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Possible involvement of the enhanced tryptophan pyrrolase activity in the corticosterone- and starvation-induced increases in concentrations of nicotinamide–adenine dinucleotides (phosphates) in rat liver

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1. Deoxycorticosterone, which does not enhance tryptophan pyrrolase activity, also fails to alter the concentrations of the NAD(P) couples in livers of fed rats, whereas corticosterone increases both pyrrolase activity and dinucleotide concentrations. 2. Starvation of rats increases serum corticosterone concentration, lipolysis, tryptophan availability to the liver, tryptophan pyrrolase activity and liver [NADP(H)]. Glucose prevents all these changes. 3. The β-adrenoceptor-blocking agent propranolol prevents the starvation-induced lipolysis and the consequent increase in tryptophan availability to the liver, but does not influence the increase in serum corticosterone concentration, liver pyrrolase activity and [NADP(H)]. 4. Actinomycin D, which prevents the starvation-induced increases in liver pyrrolase activity and [NADP(H)], does not affect those in serum corticosterone concentration and tryptophan availability to the liver. 5. Allopurinol, which blocks the starvation-induced enhancement of pyrrolase activity, also abolishes the increases in liver [NADP(H)], but not those in serum corticosterone concentration or tryptophan availability to the liver. 6. It is suggested that liver tryptophan pyrrolase activity plays an important role in NAD^+ synthesis from tryptophan in the rat.

Nicotinamide–adenine dinucleotides (phosphates) are synthesized from dietary nicotinic acid and from quinolinate produced as a result of tryptophan degradation along the hepatic kynurenine pathway, of which tryptophan pyrrolase (tryptophan 2,3-dioxygenase, EC 1.13.11.11) is the first and rate-limiting enzyme. In the presence of dietary nicotinic acid, hepatic NAD^+ synthesis has been shown (Morrison *et al.*, 1963) to be enhanced by an excess of dietary tryptophan. On the other hand, there is controversy as to whether, in the absence of dietary nicotinic acid, the synthesis of NAD^+ from tryptophan is controlled solely by liver tryptophan concentration, or, additionally, by tryptophan pyrrolase activity. Thus although changes in the latter have been implicated in the increased NAD^+ synthesis observed in rats treated chronically with either tryptophan (Powanda & Wannemacher, 1970) or carbon disulphide (Wrońska-Nofer & Obrebska, 1976), evidence was obtained for the involvement of liver tryptophan concentration alone in the increased synthesis of NAD^+ in mice treated with various agents that enhance pyrrolase activity (Powanda & Wannemacher, 1971) and in corticosteroid-treated rats rendered deficient in nicotinamide, nicotinic acid and tryptophan (Greengard *et al.*, 1966, 1968). The interpretation of these latter findings in mice and rats is complicated because of species differences in tryptophan metabolism (Monroe, 1968; Badawy & Evans, 1976*b*,*c*; Elliott *et al.*, 1977; Smith *et al.*, 1978) and the possible complex physiological changes caused in rats by the above nutritional deficiencies.

The 24h-starved rat would seem to be a simpler experimental model for studying the possible roles of liver tryptophan concentration and tryptophan pyrrolase activity in the synthesis of the NAD(P) couples because such a rat exhibits the following physiological changes: (1) a catecholamine-induced lipolysis (Hales & Kennedy, 1964) leading to an increase in free plasma tryptophan concentration, thus increasing the availability of the amino acid to the brain (Curzon & Knott, 1974) and the liver (Smith & Pogson, 1980); (2) an increase in plasma corticosteroid concentration (Fromweiler *et al.*, 1968; Chowers *et al.*, 1969), leading to: (3) a corticosterone-induced enhancement of liver tryptophan pyrrolase activity and; (4) a preferential

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increase in hepatic [NADP(H)], both of which are preventable by actinomycin D (Badawy, 1977). The 24h-starved rat therefore provides the opportunity of studying the influence on liver [NADP(H)] of agents capable of selectively blocking one or more of the above physiological changes. In the present work, this opportunity was utilized and experiments with two corticosteroids were performed with rats on a nutritionally adequate diet. The results of these investigations suggest that liver tryptophan pyrrolase activity plays an important role in NAD^+ synthesis.

