient kynurenine pathway and are sensitive to the toxicity of tryptophan. 6. It is suggested that these species are not suitable as models for studying human tryptophan metabolism. 7. The possible significance of these findings in relation to veterinary and human neonatal care is discussed.

Tryptophan pyrrolase [L-tryptophan–oxygen 2,3-oxidoreductase (decyclizing), EC 1.13.11.11] is the haem-dependent liver cytosol enzyme that catalyses the conversion of L-tryptophan into formylkynurenine. This reaction represents the first and rate-limiting step in the quantitatively most important (kynurenine–nicotinic acid) pathway of tryptophan degradation in the rat. The pyrrolase has been most extensively studied in rat liver, where at least two types of activity exist. The already active reduced holoenzyme does not require the addition of exogenous haematin for demonstration of its activity *in vitro* (Feigelson & Greengard, 1961), whereas the haem-free predominant form or apoenzyme does. By using the catalytic property of the rat liver enzyme, labelled amino acid incorporation into its purified protein and immunological titration techniques (Schimke *et al.*, 1965; Knox, 1966; Schimke, 1969), it has been possible clearly and conclusively to distinguish two mechanisms by which the pyrrolase activity is regulated. Glucocorticoids cause a hormonal-type induction involving the synthesis of new apoenzyme, whereas tryptophan produces a substrate-type enhancement consisting of decreased degradation of pre-existing apoenzyme in the presence of the normal rate of its synthesis. Rat liver tryptophan pyrrolase is also regulated by its cofactor haem and by NAD(P)H (Badawy & Evans, 1975*c*, 1976).

In an investigation of the substrate and hormonal mechanisms, Knox (1966) reported that adrenalectomized rats did not survive repeated injections of tryptophan, and suggested that in the absence of adrenocorticosteroid-mediated apo-(tryptophan pyrrolase) synthesis, the enzyme in livers of adrenalectomized rats cannot cope with the excess of tryptophan, which is then metabolized by other pathways, the products of which are toxic. In support of this suggestion, Knox (1966) found that treatment of adrenalectomized rats with a single dose of cortisol protects against death from repeated injections of tryptophan. That at least one of the metabolites of tryptophan responsible for the toxicity is 5-hydroxytryptamine is suggested by the finding (Horita & Carino, 1970) that the toxicity of a large dose of the amino acid (as assessed in terms of the number of deaths) is increased by pretreatment of intact rats with the monoamine oxidase inhibitor pargyline, whereas pretreatment with the tryptophan hydroxylase inhibitor *p*-chlorophenylalanine decreases the toxicity in rats given tryptophan plus pargyline.

Death from repeated doses of tryptophan has also been reported in the intact guinea pig (Hvitfelt & Santti, 1972), channel (American) catfish, *Ictalurus punctatus* (Brown & Dodgen, 1968) and bovine (steer) (Johnson & Dyer, 1966). The Mongolian gerbil (*Meriones unguiculatus*) survives for at least 4h after a single dose of tryptophan, but shows signs of poisoning during this period (Baughman & Franz, 1971). A feature common to all these tryptophan-sensitive species [and also frog, *Rana pipiens* (Spiegel, 1961) and cat (Leklem *et al.*, 1969)] is the absence of the hormonal induction mechanism. In addition, it has been reported (Hvitfelt & Santti, 1972; Badawy & Evans, 1974) that guinea-pig liver tryptophan pyrrolase exists only as holoenzyme, with no detectable apoenzyme activity. By contrast, human liver tryptophan pyrrolase most likely exists in both forms and can be induced by cortisol administration (Altman & Greengard, 1966), and man is able to survive repeated doses of tryptophan during treatment of depressive illness (for doses, see Young

& Sourkes, 1974). The mouse occupies the other extreme position, because, although indirect evidence suggests that it possesses both forms of the pyrrolase, adrenalectomy is not associated with tryptophan toxicity (Monroe, 1968).

Except for mouse, rat, guinea pig and man, there is little information on the form(s) of tryptophan pyrrolase in livers of various animal species, particularly those known to be sensitive to the toxicity of tryptophan. This information is desirable in the choice of animal models suitable for studying tryptophan metabolism (and particularly the relationship between tryptophan pyrrolase activity and brain 5-hydroxytryptamine concentration and/or indolylamine synthesis in general), in mood disorders, in drug-dependence and other psychological conditions. For example, unlike man (Hankes *et al.*, 1967), the bovine, with a low tryptophan pyrrolase activity (Carlson & Dyer, 1970) and a poor kynurenine pathway, metabolizes tryptophan largely via the indolylamine routes (Yang & Carlson, 1972). Experiments were therefore designed to examine: (1) the form(s) of liver tryptophan pyrrolase in various animal species; (2) the relationship(s) between the apoenzyme, the hormonal induction mechanism and the toxicity of tryptophan; (3) the concentrations of tryptophan in liver and serum and the binding of tryptophan to serum proteins in relation to one or more of the above phenomena. The present paper describes and discusses the results of these experiments. The absence of apo-(tryptophan pyrrolase) activity and the failure of chronic ethanol administration to inhibit the holoenzyme in livers of golden hamsters have been reported (Badawy & Evans, 1975*b*).

