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# Guinea-Pig Liver Tryptophan Pyrrolase

## ABSENCE OF DETECTABLE APOENZYME ACTIVITY AND OF HORMONAL INDUCTION BY CORTISOL AND POSSIBLE REGULATION BY TRYPTOPHAN

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1. When assayed in fresh homogenates, guinea-pig liver tryptophan pyrrolase exists only as holoenzyme. It does not respond to agents that activate or inhibit the rat liver enzyme *in vitro*. Only by aging (for 30min at 5°C) does the guinea-pig enzyme develop a requirement for ascorbate. 2. The guinea-pig liver enzyme is activated by the administration of tryptophan but not cortisol, salicylate, ethanol or 5-aminolaevulinate. 3. The tryptophan enhancement of the guinea-pig liver pyrrolase activity is prevented by 0, 34 and 86% by pretreatment with actinomycin D, cycloheximide or allopurinol respectively. 4. The guinea-pig liver tryptophan pyrrolase is more sensitive to tryptophan administration than is the rat enzyme. On the other hand, the concentrations of tryptophan in sera and livers of guinea pigs are 45–52% less than those in rats. 5. It is suggested that tryptophan may regulate the activity of guinea-pig liver tryptophan pyrrolase by mobilizing a latent form of the enzyme whose primary function is the detoxication of its substrate.

Liver tryptophan pyrrolase (L-tryptophan–$O_2$ oxidoreductase, EC 1.13.11.11) has been the subject of many investigations that have led to our present understanding of some of the mechanisms involved in enzyme regulation. The pyrrolase has been most extensively studied in rat liver, where two types of activity exist. The already active holoenzyme does not require the addition of exogenous haematin for demonstration of its activity (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 (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- or cofactor-type enhancement consisting of decreased degradation of pre-existing apoenzyme in the presence of the normal rate of its synthesis. The regulation of rat liver tryptophan pyrrolase activity by liver haem has been reported (Badawy & Evans, 1973*e*).

Hvitfelt & Santti (1972) made the noteworthy observation, which we have now confirmed, that guinea-pig liver tryptophan pyrrolase exists only as the holoenzyme, whose activity can be enhanced by treatment with tryptophan but not with cortisol or prednisolone. Since the chronic administration of ethanol or phenobarbitone (Badawy & Evans, 1973*a*,*b*,*d*) inhibits the activity of rat liver apotryptophan pyrrolase, and subsequent withdrawal of either drug enhances both holoenzyme and apoenzyme activities, the guinea pig, which lacks the apoenzyme, may be a useful tool in finding out if the above inhibition is involved in the mechanism(s) of dependence on these two drugs. As regards ethanol, Arvola & Forsander (1961) found that of six animal species, the guinea pig shows the least preference to a 10% (v/v) solution of ethanol over drinking water. While these questions are being examined, we now report some further experiments on the properties of guinea-pig liver tryptophan pyrrolase and its rapid activation by tryptophan. The results are also discussed in relation to the toxicity of tryptophan in some species, most of which lack the hormonal mechanism for induction of liver tryptophan pyrrolase activity.

## Materials and Methods

### *Chemicals*

Actinomycin D and allopurinol (4-hydroxypyrazolo[3,4-*d*]pyrimidine) were gifts respectively from Merck, Sharp and Dohme Ltd., Hoddesdon, Herts., U.K. and the Wellcome Foundation Ltd., London NW1 2BP, U.K. 5-Aminolaevulinate hydrochloride, 2,2′-bipyridyl, cortisol 21-acetate, cycloheximide, dimethylformamide, haemin (haematin hydrochloride), $NAD^+$, NADH and L-tryptophan were from

Sigma (London) Chemical Co., Kingston-upon-Thames, Surrey, U.K. Ethanol (99.7–100%) and all other chemicals (from BDH Chemicals Ltd., Poole, Dorset, U.K.) were of AnalaR grade, except for sodium salicylate, which was of reagent grade.