Materials and methods

Chemicals

Actinomycin D, allopurinol (4-hydroxypyrazolo-[3,4-*d*]pyrimidine) and the β-adrenoceptor-blocking agent propranolol [1-isopropylamino-3-(1-naphthyloxy)propan-2-ol] were gifts from Merck, Sharp and Dohme (Hoddesdon, Herts., U.K.), the Wellcome Foundation (London NW1 2BP, U.K.) and Imperial Chemical Industries (Alderley Edge, Macclesfield, Cheshire, U.K.) respectively. All other chemicals were purchased from BDH Chemicals and Sigma (London) Chemical Co. (both of Poole, Dorset, U.K.) and were of the purest commercially available grades.

Animals and treatments

Male Wistar rats were locally bred and weighed between 200 and 220g. The animals were maintained on cube diet 41B (Oxoid, Basingstoke, Hants., U.K.) and water. The animals were killed between 13:30 and 14:00h by stunning and cervical dislocation. Most rats were starved for 24h, whereas some were starved for longer time-intervals (up to 96h) before death.

Glucose was administered in drinking water (10%, w/v) *ad libitum*, whereas all other chemicals were given intraperitoneally. Actinomycin D (0.7mg/kg), allopurinol (20mg/kg) or propranolol (10mg/kg) was dissolved in 0.9% (w/v) NaCl (2–4ml/kg) and control rats received equal volumes of 0.9% NaCl. The allopurinol solution was prepared as described by Badawy & Evans (1973*a*). Corticosterone acetate and deoxycorticosterone acetate (50mg/kg each) were dissolved in dimethylformamide, and control rats received an equal volume (1ml/kg) of this solvent.

Chemical, enzymic and other determinations

Tryptophan pyrrolase activity was determined in fresh liver homogenates (Badawy & Evans, 1975) either in the absence (holoenzyme activity) or in the presence (total enzyme activity) of added (2μM) 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 the apoenzyme with haem (see also Badawy, 1979). Concentrations of nicotinamide–adenine dinucleotides (phosphates) were determined in frozen liver by a minor modification (Punjani *et al.*, 1979) of the method of Slater *et al.* (1964).

Frozen liver was also used to determine tryptophan concentration. This and concentrations of free (ultrafiltrable) serum and total (acid-soluble) serum tryptophan were determined by standard procedures (for references, see Badawy *et al.*, 1979). Serum corticosterone concentration was determined fluorimetrically by the method of Glick *et al.* (1964). Statistical analysis of results was performed by using Student's *t* test.

Results and discussion

Preferential increase in rat liver [NADP(H)] and enhancement of tryptophan pyrrolase activity by starvation and their prevention by glucose

As shown in Table 1, liver [NADP(H)] were increased by starvation of rats. Thus [$NADP^+$] was increased by 54, 137, 127 and 81% by starvation for 1, 2, 3 and 4 days respectively, whereas the corresponding increases in [NADPH] were 20, 21, 13 and −7% respectively. The above periods of starvation therefore increased the sum of concentrations of the NADP couple by 26, 42, 34 and 9% respectively. In experiments not shown here, it was found that [NAD(H)] were not significantly altered by starvation for 1–4 days. It may therefore be concluded that starvation causes a preferential increase in concentrations of the hepatic NADP couple (which may be of physiological significance in view of the increased requirement for synthetic reactions in starvation) that is most probably produced by increased synthesis, although decreased degradation cannot be ruled out. Because the NADP couple is formed from NAD^+, it may be suggested that starvation enhances NAD^+ phosphorylation. The results in Table 1 also show that glucose prevented the starvation-induced increases in liver [NADP(H)].