Materials and Methods

Chemicals

Actinomycin D and allopurinol (4-hydroxypyrazolo[3,4-*d*]pyrimidine) were gifts respectively from Merck, Sharp and Dohme, Hoddesdon, Herts., U.K., and the Wellcome Foundation, London NW1 2BP, U.K. 5-Aminolaevulinate hydrochloride, 2,2′-bipyridyl, cortisol 21-acetate, cycloheximide, NAD^+, NADH and L-tryptophan were from Sigma (London) Chemical Co., Kingston-upon-Thames, Surrey, U.K. Ethanol (99.7–100%), formaldehyde solution (37–40%, w/v) and all other chemicals (from BDH Chemicals, Poole, Dorset, U.K.) were of AnalaR grade, except for dimethylformamide, haemin (haematin hydrochloride) and sodium salicylate, which were of reagent grade.

Animal species

Only adult animals were used, and, except for frogs and sheep, all species tested were of the male sex. Rats (Wistar), mice (CBA/CA), guinea pigs (Hartley–Dunkin) and Mongolian gerbils were locally bred. Samples of ox, pig and sheep liver and blood were freshly obtained from the Cardiff Abattoir, and those from a cat were obtained from a nearby University Department. Livers of these species were packed on ice during transport to this Laboratory and were analysed within 30–40min of death. Frogs (*Rana temporaria*) were from T. Gerrard and Co., East Preston, West Sussex, U.K., and New Zealand rabbits came from Cheshire Rabbit Farm, Tarporley, Cheshire, U.K. Golden (Syrian) hamsters (*Mesocricetus auratus*) were purchased from Wrights of Essex, Latchingdon, Chelmsford, Essex, U.K., and chickens and turkeys were from Orchards Farm, Little Kingshill, Great Missenden, Bucks., U.K. All species were given standard laboratory or farm diets and water *ad libitum*, and none had been treated with drugs or anabolic substances. The cat liver and blood samples were, however, obtained under pentobarbitone anaesthesia.

Doses, methods of preparation and administration of various compounds and additions *in vitro* have previously been described (Badawy & Evans, 1973, 1974, 1975*a*,*c*).

Determination of liver tryptophan pyrrolase activity

The activity of the enzyme was determined in liver homogenates by measuring the formation of kynurenine from L-tryptophan (Feigelson & Greengard, 1961) in either the absence (holoenzyme activity) or presence (total enzyme activity) of added haematin. The assay of the rat liver enzyme has previously been described in detail (see, e.g., Badawy & Evans, 1975*c*). The same procedure was used to determine the enzyme activity in other animal species, except that in some cases a more concentrated homogenate (1g liver wet wt. plus 2.75ml of homogenizing medium) was used, and in species with liver wet weights of 1g or less, the volume of the incubation mixture was scaled down by a half and/or livers from two or three animals were pooled for each assay. In species other than rat, mouse or pig, the E_{321} (for formylkynurenine) was also measured.

Determination of liver and serum tryptophan concentrations and tryptophan binding to serum proteins

The concentrations of liver, serum free (ultrafiltrable) and serum total (acid-soluble) tryptophan were essentially determined by a modification (Bloxam & Warren, 1974) of the method of Denckla & Dewey (1967).

(*a*) *Liver tryptophan.* Liver (1g) was homogenized in 7ml of ice-cold 12.5% (w/v) trichloroacetic acid. The homogenate was centrifuged at 3000*g* for

10min after being left at room temperature (22°C) for a similar time. Duplicate portions (0.2ml) of the supernatant were each mixed with 1.8ml of the 12.5% trichloroacetic acid in graduated (10ml) glass conical centrifuge tubes.

(*b*) *Serum total tryptophan.* Duplicate samples of serum (0.05ml) were each mixed with 2.0ml of the 12.5% trichloroacetic acid. The mixture was centrifuged at 3000*g* for 10min after standing at room temperature for a similar time. The supernatant was then quantitatively decanted into assay tubes.

(*c*) *Serum free tryptophan.* Serum (1.5, 1.0 or 0.5ml, depending on species) was ultrafiltered on Centriflo CF-50A membrane cones (Amicon Ltd., High Wycombe, Bucks., U.K.) by centrifugation at 3000*g* for 3–7min at room temperature. Samples (0.1 or 0.2ml) of each ultrafiltrate were mixed with 1.9 or 1.8ml of the 12.5% trichloroacetic acid.

(*d*) *Spectrofluorimetric determination.* Each of the above extracts was treated with 0.2ml of 2% (w/v) formaldehyde, followed by 0.1ml of 0.3mM-$FeCl_3$ in 10% (w/v) trichloroacetic acid. After mixing, the tubes were covered with glass marbles and then placed in a boiling-water bath for 1h. After cooling, the volume of fluid in each tube (which was decreased by evaporation) was adjusted to the 2ml mark with 10% trichloroacetic acid and the solution was thoroughly mixed. Fluorescence was measured with an Aminco–Bowman spectrophotofluorimeter (uncorrected excitation and emission wavelengths were 373 and 452nm respectively). Test samples were read against similarly treated water blanks, and amounts were measured by reference to standard solutions containing known concentrations of tryptophan.

Results

Form(s) of tryptophan pyrrolase in various animal species

Tryptophan pyrrolase activity was determined in liver homogenates of various animal species (Table 1). The holoenzyme (that measured in the absence of added haematin) was present in all species tested. The optimum concentration of haematin required to demonstrate the apoenzyme activity in rat liver homogenates is 2μM (Badawy & Smith, 1971). By using concentrations of haematin of 2–8μM, the apoenzyme activity was also demonstrated in liver homogenates from mice, pigs, chickens and turkeys. By constrast, there was no detectable apo-(tryptophan pyrrolase) activity in guinea pig, golden hamster, gerbil, cat, rabbit, frog, ox or sheep. No formylkynurenine accumulated during incubation of liver homogenates from these species, as there was no increase in the E_{321}.