*Animals and injections*

Male Hartley–Duncan albino guinea pigs (326–842g) were maintained on diet TR2 and hay, whereas some male Wistar rats (150–250g) received cube diet M.R.C. no. 41B *ad libitum*. All animals were killed between 12:00 and 15:00h by stunning and cervical fracture. All the chemicals were given intraperitoneally at various times before death. Cortisol acetate was dissolved in dimethylformamide and 0.1ml (2mg/100g body wt.) was injected; the corresponding control animals received an equal quantity of the solvent, which gave results identical with other control groups that had received 0.9% (w/v) NaCl. Tryptophan (50–500mg) and allopurinol (20mg) were dissolved in the minimum amounts of 1M-NaOH and diluted to 20ml or 4ml respectively after the pH had been adjusted to 7.3–7.8 with 1M-HCl; each animal received the above volumes/kg body wt. Actinomycin D (0.7mg/kg), cycloheximide (50mg/kg) and sodium salicylate (400mg/kg) were dissolved in 0.9% NaCl; each animal received 0.1ml/100g body wt. Ethanol (5ml/kg) was given as a 25% (v/v) solution in 0.9% NaCl, whereas 5-aminolaevulinate hydrochloride (dissolved in 0.9% NaCl) was given in four hourly doses (15mg/kg body wt. each) and the animals were killed at 1h after the last injection.

*Preparation of homogenates*

The liver was removed within 30s of the death of the animal and was homogenized for 1min at 1100rev./min in 6.5vol. (for rats) or 2.75vol. (for guinea pigs) of 140mM-KCl–2.5mM-NaOH at 0°C in a glass homogenizer with a loose-fitting Teflon pestle. The homogenates were used within 8min of preparation. Some guinea-pig liver homogenates were flushed with $O_2$ and stored at 5°C for 30min before being used for studies on the effect of aging of homogenates on tryptophan pyrrolase activity.

*Determination of tryptophan pyrrolase activity*

The activity of the enzyme was determined in liver homogenates of rats and guinea pigs by measuring the formation of kynurenine from L-tryptophan (Feigelson & Greengard, 1961) either in the absence (holoenzyme activity) or in the presence (total enzyme activity) of added haematin. The apoenzyme activity was calculated by difference. Samples (15ml) of the homogenates were added to a solution containing 5ml of 0.03M-L-tryptophan, 15ml of 0.2M-sodium phosphate buffer, pH7.0, and 25ml of water at 0°C. Where necessary, haematin hydrochloride was dissolved in 0.1M-NaOH and 0.1ml or less was included in the overall mixture to give final concentrations of 2–8$\mu$M. Samples (3ml) of the mixture were incubated with shaking at 37°C in stoppered 25ml conical flasks in an atmosphere of $O_2$ for appropriate time-intervals (each of 10min for the guinea-pig and 15min for the rat liver enzyme) up to 50 or 90min respectively. The reaction was stopped by the addition of 2ml of 0.9M-trichloroacetic acid; the flasks and contents were shaken for a further 2min and then filtered on Whatman no. 1 filter paper. To a measured portion of the filtrate (2.5ml) was added 1.5ml of 0.6M-NaOH and the kynurenine present was determined by measuring the $E_{365}$ with a Unicam SP.500 spectrophotometer and by using $\epsilon$ 4540 litre$\cdot$mol$^{-1}\cdot$cm$^{-1}$. Tryptophan pyrrolase activity was calculated from the increase in the $E_{365}$ with time during the linear phase. In the rat, the latter was preceded by a lag phase that persisted for 30–45min with the basal enzyme and the enzyme induced by the administration of cortisol. The enzyme activated by the administration of tryptophan and other cofactor-type activators, or by the addition *in vitro* of ascorbate and other stimulators, had the lag phase either abolished or shortened to 15min. The guinea-pig liver enzyme had a shorter lag phase (10 or sometimes 20min) that was also abolished by the above treatments. For an extinction range (at 365nm) of 0.1–0.8, the line representing the linear phase with both preparations exactly covered points at three to four consecutive time-intervals. In the experiments *in vitro*, ascorbic acid, ethanol, $NAD^+$, NADH, sodium salicylate, 2,2'-bipyridyl and allopurinol were dissolved in the sodium phosphate buffer with or without a little 1M-NaOH, neutralized to pH7.3 with 1M-HCl and added immediately before incubation.