Liver tryptophan pyrrolase activity was also enhanced by starvation (Table 1). The increases in the holoenzyme and total pyrrolase activities observed at 1 or 2 days of starvation were proportionate, and therefore did not alter the haem-saturation ratio of the pyrrolase. This type of enhancement is characteristic of the hormonal-induction mechanism (see also Badawy, 1979) and this is further suggested by the finding (Badawy, 1977) that actinomycin D blocks the enhancement in 24h-starved rats. The enhancement of pyrrolase activity in 48h-starved rats has also previously been reported (Badawy & Evans, 1973*b*). By contrast, the results in Table 1 of

Table 1. *Prevention by glucose of the starvation-induced increases in rat liver tryptophan pyrrolase activity and concentrations of the NADP couple*

Rats were starved for up to 4 days before being killed. Some starved rats were also allowed free access to a solution of glucose (10%, w/v) in drinking water. Pyrrolase activities and [NADP(H)] were determined as described in the Materials and methods section. Values are means ± S.E.M. for each group of four rats (pyrrolase activity, expressed in μmol of kynurenine formed/h per g wet wt. of liver) or of six rats (all other determinations, expressed in μg/g wet wt. of liver). The values in fed rats are included for comparison with all other values, and the significance of differences is indicated as follows: ††$P<0.025$; †††$P<0.02$; *$P<0.01$; **$P<0.005$; ***$P<0.001$. Concentrations of the NADP couple were determined in 1976.

Starvation (days)	Treatment	Dinucleotide concentrations [NADP+]	[NADPH]	Sum	Tryptophan pyrrolase activity Holoenzyme	Total enzyme
0	Nil	48 ± 1	216 ± 5	264 ± 6	2.4 ± 0.13	5.2 ± 0.12
1	Nil	74 ± 5***	260 ± 7***	334 ± 10***	5.2 ± 0.39***	11.7 ± 0.28***
	Glucose	48 ± 2	207 ± 11	255 ± 12	2.4 ± 0.08	3.1 ± 0.23***
2	Nil	114 ± 6***	262 ± 9**	376 ± 12***	5.5 ± 0.59**	11.9 ± 1.42**
	Glucose	53 ± 1*	215 ± 3	268 ± 5	2.6 ± 0.25	4.5 ± 0.20††
3	Nil	109 ± 1***	244 ± 4**	353 ± 5***	8.1 ± 0.59***	12.8 ± 0.48***
	Glucose	48 ± 2	211 ± 5	259 ± 6	2.1 ± 0.12	3.9 ± 0.16***
4	Nil	87 ± 2***	201 ± 5	288 ± 6†††	8.1 ± 0.89***	12.3 ± 1.23***
	Glucose	44 ± 2	213 ± 6	257 ± 7	2.1 ± 0.11	3.0 ± 0.12***

the present work show that starvation for 3 or 4 days enhanced the pyrrolase activity by a mechanism involving an increased saturation of the apoenzyme with haem, as is suggested by the relatively larger increase in the holoenzyme activity. This latter type of enhancement is caused by either the substrate (tryptophan) or the cofactor (haem) (see Badawy & Evans, 1975). In the present instance, it is more likely that tryptophan is involved, because of the possibility that this amino acid may be released in excessive amounts by such prolonged periods of starvation, but further work is required to examine this possibility. The results in Table 1 also show that glucose prevented the starvation-induced enhancement of pyrrolase activity. The mechanism of this prevention is not fully understood at present. Since starvation enhances pyrrolase activity at least at 1–2 days by a hormonal-type mechanism, it could be said that the preventive action of glucose is caused by the ability of the sugar to prevent the starvation-induced increase in serum corticosterone concentration (see Table 2). However, it has been found (A. A.-B. Badawy, C. J. Morgan & A. N. Welch, unpublished work) that phenazine methosulphate, which oxidizes liver NAD(P)H, reverses the glucose-induced prevention of the enhancement by starvation of pyrrolase activity. Phenazine methosulphate has previously been shown (Badawy & Evans, 1976*a*) to reverse the glucose-induced inhibition of the activity of the enzyme in fed rats, which was then suggested to involve an increase in concentrations of the allosteric inhibitors NAD(P)H. Further work is therefore required to examine the mechanisms of action of glucose on pyrrolase activity of starved rats.