Properties of golden-hamster liver tryptophan pyrrolase

Golden-hamster liver tryptophan pyrrolase was then examined in some detail, since, as far as we could ascertain, it has not previously been studied, and also because it was of interest to determine whether the absence of the apoenzyme is associated with a lack of the hormonal induction mechanism.

Table 1. *Tryptophan pyrrolase activity in liver homogenates of various animal species*

The enzyme activity was determined, as described in the Materials and Methods section, by incubating liver homogenates in the absence or presence of various concentrations of haematin. Only one result for the cat is shown. Values for other species are means ± S.E.M. for each group of four experiments, each of which was performed with one animal liver, except in mice and hamsters where three livers were pooled.

Species	Concn. of haematin (μM)	Kynurenine formed (μmol/h per g wet wt. of liver)				
		0	2	4	6	8
Rat		1.6±0.1	3.8±0.2	3.8±0.2	3.6±0.3	3.3±0.2
Mouse		1.4±0.1	2.8±0.2	2.8±0.2	2.5±0.3	2.2±0.2
Pig		2.4±0.2	4.1±0.2	4.3±0.6	3.9±0.2	4.1±0.3
Turkey		2.0±0.1	3.4±0.3	3.3±0.2	3.1±0.3	2.9±0.2
Chicken		1.9±0.2	3.3±0.2	3.2±0.2	2.7±0.1	2.7±0.2
Cat		0.84	0.82	0.78	0.82	0.79
Frog		2.5±0.2	2.4±0.1	2.3±0.1	2.3±0.2	2.3±0.1
Gerbil		1.2±0.1	1.2±0.1	1.2±0.1	1.1±0.1	1.2±0.1
Guinea pig		2.3±0.2	2.1±0.1	2.2±0.2	2.3±0.2	2.0±0.2
Hamster		2.4±0.2	2.4±0.3	2.3±0.3	2.1±0.2	2.0±0.4
Ox		0.8±0.1	0.8±0.1	0.8±0.1	0.8±0.1	0.7±0.1
Sheep		0.8±0.1	0.8±0.1	0.8±0.1	0.8±0.1	0.8±0.1
Rabbit		1.3±0.1	1.2±0.1	1.3±0.1	1.3±0.1	1.3±0.1

Table 2. *Comparison of the effects of activators and inhibitors on tryptophan pyrrolase activity in liver homogenates of rats and golden hamsters*

The results are given as the mean ± S.E.M. of homogenates from four separate animals incubated in the absence of added haematin. The final concentrations of the compounds tested are as follows: ascorbate (5mM); ethanol (10mM); NAD^+ (2mM); NADH (5mM); allopurinol (0.05 mM); sodium salicylate (2mM); 2,2'-bipyridyl (0.1mM). In the experiments with allopurinol, (*a*) indicates the absence and (*b*) the presence of added haematin (2μM).

	Kynurenine formed (μmol/h per g wet wt. of liver)			
	Rat		Hamster	
Addition	Control	Test	Control	Test
Ascorbate	1.5±0.2	3.0±0.2	2.5±0.1	2.2±0.1
NADH	1.5±0.3	3.2±0.1	2.5±0.1	2.5±0.1
Ethanol+NAD^+	1.5±0.2	3.0±0.2	2.5±0.1	2.4±0.2
Allopurinol (*a*)	1.3±0.2	1.3±0.2	2.5±0.2	2.5±0.3
Allopurinol (*b*)	3.3±0.3	1.5±0.2	2.5±0.1	2.4±0.2
Salicylate	1.4±0.1	0.0	2.5±0.2	2.4±0.3
2,2'-Bipyridyl	1.5±0.1	0.7±0.1	2.5±0.2	2.5±0.2

Table 3. *Effects of administration of various compounds on liver tryptophan pyrrolase activity in golden hamsters and frogs*

Each hamster received an intraperitoneal injection of dimethylformamide (1ml/kg), cortisol acetate (20mg/kg), 0.9% NaCl (1ml/kg), tryptophan (200mg/kg), sodium salicylate (400mg/kg) or ethanol (5ml/kg) 4h before being killed. 5-Aminolaevulinate hydrochloride was given in four doses (15mg/kg) at 1h intervals and the animals were killed 1h after the last injection. Frogs received the same doses of the first four compounds by injections into the dorsal lymph glands. The enzyme activity was determined, as described in the Materials and Methods section, in the absence of added haematin. Values are means ± S.E.M. of each group of four animals.

	Kynurenine formed (μmol/h per g wet wt. of liver)	
Injection(s)	Golden hamster	Frog
Dimethylformamide	2.2±0.2	2.4±0.2
Cortisol	2.2±0.1	2.4±0.1
0.9% NaCl	2.4±0.1	2.0±0.1
Tryptophan	8.2±0.6	7.1±0.5
Cortisol+tryptophan	6.6±0.4	—
Tryptophan	6.8±0.9	—
Salicylate	2.6±0.2	—
Ethanol	2.7±0.1	—
5-Aminolaevulinate	2.6±0.3	—

The hamster enzyme was assayed in the absence and in the presence of compounds known either to activate or to inhibit the rat liver enzyme *in vitro* (Table 2). No activation of the hamster enzyme was produced by ascorbate, NADH or ethanol plus NAD^+. Allopurinol, which inhibits the haematin activation of the apoenzyme of rat liver, did not alter the activity of the hamster enzyme whether the incubation mixture lacked or contained added haematin. Salicylate and 2,2'-bipyridyl, which have been shown to inhibit the rat liver holoenzyme activity (Badawy & Smith, 1971), were also without effect on the golden-hamster enzyme. The inability of all the above agents to affect the hamster enzyme activity was not changed by the presence of added haematin.