*Determination of serum and liver tryptophan*

This was performed fluorimetrically by the method of Hess & Udenfriend (1959) on acid extracts of sera and livers of rats and guinea pigs.

(*a*) *Serum tryptophan.* Serum (1ml) was treated with 3ml of water and 2ml of 20% (w/v) trichloroacetic acid, homogenized, left for 10min at room temperature and then centrifuged at 5000*g* for 10min.

(*b*) *Liver tryptophan.* Liver (1g) was heated with 3.2ml of water in a boiling-water bath for 2min, cooled, homogenized with the addition of 2ml of 20% trichloroacetic acid and centrifuged at 5000*g* for 10min after being left for a similar time at room temperature.

(*c*) *Spectrofluorimetric determination.* Samples (0.3ml) of the above supernatants were mixed with 1.8ml of water, 0.9ml of 20% trichloroacetic acid and 0.1ml of 20% formaldehyde and heated in a boiling-water bath for 25min. The contents of each tube then

Table 1. *Comparison of the effects of activators and inhibitors of tryptophan pyrrolase activity in liver homogenates of rats and guinea pigs*

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 (0.05mM); 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 | | Guinea pig | |
| Addition | Control | Test | Control | Test |
| Ascorbate | 1.5±0.2 | 3.0±0.6 | 2.2±0.2 | 2.2±0.2 |
| NADH | 1.5±0.3 | 3.2±0.1 | 2.2±0.2 | 2.1±0.2 |
| Ethanol+$NAD^+$ | 1.5±0.2 | 3.0±0.2 | 2.2±0.2 | 2.1±0.1 |
| Allopurinol (*a*) | 1.3±0.2 | 1.3±0.2 | 2.4±0.2 | 2.2±0.2 |
| Allopurinol (*b*) | 3.3±0.3 | 1.5±0.3 | 2.5±0.1 | 2.5±0.1 |
| Salicylate | 1.4±0.1 | 0.0 | 2.3±0.5 | 2.8±0.6 |
| 2,2′-Bipyridyl | 1.5±0.1 | 0.7±0.1 | 2.1±0.3 | 2.0±0.1 |

received 0.1ml of 6% (w/v) $H_2O_2$ and heating was continued for a further 25min period. After cooling the fluorescence was measured against water blanks with an Aminco–Bowman spectrophotofluorimeter (activation and emission wavelengths were 365 and 440 respectively). A freshly prepared solution of tryptophan of a known concentration, treated exactly as the serum and liver extracts, was used as a standard. The results were expressed in μg of tryptophan/ml of serum or per g wet wt. of liver, ±S.E.M. of each group of eight to ten animals.

## Results

### *Effects of activators and inhibitors on rat and guinea-pig liver tryptophan pyrrolase activities*

The effects of various concentrations of added haematin on liver tryptophan pyrrolase activity are shown in Fig. 1. At 2μM, haematin caused maximum activation of the rat liver enzyme. The guinea-pig liver enzyme, on the other hand, was not activated by concentrations of haematin of up to 8μM.

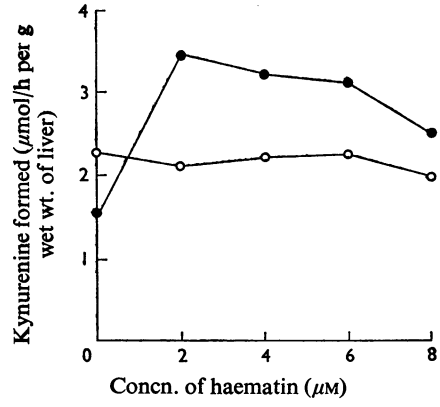


Fig. 1. *Effects of haematin on tryptophan pyrrolase activity in liver homogenates from rats and guinea pigs*

The enzyme activity was determined, as described in the Materials and Methods section, in the absence or in the presence of various concentrations of added haematin. Each point represents the mean value for four rats (●) or for four guinea pigs (○).