Prevention by glucose of the starvation-induced increases in serum corticosterone concentration and tryptophan availability to the liver

As shown in Table 2 (and also subsequently in the present paper), starvation of rats for 24 h increased the concentrations of serum corticosterone and liver tryptophan. This latter increase is most probably the result of an increased availability of circulating free tryptophan to the liver, as is the case in relation to the starvation-induced increase in brain tryptophan concentration (Curzon & Knott, 1974). These latter authors found that this increased availability of tryptophan is caused by a catecholamine-induced increase in plasma non-esterified fatty acid concentration causing displacement of plasma protein-bound tryptophan, thus increasing the concentration of the free plasma amino acid. This tryptophan displacement leads to an increase in the percentage of free plasma (or serum) tryptophan, and is associated with a moderate decrease in total plasma tryptophan concentration. All these changes are confirmed by the results in Table 2, and have previously been demonstrated (Badawy & Evans, 1976*c*) in fed rats, in which acute ethanol administration also enhances the availability of free serum tryptophan to liver (and brain) by the same lipolysis-dependent mechanism. The results in Table 2 also show that glucose administration to starved rats prevented the increases in serum corticosterone concentration and tryptophan availability to the liver. Glucose administration to fed rats decreased free serum, total serum and liver tryptophan concentrations, presumably by inhibiting lipolysis (see, e.g., Madras *et al.*, 1973). Although the latter

Table 2. *Effects of glucose on the starvation-induced changes in rat serum corticosterone concentration and tryptophan disposition*

Rats were either fed or starved for 24h. Both fed and starved rats were allowed free access to either drinking water or a solution of glucose (10%, w/v) in drinking water for 24h before death. Concentrations of tryptophan (in μg/ml of serum or μg/g wet wt. of liver) and corticosterone (in μg/l of serum) were determined as described in the Materials and methods section. Values are means ± S.E.M. for each group of six rats. The values in columns (1) and (4) are compared with those in column (3), whereas those in column (2) are compared with those in column (4). The significance of differences is indicated as follows: †$P < 0.05$; *$P < 0.01$; ***$P < 0.001$.

		Serum corticosterone and tryptophan metabolism parameters			
		Starved rats		Fed rats	
Determination	Treatment ...	Nil (1)	Glucose (2)	Nil (3)	Glucose (4)
Free serum tryptophan		2.05 ± 0.09***	1.37 ± 0.03***	1.53 ± 0.05	1.09 ± 0.03***
Total serum tryptophan		19.75 ± 0.52***	25.67 ± 0.69***	25.02 ± 0.86	21.70 ± 0.49*
Free serum tryptophan (%)		10.38 ± 0.46***	5.34 ± 0.08	6.11 ± 0.36	5.02 ± 0.22†
Liver tryptophan		7.09 ± 0.15***	4.46 ± 0.13	5.84 ± 0.17	4.29 ± 0.13***
Serum corticosterone		56.00 ± 2.17***	35.30 ± 1.08†	37.20 ± 1.62	38.80 ± 0.79

authors found that glucose increases brain tryptophan concentration (by an insulin-mediated decrease in concentrations of plasma neutral amino acids that compete with tryptophan for the same cerebral uptake mechanism), the sugar-induced decrease in liver tryptophan concentration (Table 2) must be caused by the decreased availability of the circulating amino acid to the liver.

The results in Tables 1 and 2 therefore demonstrate the ability of glucose to prevent the starvation-induced increases in liver [NADP(H)], tryptophan pyrrolase activity, tryptophan availability to the liver and serum corticosterone concentration. They, however, do not throw light on the mechanism(s) of the increases in liver [NADP(H)]. The effects of agents that do so will now be described.

Failure of propranolol to prevent the starvation-induced increases in liver [NADP(H)] and tryptophan pyrrolase activity despite prevention of the increased availability of tryptophan to the liver