The effects of various treatments *in vivo* on golden-hamster (and also frog) liver tryptophan pyrrolase activity are shown in Table 3. The activity of the hamster enzyme was not altered at 4h after the administration of dimethylformamide, cortisol, salicylate, ethanol or 5-aminolaevulinate. There was no difference in activity in the absence or presence of added haematin. Only tryptophan increased the enzyme activity in hamster liver, by 3.4-fold. The combined injection of tryptophan plus cortisol gave results, with the hamster enzyme, similar to those observed after the administration of tryptophan alone. The frog liver enzyme activity was increased at 4h after the administration of tryptophan (by 3.5-fold), but was unaltered by treatment with cortisol.

The time-course of the tryptophan-induced rise in golden-hamster liver tryptophan pyrrolase activity is shown in Fig. 1. As early as 30min after the injection of tryptophan (200mg/kg body wt.), there was a 150% ($P < 0.005$) increase in the enzyme activity. The activity continued to rise, reached a maximum (3.4 times the basal activity) at 4h and finally fell to normal at 6–7h. During the entire time-course of this enhancement, no further activation of the enzyme was observed when haematin was added to the incubation mixtures *in vitro*.

The increase in the pyrrolase activity observed at 4h after administration of tryptophan was inhibited by 2, 38 and 86% by pretreatment of golden hamsters with actinomycin D, cycloheximide or allopurinol respectively (Table 4). Pretreatment with allopurinol also strongly (90%) inhibited the rise in the enzyme activity observed at 30min after tryptophan administration.

The early responses of rat, golden-hamster and frog liver tryptophan pyrrolases were compared at 30min after the injection of various doses of tryptophan (Fig. 2). A 100mg/kg dose of tryptophan was sufficient to produce maximum or near-maximum increases (2.1–2.7-fold; $P < 0.001$) in the activity of the hamster, frog and also guinea pig (included for comparison; see Badawy & Evans, 1974) enzyme, whereas doses of up to 500mg/kg were without effect on the rat enzyme.

The addition of NAD^+ (2mM) to guinea-pig liver homogenates or the administration of phenazine methosulphate (10mg/kg) to guinea pigs 1h before death did not alter the holoenzyme activity (assayed in the absence of added haematin), and did not reveal any extra activity when haematin (2μM) was included in the incubation mixtures.

Toxicity of tryptophan in frogs and golden hamsters

Four injections (at doses of tryptophan of 500mg/kg each) were administered, at 2h intervals into 10 frogs and 10 golden hamsters. Similar numbers of animals of each species received equal volumes of 0.9% NaCl in similar regimens. Animals receiving the 0.9% NaCl did not die or show any signs of poisoning. Of the tryptophan-treated frogs, one died after two injections, another died after three injections and the remaining eight died after four injections (within 16h of the first injection). None of the tryptophan-treated hamsters died. All of them, however, showed signs of poisoning as early as 1h after the first injection. They experienced difficulty in walking, becoming limp and listless.

Liver and serum tryptophan concentrations and tryptophan binding to serum proteins in various animal species

The concentrations of tryptophan in livers and sera of various animal species are shown in Table 5. According to concentration, the species have been

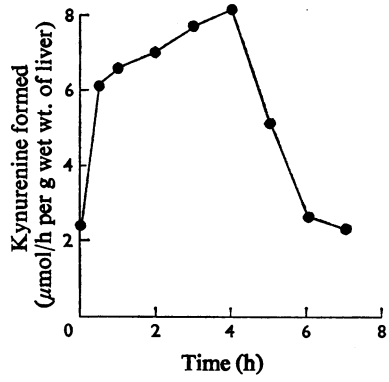

Fig. 1. *Time-course of the effect of intraperitoneal administration of tryptophan on the activity of golden-hamster liver tryptophan pyrrolase*

Tryptophan (200mg/kg body wt.) was injected at zero time. Each point represents the mean value for four hamsters. The enzyme activity was determined, as described in the Materials and Methods section, in the absence of added haematin.

Table 4. *Effects of pretreatment of golden hamsters with allopurinol, cycloheximide or actinomycin D on the enhancement of liver tryptophan pyrrolase activity by tryptophan*

Each hamster received an intraperitoneal injection of tryptophan (200mg/kg) or an equal volume of 0.9%NaCl either at 30min or at 4h before being killed. Actinomycin D (0.7mg/kg) or cycloheximide (50mg/kg) were administered at 1h, whereas allopurinol (20mg/kg) was injected at 30min, before tryptophan or 0.9%NaCl. The enzyme activity was determined, as described in the Materials and Methods section, in the absence of added haematin. Values are means ± S.E.M. of each group of four animals.

Time after injection	Pretreatment	Kynurenine formed (μmol/h per g wet wt. of liver) 0.9%NaCl	Kynurenine formed (μmol/h per g wet wt. of liver) Tryptophan	Inhibition of enhancement (%)
30min	Nil	2.3±0.3	6.1±0.7	—
	Allopurinol	2.9±0.3	3.3±0.3	90
4h	Nil	2.4±0.1	8.2±0.6	—
	Actinomycin D	2.4±0.5	8.1±0.9	2
	Cycloheximide	2.4±0.3	6.0±0.3	38
	Allopurinol	2.8±0.1	3.6±0.3	86

divided into two groups. In the first group (comprising rat, mouse, pig, turkey and chicken), the concentrations of liver, serum free and serum total tryptophan were, in general, higher than those in animals of the second group. Exceptions in these differences were: (1) the higher total tryptophan concentration in gerbil serum; (2) the high serum free tryptophan concentrations in frog, golden hamster and sheep. The mean liver tryptophan and serum free and total tryptophan concentrations in species of group 2 were 43–51% lower ($P = 0.005$–0.001) than the corresponding values in animals of group 1. The frog was exceptional in having a very high (83%) proportion of serum tryptophan in the free (ultrafiltrable) state. By omitting this value for the frog, there was no significant difference between the two groups of species in the proportion of serum tryptophan present in the free state (group 1: 15.79±2.4%; group 2: 14.66±2.7%; $P > 0.10$).