Table 2. *Effect of aging of homogenates on the activity of guinea-pig liver tryptophan pyrrolase*

The results are given as the mean±S.E.M. of homogenates from four separate animals incubated in the presence or the absence of ascorbate (5mM), in the presence or the absence of haematin (2μM) or in the presence or the absence of the two together. Fresh homogenates were incubated directly after preparation, whereas aged homogenates were prepared by flushing fresh ones with $O_2$ and storing them at 5°C for 30min before being tested.

| | Kynurenine formed (μmol/h per g wet wt. of liver) | |
|---|---|---|
| Treatment | Fresh homogenates | Aged homogenates |
| Control | 1.9±0.2 | 0.7±0.1 |
| Ascorbate | 1.8±0.1 | 1.8±0.2 |
| Haematin | 1.7±0.1 | 0.7±0.1 |
| Ascorbate+haematin | 1.7±0.1 | 1.8±0.1 |

The guinea-pig liver tryptophan pyrrolase was assayed in the absence and in the presence of compounds known to either activate or to inhibit the rat liver enzyme *in vitro* (Table 1). No activation of the guinea-pig liver enzyme was produced by ascorbate, NADH or ethanol plus $NAD^+$. The rat liver apoenzyme inhibitor allopurinol did not alter the activity of guinea-pig tryptophan pyrrolase when tested in either the absence or the presence of added

Table 3. *Effects of administration of cortisol, salicylate, ethanol, 5-aminolaevulinate and tryptophan on the activity of guinea-pig liver tryptophan pyrrolase*

Each guinea pig received an intraperitoneal injection of 0.9% (w/v) NaCl, cortisol acetate (20mg/kg), tryptophan (500mg/kg), sodium salicylate (400mg/kg) or ethanol (5ml/kg body wt.) 4h before being killed. 5-Aminolaevulinate hydrochloride was given in four hourly doses (15mg/kg each) and the animals were killed at 1h after the last injection. The enzyme activity was determined as described in the Materials and Methods section in either the absence or the presence of added haematin (2$\mu$M), ascorbic acid (5mM) or both. The results are given as the mean±S.E.M. of each group of four guinea pigs.

| | Kynurenine formed ($\mu$mol/h per g wet wt. of liver) | | | |
|---|---|---|---|---|
| | No haematin | | +Haematin | |
| Injection(s) | Nil | +Ascorbate | Nil | +Ascorbate |
| 0.9% NaCl | 2.4±0.2 | 2.6±0.3 | 2.3±0.2 | 2.7±0.2 |
| Cortisol | 2.4±0.2 | 2.3±0.2 | 2.4±0.2 | 2.6±0.2 |
| Salicylate | 2.3±0.1 | 2.5±0.1 | 2.6±0.1 | 2.2±0.2 |
| Ethanol | 2.1±0.2 | 2.2±0.2 | 2.4±0.3 | 2.3±0.1 |
| 5-Aminolaevulinate | 2.6±0.4 | 2.6±0.2 | 2.5±0.2 | 2.5±0.1 |
| Tryptophan | 8.0±0.5 | 7.1±0.4 | 7.8±0.7 | 7.6±0.6 |

haematin. Salicylate and 2,2'-bipyridyl, which inhibit the holoenzyme activity in rat liver homogenates, were, however, without effect on the guinea-pig liver enzyme. The absence of any effect of the above compounds on guinea-pig tryptophan pyrrolase activity was not changed by the presence of added haematin.

The effect of aging of the homogenates (for 30min at 5°C) on the activity of guinea-pig liver tryptophan pyrrolase is shown in Table 2. Aging decreased the enzyme activity by 63% ($P<0.001$), and ascorbate but not haematin was effective in abolishing this inhibition.

*Effects of various treatments in vivo on the activity of guinea-pig liver tryptophan pyrrolase*

The activity of the guinea-pig liver enzyme was not altered at 4h after the administration of cortisol, salicylate, ethanol or 5-aminolaevulinate (Table 3), whether or not the homogenates were supplemented with ascorbate and/or haematin. Only tryptophan increased the enzyme activity, by almost fourfold. Neither haematin nor ascorbate caused any further increase *in vitro* in the activity of the enzyme from tryptophan-treated guinea pigs.