In investigating the lipolytic effects of starvation and the stress induced in starved rats by their removal from cages, both Brodie *et al.* (1969) and Curzon & Knott (1975) reported that the β-adrenoceptor-blocking agent propranolol does not reverse the starvation-induced lipolysis. The possibility that propranolol was ineffective because the above authors administered it only 1h, and not, e.g., 4h, before death was raised by the finding (Badawy, 1977) that actinomycin D administered 4h before death (i.e. at 20h after the start of starvation) is capable of preventing the corticosterone-mediated enhancement of liver tryptophan pyrrolase activity, thus suggesting that the release of corticosterone (and possibly also catecholamines) may occur at approx. 20h after the start of starvation. The results in Table 3 show that propranolol given as suggested above prevented the starvation-induced changes in free serum, total serum and liver tryptophan concentrations, thus suggesting that these effects of lipolysis can be prevented by adrenoceptor blockade. A detailed time-course of the effects of starvation on lipolysis may therefore be of interest. By contrast, propranolol failed to prevent the starvation-induced increases in serum corticosterone concentration, liver tryptophan pyrrolase activity and [NADP(H)]. The following suggestions may be made as a result of these findings: (1) the 24h-starvation-induced enhancement of pyrrolase activity is not caused by the increase in liver tryptophan concentration, but is most probably produced by hormonal induction of pyrrolase synthesis, as discussed above; (2) the increase in liver tryptophan concentration is not caused by the starvation-induced release of corticosterone; (3) the starvation-induced increase in liver [NADP(H)] occurs irrespective of whether or not liver tryptophan concentration is increased, and may therefore be caused by the enhancement of pyrrolase activity.

The results in Table 3 also show that administration of propranolol to fed rats decreased liver tryptophan concentration, presumably by decreasing lipolysis (as is suggested by the decreases in concentration and percentage of free serum tryptophan), but did not exert any significant effects on any of the other aspects examined. Furthermore, the ability of propranolol to decrease liver tryptophan concentration, but not those of liver [NADP(H)], in fed rats provides additional evidence that these two functions are not related under these conditions.

Table 3. *Effects of propranolol on the starvation-induced changes in rat liver tryptophan pyrrolase activity and [NADP(H)] and in tryptophan disposition and serum corticosterone concentration*

Both fed and 24h-starved rats received, 4h before death, an intraperitoneal injection of either propranolol (10mg/kg) or an equal volume (2ml/kg) of 0.9% NaCl. The above parameters were determined as described in the Materials and methods section and are expressed as in Tables 1 and 2. Values are means ± S.E.M. for each group of four (pyrrolase activity) or of six rats (all other determinations). The values in columns (1) and (4) are compared with those in column (3), whereas those in column (2) are compared with those in column (4). The significance of differences is indicated as follows: †$P<0.05$; †††$P<0.02$; *$P<0.01$; **$P<0.005$; ***$P<0.001$.

	Corticosterone, dinucleotide and tryptophan metabolism parameters			
	Starved rats		Fed rats	
Determination Treatment ...	0.9% NaCl (1)	Propranolol (2)	0.9% NaCl (3)	Propranolol (4)
Holo-(tryptophan pyrrolase)	2.70 ± 0.40†	2.60 ± 0.15***	1.50 ± 0.16	1.50 ± 0.05
Total tryptophan pyrrolase	7.70 ± 0.94*	7.40 ± 0.10***	3.90 ± 0.17	4.00 ± 0.29
Apo-(tryptophan pyrrolase)	5.00 ± 0.56**	4.80 ± 0.09***	2.40 ± 0.11	2.50 ± 0.18
Free serum tryptophan	2.02 ± 0.03***	1.53 ± 0.04***	1.51 ± 0.03	1.18 ± 0.02***
Total serum tryptophan	17.98 ± 0.23**	22.10 ± 0.59	20.25 ± 0.52	20.55 ± 0.53
Free serum tryptophan (%)	11.23 ± 0.27***	6.92 ± 0.28***	7.46 ± 0.20	5.74 ± 0.15***
Liver tryptophan	7.40 ± 0.18***	5.38 ± 0.21*	5.67 ± 0.13	4.47 ± 0.16***
Serum corticosterone	59.80 ± 1.83***	59.70 ± 1.69***	39.30 ± 0.50	36.70 ± 1.80
Liver [$NADP^+$]	62 ± 1***	60 ± 1***	37 ± 1	41 ± 1†††
Liver [NADPH]	261 ± 5***	258 ± 10†	212 ± 6	221 ± 10
Sum of NADP couple	323 ± 6***	318 ± 15†††	249 ± 6	262 ± 11