Discussion

Form(s) and properties of tryptophan pyrrolase in animal species

Investigators of tryptophan pyrrolase had only measured the activity of the holoenzyme, until Feigelson & Greengard (1961) demonstrated the presence of the apoenzyme in homogenates of rat

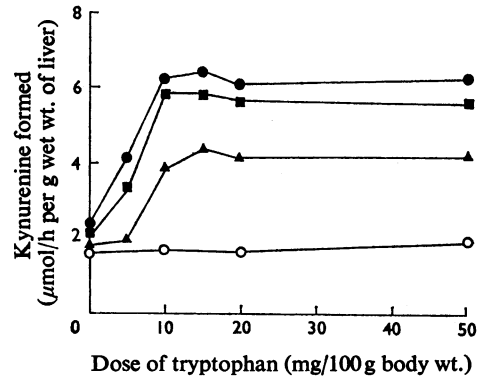

Fig. 2. *Comparison of the 30min effect of intraperitoneal or intraglandular administration of various doses of tryptophan on the activity of liver tryptophan pyrrolase in rats, golden hamsters, guinea pigs and frogs*

Rats, hamsters, guinea pigs (intraperitoneal injection) or frogs (intraglandular injection) received various doses of tryptophan or equal volumes of 0.9% NaCl 30min before being killed. Each point represents the mean value for four animals, except that the results for the frog are means of four experiments, each of which has been performed on two frog livers. The enzyme activity was determined, as described in the Materials and Methods section, in the absence of added haematin. ○, Rat; ●, hamster; ■, guinea pig; ▲, frog.

Table 5. *Tryptophan concentrations in livers and sera of various animal species*

The concentrations of liver, serum free (ultrafiltrable) and serum total (acid-soluble) tryptophan were determined as described in the Materials and Methods section. Values are means±S.E.M. of the numbers of animals indicated in parentheses. Determinations of serum tryptophan concentrations in mice, hamsters and frogs involved pooling samples from two to four animals for each measurement. Only one cat was tested.

Species	Concentration (μg/g wet wt. of liver or per ml of serum)		
	Liver tryptophan	Serum free tryptophan	Serum total tryptophan
Group 1			
Rat	10.23±0.46 (8)	1.72±0.08 (8)	18.27±0.84 (8)
Mouse	11.28±0.37 (6)	2.41±0.19 (4×3)	18.70±0.57 (4×3)
Pig	10.17±0.41 (6)	2.55±0.12 (6)	17.43±0.79 (6)
Turkey	11.51±0.51 (4)	3.43±0.23 (4)	18.89±0.34 (4)
Chicken	14.47±0.91 (4)	4.40±0.11 (4)	18.42±1.05 (4)
Group 2			
Cat	5.40	1.09	5.87
Frog	7.19±0.38 (6)	2.98±0.08 (4×4)	3.60±0.14 (4×4)
Guinea pig	5.06±0.23 (10)	1.03±0.04 (10)	11.33±0.51 (10)
Hamster	7.37±0.15 (5)	1.94±0.05 (5)	7.35±0.30 (5)
Gerbil	7.64±0.13 (4)	1.13±0.09 (4)	17.85±0.61 (4)
Ox	6.42±0.03 (5)	0.45±0.01 (5)	5.06±0.20 (5)
Sheep	8.76±0.34 (6)	1.70±0.14 (6)	8.40±0.17 (6)
Rabbit	4.75±0.33 (4)	1.63±0.06 (4)	12.70±0.99 (4)

liver incubated with haematin. Except for the indirect demonstration (Monroe, 1968) of the mouse liver apoenzyme by the use of globin to prevent its conjugation with haematin (Feigelson & Greengard, 1961), most measurements of the pyrrolase activity in animal species have been carried out in either the absence (Spiegel, 1961; Johnson & Dyer, 1966; Calandra *et al.*, 1972) or the presence (Brown & Dodgen, 1968; Leklem *et al.*, 1969; Carlson & Dyer, 1970; Baughman & Franz, 1971; Green *et al.*, 1975) of added haematin. Simultaneous measurements of both activities are important not only to demonstrate the absence or presence of the apoenzyme, but also to test which form a compound may activate (Badawy & Evans, 1975*c*) or inhibit (Madras & Sourkes, 1968; Badawy & Smith, 1971; Badawy & Evans, 1973, 1975*a*,*b*).

Table 1 shows such simultaneous measurements. The presence of the apoenzyme in rat liver is well known. The activation of the mouse liver enzyme by haematin *in vitro* provides a direct confirmation of previous indirect evidence (Monroe, 1968) suggesting the presence of the apoenzyme in this species. In addition to the rat and mouse, the apoenzyme is also present in the pig, turkey and chicken. By contrast, a larger number of animal species do not possess any detectable apo-(tryptophan pyrrolase) activity (Table 1). These are the golden hamster, gerbil, rabbit, cat, frog, ox, sheep and guinea pig, which has previously been examined. The guinea-pig and rat liver enzymes have also been shown to differ in other respects. The former appears to be tightly bound to its haem activator (Hvitfelt & Santti, 1972) and does not respond to agents that activate or inhibit the rat liver enzyme *in vitro* (Badawy & Evans, 1974). Lack of response to these agents is also observed with the golden-hamster enzyme (Table 2) and conclusions reached from experiments with the guinea pig also apply to this species.