The time-course of the tryptophan-induced rise in guinea-pig liver tryptophan pyrrolase activity is shown in Fig. 2. As early as 30min after the injection of tryptophan (500mg/kg body wt.), there was a 130% ($P<0.001$) increase in the pyrrolase activity. The activity continued to rise, reaching a maximum value (4.2 times the basal activity) at 5h and finally fell to the normal value after 8h. During the entire time-course of this enhancement, no further activation of the enzyme was observed when haematin and/or ascorbate were added to the incubation mixture *in vitro*.

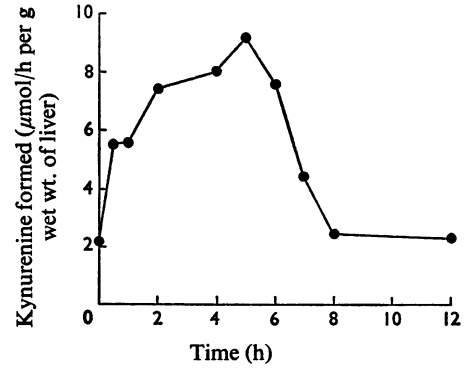


Fig. 2. *Time-course of the effect of intraperitoneal administration of tryptophan (500mg/kg body wt.) on the activity of guinea-pig liver tryptophan pyrrolase*

Tryptophan (500mg/kg body wt.) was injected at zero time. Each point represents the mean value for four guinea pigs. The enzyme activity was determined, as described in the Materials and Methods section, without added haematin or ascorbate.

The increase in tryptophan pyrrolase activity observed at 4h after the administration of tryptophan was prevented by 0, 34 and 86% by pretreatment of guinea pigs with actinomycin D, cycloheximide or allopurinol respectively (Table 4). Allopurinol pretreatment also strongly (85%) prevented the rise in the enzyme activity observed at 30min after tryptophan administration.

The early responses of rat and guinea-pig liver tryptophan pyrrolases were compared at 30min after the injection of various doses of tryptophan (Fig. 3). A 59% increase ($P<0.02$) of the guinea-pig liver

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

Each guinea pig received an intraperitoneal injection of tryptophan (500mg/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 body wt.) 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 both haematin and ascorbate. The results are given as the mean±S.E.M. of each group of four guinea pigs.

| 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 | 2.1±0.3 | 5.5±0.2 | — |
| | Allopurinol | 2.2±0.0 | 2.7±0.4 | 85 |
| 4h | Nil | 2.4±0.2 | 8.0±0.5 | — |
| | Actinomycin D | 2.6±0.3 | 8.2±0.5 | 0 |
| | Cycloheximide | 1.7±0.1 | 5.4±0.0 | 34 |
| | Allopurinol | 2.5±0.2 | 3.3±0.2 | 86 |

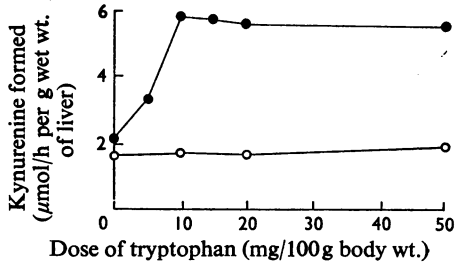


Fig. 3. *Comparison of the 30min effect of intraperitonea administration of various doses of tryptophan on the activity of liver tryptophan pyrrolase in guinea pigs and rats*

Guinea pigs or rats 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. The enzyme activity was determined, as described in the Materials and Methods section, in the absence of added haematin or ascorbate. ○, Activity in rats; ●, activity in guinea pigs.

enzyme activity was produced by a 50mg dose of tryptophan/kg, and maximum activation was obtained by double the above dose. The rat liver enzyme activity, on the other hand, was not significantly altered ($P>0.1$) by doses of tryptophan of 50–500mg/kg body wt.

The concentrations of tryptophan in sera and livers of rats were (in μg/ml of serum or in μg/g wet wt. of liver, ±S.E.M. of ten rats) 14.91±0.62 and 13.01±0.27 respectively. The corresponding mean concentrations in eight guinea pigs were 7.12±0.23 and 7.19±0.17 respectively.