Prevention by actinomycin D and allopurinol of the starvation-induced increases in liver tryptophan pyrrolase activity and [NADP(H)] and their failure to prevent the increased availability of tryptophan to the liver

The above findings with propranolol suggest that the starvation-induced increases in liver [NADP(H)] can still be observed in the absence of an increased liver tryptophan concentration. It is equally important to find out if, in the presence of an elevated liver tryptophan concentration, the increases in [NADP(H)] could be abolished by blockade of the enhancement of liver tryptophan pyrrolase activity. An agent that prevents the starvation-induced increases in liver [NADP(H)] and pyrrolase activity is actinomycin D (Badawy, 1977), whose action on the latter enzyme involves inhibition of synthesis at the mRNA step (Greengard *et al.*, 1963). This agent should not therefore prevent the starvation-induced increase in serum corticosterone concentration (or possibly also the accompanying lipolysis). That this is so is shown in Table 4. Administration of actinomycin D to 24h-starved rats did not exert any significant effects on the starvation-induced changes in concentration of serum corticosterone or in those of free serum, total serum and liver tryptophan. The only effect of actinomycin D on fed rats was the 32% increase in serum corticosterone concentration. These results and those previously reported (Badawy, 1977) therefore suggest that prevention of the starvation-induced increases in liver [NADP(H)] by actinomycin D is not caused by any change in liver tryptophan concentration, but must involve the blockade of the enhancement of tryptophan pyrrolase activity.

Another agent that is capable of blocking the starvation-induced enhancement of pyrrolase activity is allopurinol (see Morgan & Badawy, 1980). This drug acts in fed rats specifically by preventing the conjugation of the apoenzyme with its cofactor haem (Badawy & Evans, 1973*a*). The results in Table 5 show that administration of allopurinol blocked the starvation-induced increases in liver tryptophan pyrrolase activity and [NADP(H)], but did not influence the changes in concentrations of serum corticosterone, free serum, total serum and liver tryptophan. These results therefore provide additional evidence that the starvation-induced increases in liver [NADP(H)] are not caused by the increase in liver tryptophan concentration, but are most likely the result of the enhancement of tryptophan pyrrolase activity.

In fed rats, allopurinol prevented the conjugation of apo-(tryptophan pyrrolase) with haematin added *in vitro* (as is suggested by the decreases in activities of the total enzyme and apoenzyme, but not in that of the holoenzyme) (Table 5). The failure of allopurinol to alter, in fed rats, the activity of the holoenzyme (which is presumably the active form *in vivo*) may therefore explain the inability of the drug to alter liver [NADP(H)] in such rats. Ad-

Table 4. *Effects of actinomycin D on the starvation-induced changes in rat serum corticosterone concentration and tryptophan disposition*

Both fed and 24h-starved rats received, 4h before death, an intraperitoneal injection of either actinomycin D (0.7mg/kg) or an equal volume (2ml/kg) of 0.9% NaCl. The above parameters were determined as described in the Materials and methods section and are expressed as in Table 3. Values are means ± S.E.M. for each group of six rats. The results in columns (1) and (4) are compared with those in column (3), whereas those in column (2) are compared with those in column (4). The significance of differences is indicated as follows: $*P < 0.01$; $**P < 0.005$; $***P < 0.001$.

	Serum corticosterone and tryptophan metabolism parameters			
	Starved rats		Fed rats	
Determination Treatment ...	0.9% NaCl (1)	Actinomycin D (2)	0.9% NaCl (3)	Actinomycin D (4)
Free serum tryptophan	1.61 ± 0.04***	1.61 ± 0.05***	1.13 ± 0.05	1.18 ± 0.05
Total serum tryptophan	17.90 ± 0.70**	18.82 ± 0.63*	22.17 ± 0.72	22.71 ± 1.02
Free serum tryptophan (%)	8.99 ± 0.48***	8.55 ± 0.50***	5.10 ± 0.18	5.20 ± 0.30
Liver tryptophan	6.82 ± 0.10***	6.82 ± 0.28***	5.26 ± 0.18	4.97 ± 0.12
Serum corticosterone	55.50 ± 1.82***	60.70 ± 1.23**	39.80 ± 2.23	52.60 ± 1.31***