An attempt has been made to test the possibility that the absence of the apoenzyme from guinea-pig liver is due to a permanent inhibition caused by the higher state of reduction of the NAD^+ couple (Rawat, 1973). NADH inhibits the activity of the purified rat liver enzyme (Cho-Chung & Pitot, 1967), whereas in homogenates it exerts a biphasic effect; the initial stimulation (see, e.g., Table 2) is followed by inhibition (A. A.-B. Badawy, unpublished work). It is also known (Badawy & Evans, 1975*a*, 1976) that the inhibition of the rat liver apoenzyme by the administration of agents that increase [NADH] is reversed by the addition of NAD^+ *in vitro* or by the administration of phenazine methosulphate, which rapidly reoxidizes NADH (Katz & Wals, 1970). Neither treatment causes any extra activity to appear when guinea-pig liver homogenates are incubated with or without added haematin (see the text). This suggests that the apoenzyme is either permanently absent from this species, or, if present, cannot be demonstrated by such short-term treatments.

Regulation of tryptophan pyrrolase activity in various animal species and the relationship between the apoenzyme and the hormonal induction mechanism

The regulation of the enzyme activity has been most extensively studied in rat liver, where at least four mechanisms may be involved (see the introduction). As in the rat, the mouse liver enzyme responds to induction by cortisol and activation by tryptophan (Monroe, 1968). In man, tryptophan has not been tested, but cortisol causes induction (Altman & Greengard, 1966). By contrast, the administration of corticosteroids fails to enhance liver tryptophan pyrrolase activity in frog, *Rana pipiens* (Spiegel, 1961), catfish (Brown & Dodgen, 1968), cat (Leklem *et al.*, 1969), steer or calf (Carlson & Dyer, 1970), gerbil (Baughman & Franz, 1971; Green *et al.*, 1975), guinea pig (Hvitfelt & Santti, 1972; Badawy & Evans, 1974), frog, *Rana temporaria*, and golden hamster (Table 3); only a moderate response is observed in sheep (Carlson & Dyer, 1970). Since these species (except possibly the catfish, which has not been tested) lack the apoenzyme (Table 1), it may be concluded that the absence of apo-(tryptophan pyrrolase) is directly related to the absence of the hormonal induction mechanism.

Tryptophan, on the other hand, enhances the pyrrolase activity in these species, except the catfish (Brown & Dodgen, 1968) and steer (Carlson & Dyer, 1970), although the latter species has been reported (Johnson & Dyer, 1966) to show inhibition at a later time-interval after tryptophan administration. The nature of the tryptophan enhancement of the pyrrolase activity in some of these species is different from that in the rat. Thus, although the overall period of enhancement is similar in rat (Badawy & Evans, 1975*c*), guinea pig (Badawy & Evans, 1974) and golden hamster (Fig. 1), the enzymes from the latter two species show a more rapid response to tryptophan. Also in agreement with studies in the guinea pig (Badawy & Evans, 1974), the tryptophan activation of the hamster enzyme is not affected by actinomycin D and is only moderately inhibited by cycloheximide (Table 4). This suggests that tryptophan activates the enzyme in either species by a mechanism in which the synthesis of mRNA and/or protein does not play a major role. The tryptophan enhancement of the guinea-pig (Badawy & Evans, 1974) or hamster (Table 4) enzyme is, however, blocked by allopurinol. This drug is a specific inhibitor of apoenzyme activation in the rat, where it also prevents the tryptophan activation of the pyrrolase (Badawy &

Evans, 1973). A similar effect in the guinea pig or golden hamster therefore suggests that tryptophan activates the pyrrolase in these species by promoting the rapid conjugation with haem of a normally undetectable (Table 1) form of apo-(tryptophan pyrrolase). Moreover, the rapidity of this effect (Fig. 1) suggests that this form may be released from a latent condition rather than being either synthesized or activated by a mechanism similar to that in rat liver. The latter requires 1–2h before an appreciable increase in the enzyme activity can be demonstrated (Badawy & Evans, 1975*c*). Also, large doses of tryptophan do not cause an early activation of the rat liver enzyme, whereas smaller doses do so in the frog, guinea pig and golden hamster (Fig. 2). This early effect of tryptophan requires further investigation.

Concentrations of tryptophan in liver and serum and tryptophan binding to serum proteins in various animal species

Tryptophan is the only circulating amino acid that is protein-bound (McMenamy *et al.*, 1957). The results in Table 5 show that such binding occurs in various animal species, with frog (cold-blooded) serum exhibiting the least binding. Similar findings have already been reported (Fuller & Roush, 1973). Our studies (Table 5) show a distinction between species lacking apo-(tryptophan pyrrolase) and those possessing it. The concentrations of tryptophan in livers and sera of the former species are 43–51% lower than in species possessing the apoenzyme. Similar conclusions about serum tryptophan concentrations can be drawn from the data of Fuller & Roush (1973). The extent of binding of tryptophan to serum proteins does not differ between the two groups of species (the value in the frog is exceptionally low). The reason(s) why liver and serum tryptophan concentrations are lower in species lacking the apoenzyme is not clear at present. However, factors such as the nature of tryptophan pyrrolase or the dietary habits of these species (except the cat, which is carnivorous) may be involved.