## Discussion

A very striking difference between guinea-pig and rat liver tryptophan pyrrolases is that the former is not activated by exogenous haematin (Fig. 1). This finding supports the previous conclusion (Hvitfelt & Santti, 1972) that guinea-pig liver tryptophan pyrrolase exists only as holoenzyme. The activation of rat liver tryptophan pyrrolase by ascorbate as well as by NADH or ethanol plus $NAD^+$, which has been suggested to be due to the conversion into the active enzyme of a latent form or of an inactive subunit (Knox, 1966; Cho-Chung & Pitot, 1967; Badawy & Evans, 1973*c*), was not observed in fresh guinea-pig liver homogenates (Table 1). This suggests the absence from the latter animal liver of an inactive form of the enzyme that could be released or activated by the above compounds. This is also supported by the finding (Hvitfelt & Santti, 1972) that preincubation of the enzyme with tryptophan, ascorbate and methaemoglobin, a treatment that releases the rat liver latent form of the enzyme (Knox, 1966), actually inactivates guinea-pig liver tryptophan pyrrolase. The absence of inhibition of the latter enzyme activity (Table 1) by allopurinol, a specific apoenzyme inhibitor in the rat (Badawy & Evans, 1973*c*), is consistent with the apoenzyme being absent from guinea-pig liver. The lack of inhibition (Table 1) of the guinea-pig liver enzyme activity by the rat liver holoenzyme inhibitors salicylate and 2,2′-bipyridyl (Badawy & Smith, 1971) seems at first surprising, but could be explained by the enzyme in guinea-pig liver being tightly bound to its haem activator, with which the above inhibitors are presumed to interact. This explanation is supported by the finding (Hvitfelt & Santti, 1972) that treatments such as aging, dialysis or preincubation do not cause deconjugation of the guinea-pig enzyme–haem complex, thus reflecting poor reversibility of the conjugation. This latter point is also indicated by the finding (Table 2) that haematin does not reverse the inhibition of the guinea-pig liver tryptophan pyrrolase activity caused by aging. The effectiveness of ascorbate in abolishing this inhibition may be explained by reduction of the oxidized to the active holoenzyme.

The enhancement of guinea-pig liver tryptophan pyrrolase activity by tryptophan but not by cortisol administration (Table 3) confirms previous findings (Hvitfelt & Santti, 1972) except that the activity of the enzyme from tryptophan-treated guinea pigs is not further increased by the addition *in vitro* of ascorbate (or haematin). The nature of the above enhancement was investigated by pretreatment of the animals with various inhibitors (Table 4). The tryptophan effect was not prevented by actinomycin D, thus suggesting the lack of involvement of RNA synthesis in this process. The moderate prevention of the tryptophan-induced rise in the enzyme activity caused by cycloheximide (Table 4) suggests that ongoing protein synthesis does not play a major role in the tryptophan effect. Results of experiments using inhibitors of protein synthesis such as cycloheximide or puromycin should, however, be cautiously interpreted, since these compounds may possess other properties, and also because although the tryptophan activation of the rat liver enzyme does not involve the synthesis of new apoenzyme (Schimke, 1969), both puromycin (Greengard *et al.*, 1963) and cycloheximide (Badawy & Smith, 1971) prevent the tryptophan effect.

The enhancement of guinea-pig liver tryptophan pyrrolase activity by tryptophan administration is, however, blocked by allopurinol (Table 4). Allopurinol is a specific apoenzyme inhibitor in the rat, where it also completely prevents the tryptophan enhancement of the pyrrolase activity (Badawy & Evans, 1973*c*). A similar effect in the guinea pig therefore suggests that tryptophan may activate the enzyme by promoting the rapid conjugation of an apotryptophan pyrrolase protein with its haem activator. Moreover, the rapidity of this effect (Fig. 2) suggests that this form of the enzyme is 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 could be demonstrated (Civen & Knox, 1959; Greengard & Feigelson, 1961; Knox, 1966). Also large doses of tryptophan do not cause an early activation of the rat enzyme, whereas smaller doses do so in the guinea pig (Fig. 3).