Table 5. *Effects of allopurinol on the starvation-induced changes in rat liver tryptophan pyrrolase activity and [NADP(H)] and in tryptophan disposition and serum corticosterone concentration*

Both fed and 24h-starved rats received, 2h before death, an intraperitoneal injection of either allopurinol (20mg/kg) or an equal volume (4ml/kg) of 0.9% NaCl. The above parameters were determined as described in the Materials and methods section and are expressed as in Tables 1 and 2. Values are means ± S.E.M. for each group of four (pyrrolase activity) or of six rats (all other determinations). The values in columns (1) and (4) are compared with those in column (3), whereas those in column (2) are compared with those in column (4). The significance of differences is indicated as follows: $\dagger P < 0.05$; $\dagger\dagger\dagger P < 0.02$; $**P < 0.005$; $***P < 0.001$.

	Corticosterone, dinucleotide and tryptophan metabolism parameters			
	Starved rats		Fed rats	
Determination Treatment ...	0.9% NaCl (1)	Allopurinol (2)	0.9% NaCl (3)	Allopurinol (4)
Holo-(tryptophan pyrrolase)	3.40 ± 0.37**	1.80 ± 0.12	1.50 ± 0.09	1.50 ± 0.05
Total tryptophan pyrrolase	7.60 ± 0.66**	3.10 ± 0.22***	3.80 ± 0.10	1.70 ± 0.00***
Apo-(tryptophan pyrrolase)	4.20 ± 0.42**	1.30 ± 0.15***	2.30 ± 0.10	0.20 ± 0.02***
Free serum tryptophan	1.33 ± 0.02***	1.41 ± 0.05***	0.98 ± 0.04	1.00 ± 0.03
Total serum tryptophan	15.60 ± 0.18***	15.67 ± 0.40†††	18.90 ± 0.59	18.65 ± 0.86
Free serum tryptophan (%)	8.52 ± 0.13**	9.00 ± 0.67***	5.18 ± 0.32	5.36 ± 0.35
Liver tryptophan	6.73 ± 0.13***	6.94 ± 0.13***	5.45 ± 0.14	5.30 ± 0.19
Serum corticosterone	66.30 ± 3.40**	66.70 ± 2.50***	47.50 ± 2.30	45.00 ± 1.90
Liver [$NADP^+$]	43 ± 3**	29 ± 3	29 ± 2	27 ± 1
Liver [NADPH]	228 ± 13†	180 ± 7	191 ± 6	189 ± 2
Sum of NADP couple	271 ± 12**	209 ± 5	220 ± 4	216 ± 2

ministration of allopurinol to fed mice has previously been shown (Powanda & Wannemacher, 1971) to exert no effect on liver [NAD^+].

Failure of deoxycorticosterone, but not corticosterone, to increase the concentrations of the hepatic NAD(P) couples in normal fed rats

Greengard *et al.* (1966, 1968) reported that both corticosterone and deoxycorticosterone increased total plasma tryptophan concentration and prevented or reversed the decreases in liver [NAD(H)] observed in rats fed on a diet deficient in nicotinamide, nicotinic acid and tryptophan. To find out the effects of these two steroids in normal (fed) rats, experiments whose results are shown in Table 6 were set up. In confirmation of previous findings (Knox, 1962; Greengard *et al.*, 1966), deoxycorticosterone failed to enhance liver tryptophan pyrrolase activity in normal rats, whereas corticosterone increased those of the holoenzyme, total enzyme and apoenzyme by 165, 163 and 161% respectively. Of the two corticosteroids, only corticosterone increased

Table 6. *Effects of corticosterone and deoxycorticosterone on liver tryptophan pyrrolase activity and concentrations of the NAD(P) couples and on tryptophan disposition and serum corticosterone concentration in normal rats*

Normal fed rats received an intraperitoneal injection of corticosterone acetate, deoxycorticosterone acetate (50mg/kg each) or an equal volume (1ml/kg) of the solvent dimethylformamide. The above parameters were determined as described in the Materials and methods section either at 4h (pyrrolase activity) or at 6h (all other determinations) after the above injections. The results are expressed as described in Table 5. Values are means ± S.E.M. for each group of four (pyrrolase activities) or of six (all other determinations) rats. The values in rats treated with either corticosteroid are compared with those obtained in control (dimethylformamide-treated) rats, and the significance of differences is indicated as follows: †$P < 0.05$; *$P < 0.01$; **$P < 0.005$; ***$P < 0.001$.