The low serum total tryptophan concentrations in these species may explain the inability of salicylate or ethanol to activate the pyrrolase in guinea pig (Badawy & Evans, 1974) and golden hamster (Table 3). These drugs (ethanol acting via non-esterified fatty acids) activate the rat liver enzyme by displacing tryptophan from its binding sites on serum proteins (Badawy & Smith, 1971, 1972; Badawy & Evans, 1975*b*), and a possible explanation for their ineffectiveness in the former two species is that the binding sites on serum proteins are not sufficiently saturated to allow tryptophan displacement. A similar situation has been proposed in the rat (Badawy & Smith, 1972), where a non-activating dose of salicylate (200mg/kg) renders a similarly inactive dose of tryptophan (50mg/kg) capable of activating tryptophan pyrrolase. The absence of activation of the guinea-pig (Badawy & Evans, 1974) or golden-hamster (Table 3) enzyme by 5-aminolaevulinate, which increases the haem saturation of the rat liver apoenzyme (Badawy & Evans, 1975*c*), may simply be explained by the absence of the apoenzyme from the former two species. It also suggests that the cofactor mechanism of activation of the pyrrolase may be absent from guinea-pig and hamster livers.

The combined injection of tryptophan plus cortisol produces an additive effect on rat liver tryptophan pyrrolase activity, because the two agents act by different mechanisms (see, e.g., Badawy & Evans, 1975*c*). The absence of an additive effect of such a combined injection in the cat (Leklem *et al.*, 1969), gerbil (Baughman & Franz, 1971) or golden hamster (Table 3) excludes the possibility that the absence of the hormonal induction mechanism is due to low liver tryptophan concentrations in these species (Table 5).

Importance of choice of animal species as models for studying human tryptophan metabolism

Species from which apo-(tryptophan pyrrolase) and the hormonal induction mechanism are absent metabolize tryptophan differently from those possessing both aspects. Thus tryptophan is metabolized in cattle largely via the indolylamine routes (Yang & Carlson, 1972), whereas man metabolizes the amino acid mainly by the kynurenine pathway (see, e.g., Hankes *et al.*, 1967). The cat excretes more unchanged tryptophan and less kynurenine metabolites than does the rat (Leklem *et al.*, 1969). Gerbil brain 5-hydroxytryptamine synthesis and turnover are not decreased by administration of cortisol, whereas the opposite is true in the rat (Green *et al.*, 1975). These findings therefore suggest that species lacking apo-(tryptophan pyrrolase) or its hormonal induction mechanism are not suitable as models for studying human tryptophan metabolism. They may, however, be useful, as the findings by Green *et al.* (1975) show, to test certain aspects of tryptophan metabolism, particularly the inverse relationship between tryptophan pyrrolase activity and brain 5-hydroxytryptamine synthesis. Also, since the haem (cofactor) mechanism of pyrrolase activation may be absent from these species (see above), they should not be used to examine tryptophan metabolism in relation to porphyria (see Badawy & Evans, 1975*c*), although they could serve as tools for excluding tryptophan pyrrolase from playing a part in the overall utilization of haem by liver haem proteins.

Relationship between apo-(tryptophan pyrrolase) and the toxicity of tryptophan, and possible implications in veterinary and human neonatal care

The results discussed so far establish an association between the absence of apo-(tryptophan pyrrolase), the absence of the hormonal induction mechanism, the low serum and liver tryptophan concentrations and also the limited metabolism of tryptophan by the kynurenine pathway in certain animal species. We now suggest that the above aspects may be related to death or toxicity symptoms resulting from tryptophan administration into these same species (see the text for frog and hamster, and the introduction for other species). It, however, remains to be seen whether the cat or rabbit is sensitive to the toxicity of tryptophan, and whether the catfish or rabbit lacks the apoenzyme or the hormonal induction mechanism respectively. The excessive metabolism of exogenous tryptophan by the indole routes, presumably because of the limited kynurenine pathway, may be the cause of such toxicity. 5-Hydroxytryptamine could be implicated (Johnson & Dyer, 1966; Horita & Carino, 1970), although other indole derivatives may also be involved (Yang & Carlson, 1972).

The conclusions of the present paper may have important implications in relation to veterinary and human neonatal care. The sensitivity of the above species (which include farm animals and pets) to the toxicity of tryptophan stresses the need to protect such animals against tryptophan-rich feeds or diets or treatments that could further limit the capacity of the already deficient kynurenine pathway.

As regards neonatal care, the development of tryptophan pyrrolase in species lacking the apoenzyme is different from that in those possessing it. Thus, whereas the enzyme fully develops on the first day of life of the guinea pig (Nemeth & Nachmias, 1958) or rabbit (Nemeth, 1959), its activity is low in the newborn infant (Auricchio *et al.*, 1959) and appears in the rat on approximately the twelfth postnatal day (Auerbach & Waisman, 1959). The newborn-rat liver enzyme exists only as holoenzyme (Greengard & Feigelson, 1963) and its development cannot be hastened by the administration of cortisone or tryptophan (Knox & Greengard, 1965). The development of the human liver enzyme has not been studied, but, if man resembles rat (by belonging to the group of species possessing the apoenzyme), it is possible that the neonatal development of his pyrrolase may be subject to the same conditions. Moreover, newborn-infant serum may resemble that of newborn rat in binding very little tryptophan (Bourgoin *et al.*, 1974). Thus the deficiency of tryptophan pyrrolase and the possible weak binding of tryptophan to serum proteins may render the newborn infant vulnerable to the toxicity of tryptophan. Various constituents of baby foods and/or milk have been implicated in sudden deaths or other complications, but the possibility remains that these conditions may involve a toxic action by tryptophan.