The guinea-pig liver and serum concentrations of tryptophan, the first to be reported in the literature as far as we could ascertain, are 45–52% lower than those in the rat, and this may explain, at least in part, the higher sensitivity of the former animal to tryptophan treatment. A tryptophan-type enhancement of the rat liver enzyme activity can be produced by the administration of salicylate (Badawy & Smith, 1971) or ethanol (Badawy & Evans, 1973*a*,*b*). The effect of salicylate may be caused by displacement of serum and liver protein-bound tryptophan (Badawy & Smith, 1972), whereas that of ethanol may be similarly produced by non-esterified fatty acids, whose blood concentrations are increased by ethanol (Brodie *et al.*, 1961). The insensitivity of guinea-pig liver tryptophan pyrrolase to salicylate or ethanol treatment (Table 3) may be explained by the tryptophan-binding sites on serum proteins not being sufficiently saturated to allow displacement by salicylate or free fatty acids. A similar explanation 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 body wt.) capable of enhancing liver tryptophan pyrrolase activity. The absence of activation of the guinea-pig liver enzyme by repeated administration of the haem precursor 5-aminolaevulinate, which increases the haem saturation of the rat liver apoenzyme (Druyan & Kelly, 1972; Badawy & Evans, 1973*e*), may be simply explained by the absence of the apoenzyme from guinea-pig liver.

The lower serum and liver contents of tryptophan, and the rapidity and sensitivity with which tryptophan pyrrolase responds to tryptophan treatment (Figs. 2 and 3), may throw some light on the mechanism of toxicity of the amino acid in the guinea pig. There is little information on the early response of tryptophan pyrrolase to administered tryptophan in other species that lack the hormonal induction mechanism, but available information (Spiegel, 1961; Johnson & Dyer, 1966; Knox, 1966; Brown & Dodgen, 1968; Hvitfelt & Santti, 1972) on other parameters have been compiled (Table 5). All species mentioned, except the frog, which has not been tested, do not survive repeated doses of tryptophan, and all but the bovine, which was also not examined, do not possess the hormonal mechanism for induction of liver tryptophan pyrrolase activity. Apart from the inhibition of the enzyme activity in the bovine, the catfish enzyme is not activated by the administration of

Table 5. *Comparison of data on some aspects of tryptophan metabolism in five animal species*

?, Not tested; (--), inhibition; (-), does not occur physiologically; -, negative effect or absence; +, positive effect or presence.

| Parameter | Guinea pig | Frog | Catfish | Bovine | Adrenalectomized rat |
|---|---|---|---|---|---|
| Death after repeated doses of tryptophan | + | ? | + | + | + |
| Hormonal induction of tryptophan pyrrolase | - | - | - | ? | (-) |
| Tryptophan enhancement of tryptophan pyrrolase | + | + | - | (--) | + |
| Holotryptophan pyrrolase | + | + | ? | ? | + |
| Apotryptophan pyrrolase | - | ? | ? | ? | + |

tryptophan, whereas the enzymes from guinea pigs, frogs and adrenalectomized rats are. It may be relevant that the bovine metabolizes tryptophan mainly via the indoleamine pathways with very little conversion into products of the kynurenine (pyrrolase) pathway (Yang & Carlson, 1966). Tryptophan pyrrolase exists as holoenzyme in guinea pig, and as holoenzyme and apoenzyme in rat liver. The frog liver enzyme (Table 5) was assayed in the absence of added haematin and no information is therefore available as to whether it also exists as apoenzyme. The type(s) of enzyme present in the bovine or in the catfish has not been investigated.

The absence of apoenzyme activity and/or the lack of a hormonal induction mechanism may be related to the cause of death after repeated administration of tryptophan. The mechanism of toxicity of this amino acid is poorly understood, but Knox (1966) suggested that in the absence of hormonal control of tryptophan pyrrolase in the adrenalectomized rat (and possibly in some of the above species), excessive amounts of tryptophan may be metabolized via other pathways, the products of which are toxic.

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