	Corticosterone, dinucleotide and tryptophan metabolism parameters		
Determination Treatment ...	Dimethylformamide	Deoxycorticosterone	Corticosterone
Holo-(tryptophan pyrrolase)	2.00 ± 0.11	1.80 ± 0.11	5.30 ± 0.38***
Total tryptophan pyrrolase	4.30 ± 0.15	4.10 ± 0.10	11.30 ± 0.44***
Apo-(tryptophan pyrrolase)	2.30 ± 0.15	2.30 ± 0.15	6.00 ± 0.29***
Free serum tryptophan	1.20 ± 0.05	1.13 ± 0.06	0.91 ± 0.03***
Total serum tryptophan	15.23 ± 0.69	15.12 ± 0.60	11.50 ± 0.49***
Free serum tryptophan (%)	7.88 ± 0.28	7.47 ± 0.42	7.91 ± 0.44
Liver tryptophan	4.25 ± 0.16	4.28 ± 0.18	3.22 ± 0.09***
Serum corticosterone	58.60 ± 1.10	53.50 ± 2.88	43.30 ± 3.52**
Liver [NAD^+]	335 ± 10	330 ± 14	389 ± 22†
Liver [NADH]	86 ± 3	88 ± 6	105 ± 7**
Liver [$NADP^+$]	37 ± 1	36 ± 1	45 ± 2 *
Liver [NADPH]	207 ± 6	214 ± 7	279 ± 14***
Sum of dinucleotides	665 ± 13	668 ± 10	818 ± 14***

the concentrations of liver NAD^+, NADH, $NADP^+$ and NADPH (by 16, 34, 19 and 35% respectively). Neither steroid increased the total serum tryptophan concentration at 6h, and in fact only corticosterone decreased those of free serum, total serum and liver tryptophan (by 24% each). These similar decreases in free serum and total serum tryptophan concentrations resemble those caused by treatments that enhance tryptophan pyrrolase activity, such as acute administration of cortisol (Green *et al.*, 1975) or ethanol (Badawy & Evans, 1976*c*) and ethanol withdrawal, which increases the release of corticosterone (Badawy *et al.*, 1980). The findings in Table 6 therefore suggest that the corticosterone-induced increases in concentrations of the hepatic NAD(P) couples are caused by the enhancement of tryptophan pyrrolase activity, and not by an increased liver tryptophan concentration, and that the opposite conclusions reached by Greengard *et al.* (1966, 1968) apply only to animals rendered deficient in nicotinamide, nicotinic acid and tryptophan. It may also be relevant to note that the latter deficiency enhances tryptophan pyrrolase activity (Powanda & Wannemacher, 1970), an effect that is expected to enhance the conversion of tryptophan into NAD^+.

Conclusions

The present findings in starved, and in corticosteroid-treated fed, rats strongly suggest that the concentrations of liver nicotinamide–adenine dinucleotides (phosphates) are regulated by the activity of liver tryptophan pyrrolase. Evidence supporting this conclusion in the rat is provided by the finding (Wrońska-Nofer & Obrebska, 1976) that the increases in liver [$NAD(P)^+$] observed after tryptophan loading of, and the enhanced conversion of labelled tryptophan into these oxidized dinucleotides in, rats chronically treated with carbon disulphide are associated with an enhanced liver tryptophan pyrrolase activity, but not with an increased liver tryptophan concentration. The rat differs from a number of other animal species in various aspects of hepatic tryptophan metabolism, including quinolinate accumulation (for references, see the introduction) and this may therefore explain the different conclusions concerning the roles of liver tryptophan concentration and tryptophan pyrrolase activity in hepatic NAD^+ synthesis.

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