This work was supported by South Glamorgan Area Health Authority, the Medical Research Council and the Wellcome Trust. We are very grateful to Mr. P. C. Gleeson for samples of ox, pig and sheep livers and blood, Mr. D. Ritchie for similar samples from a cat, Dr. G. E. Lovatt and Mr. W. A. Slater for generous gifts of allopurinol and actinomycin D respectively, Mr. C. J. Morgan and Miss Jill C. Davis for skilful technical assistance and Mr. A. Dacey for competent handling of animals.

References

Altman, K. & Greengard, O. (1966) *J. Clin. Invest.* **45**, 1527–1534

Auerbach, V. H. & Waisman, H. A. (1959) *J. Biol. Chem.* **234**, 304–306

Auricchio, S., Guagliariello, E. & Rubino, A. (1959) *Boll. Soc. Ital. Biol. Sper.* **35**, 2206–2208

Badawy, A. A.-B. & Evans, M. (1973) *Biochem. J.* **133**, 585–591

Badawy, A. A.-B. & Evans, M. (1974) *Biochem. J.* **138**, 445–451

Badawy, A. A.-B. & Evans, M. (1975*a*) *Biochem. J.* **148**, 425–432

Badawy, A. A.-B. & Evans, M. (1975*b*) *Adv. Exp. Med. Biol.* **59**, 229–251

Badawy, A. A.-B. & Evans, M. (1975*c*) *Biochem. J.* **150**, 511–520

Badawy, A. A.-B. & Evans, M. (1976) *Biochem. J.* **156**, 381–390

Badawy, A. A.-B. & Smith, M. J. H. (1971) *Biochem. J.* **123**, 171–174

Badawy, A. A.-B. & Smith, M. J. H. (1972) *Biochem. Pharmacol.* **21**, 97–101

Baughman, K. L. & Franz, J. M. (1971) *Int. J. Biochem.* **2**, 201–211

Bloxam, D. L. & Warren, W. H. (1974) *Anal. Biochem.* **60**, 621–625

Bourgoin, S., Faivre-Bauman, A., Benda, P., Glowinski, J. & Hamon, M. (1974) *J. Neurochem.* **23**, 319–327

Brown, J. N. & Dodgen, C. L. (1968) *Biochim. Biophys. Acta* **165**, 463–469

Calandra, P., Rampichini, L. & Severini, M. (1972) *Acta Vitaminol. Enzymol.* **26**, 69–77

Carlson, J. R. & Dyer, I. A. (1970) *J. Nutr.* **100**, 94–100

Cho-Chung, Y. S. & Pitot, H. C. (1967) *J. Biol. Chem.* **242**, 1192–1198

Denckla, W. D. & Dewey, H. K. (1967) *J. Lab. Clin. Med.* **69**, 160–169

Feigelson, P. & Greengard, O. (1961) *J. Biol. Chem.* **236**, 153–157

Fuller, R. W. & Roush, B. W. (1973) *Comp. Biochem. Physiol. B* **46**, 273–276

Green, A. R., Sourkes, T. L. & Young, S. N. (1975) *Br. J. Pharmacol.* **53**, 287–292

Greengard, O. & Feigelson, P. (1963) *Ann. N.Y. Acad. Sci.* **111**, 227–232
Hankes, L. V., Brown, R. R., Lippincott, S. & Schmaeler, M. (1967) *J. Lab. Clin. Med.* **69**, 313–324
Horita, A. & Carino, M. A. (1970) *Biochem. Pharmacol.* **19**, 1521–1524
Hvitfelt, J. & Santti, R. S. (1972) *Biochim. Biophys. Acta* **258**, 358–365
Johnson, R. J. & Dyer, I. A. (1966) *Life Sci.* **5**, 1121–1124
Katz, J. & Wals, P. A. (1970) *J. Biol. Chem.* **245**, 2546–2548
Knox, W. E. (1966) *Adv. Enzyme Regul.* **4**, 287–297
Knox, W. E. & Greengard, O. (1965) *Adv. Enzyme Regul.* **3**, 247–313
Leklem, J. E., Woodford, J. & Brown, R. R. (1969) *Comp. Biochem. Physiol.* **31**, 95–109
Madras, B. K. & Sourkes, T. L. (1968) *Biochem. Pharmacol.* **17**, 1037–1047
McMenamy, R. H., Lund, C. C. & Oncley, J. L. (1957) *J. Clin. Invest.* **36**, 1672–1679
Monroe, C. B. (1968) *Am. J. Physiol.* **214**, 1410–1414
Nemeth, A. M. (1959) *J. Biol. Chem.* **234**, 2921–2923
Nemeth, A. M. & Nachmias, V. T. (1958) *Science* **128**, 1085–1086
Rawat, A. K. (1973) *Adv. Exp. Med. Biol.* **35**, 145–166
Schimke, R. T. (1969) *Curr. Top. Cell. Regul.* **1**, 77–124
Schimke, R. T., Sweeney, E. W. & Berlin, C. M. (1965) *J. Biol. Chem.* **240**, 322–331
Spiegel, M. (1961) *Biol. Bull.* **121**, 547–553
Yang, J. N. Y. & Carlson, J. R. (1972) *J. Nutr.* **102**, 1655–1665
Young, S. N. & Sourkes, T. L. (1974) *Lancet* **ii**, 897–898