the protein recognizes certain clusters of nucleotides on the DNA. Steroid molecules might, by interacting with the recognition sites on the protein, cause a partial dissociation of the latter from the DNA (Sluyser, 1971).

It is generally assumed that the acidic proteins of chromatin are specific regulators of gene activity, and that the histones are involved in a more general role in preserving nucleoprotein structure. This assumption is based on the belief that the number of different types of histones per cell is too small to allow histones to act as regulators of single genes. However, chromatographic studies have revealed that the lysine-rich histones are complex mixtures of structurally closely related molecules (Stellwagen & Cole, 1969). It seems possible that refined fractionation studies may also reveal such a micro-heterogeneity in some other types of histones as well.

In view of the possibility that histones are involved in steroid-hormone action we have investigated whether histones can bind steroid molecules. [^3H]-Cortisol was injected into rats and histones were isolated from liver nuclei by various methods. These histone preparations were found to contain a low but significant amount of radioactivity. The specific radioactivity of the liver histones increased linearly with increasing doses of injected [^3H]cortisol. The histone-[^3H]cortisol complexes could be dissociated in 40% guanidinium chloride, indicating non-covalent binding. When rat liver nuclei were incubated with receptor-[^3H]cortisol complex, radioactivity became bound to the histones. On the other hand, practically no radioactivity was bound to these histones when the nuclei were incubated with [^3H]cortisol in the absence of cytoplasmic receptor (M. Sluyser & Y. Hermes unpublished work).

The binding of steroids to rat liver histones *in vitro* is of the low-specific non-saturable type (Sluyser, 1969, 1971). However, *in vivo* specificity might be conferred on this interaction by the receptor protein that transports the hormone to the chromatin. Although it is not clear whether the binding of receptor-[^3H]cortisol complex to rat liver histones is physiologically important, these studies encourage further work to investigate the possible involvement of histones in the mechanism of steroid-hormone action.

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Enzyme Activity Changes Relevant to the Control of Tryptophan Metabolism

By G. CURZON (*Department of Neurochemistry, Institute of Neurology, London WC1N 3BG, U.K.*)

A number of important biochemical processes are under the influence of tryptophan concentration. Among these are the synthesis of NAD through a series of reactions occurring subsequent to the action of hepatic pyrrolase on tryptophan and the synthesis of the putative neurotransmitter 5-hydroxytryptamine. The rate-limiting step in the synthesis of the latter substance is the conversion of tryptophan into 5-hydroxytryptophan by tryptophan hydroxylase. Also, tryptophan concentrations appear to have a limiting role in ribosomal aggregation and protein synthesis, and administration of tryptophan is followed by increased synthesis of many enzymes (for a review see Brown, 1971).

In these circumstances the synthesis and activities of the two enzymes tryptophan pyrrolase and tryptophan hydroxylase are of special importance. Tryptophan pyrrolase has been studied in great detail, but with most attention being paid to the mechanisms by which its synthesis or activity are increased by adrenocortical hormones or substrate. Much less work has been done on the relationships between induced tryptophan pyrrolase activity and the control of physiological concentrations of tryptophan and its metabolites. The existence of specific influences on plasma tryptophan concentrations is suggested by their diurnal rhythm being out of phase with the rhythms of those of other amino acids (Fernstrom *et al.*, 1971). However, a clear relationship between the rhythms of tryptophan concentration and of tryptophan pyrrolase activity is not apparent (Harde-land & Rensing, 1968; Rapoport *et al.*, 1966). Also, although adrenalectomy abolished the rhythm of tryptophan pyrrolase activity it altered but did not abolish that of the tryptophan concentration (Rapoport *et al.*, 1966).

Major difficulties in determining the physiological significance of pyrrolase changes derive from: (i) most work on the metabolism of tryptophan *in vivo* on the tryptophan pyrrolase pathway having concerned the handling of large tryptophan loads; (ii) most measurements of tryptophan pyrrolase activity having been done *in vitro* on liver homogenates or preparations. Although activities measured by these two methods were found to correlate positively (Altman & Greengard, 1966), both are of limited physiological relevance, and two studies suggest that the apparent induction by corticoids of tryptophan pyrrolase as determined *in vitro* may not indicate altered tryptophan metabolism. Thus Powanda & Wannemacher (1971) find that tryptophan but not cortisol administration is followed by increased liver

NAD concentrations in the mouse. Kim & Miller (1969) estimated metabolism in the tryptophan pyrrolase pathway *in vivo* by measuring exhaled $^{14}\text{CO}_2$ after the injection of labelled tryptophan and found that cortisol caused only small increases of $^{14}\text{CO}_2$, although *in vitro* the tryptophan pyrrolase activity rose several-fold. However, Chytil (1971) and M. H. Joseph & G. Curzon (unpublished work) obtained relatively large increases in $^{14}\text{CO}_2$ output after the injection of cortisol.

Although the latter findings indicate that adrenocortically induced increases in tryptophan pyrrolase activity may cause increased turnover of tryptophan, evidence is against any marked effect on plasma tryptophan concentrations except in extreme circumstances. Rat plasma tryptophan concentrations decrease only transiently and to a small extent after injection of sufficient cortisol to give a large increase in tryptophan pyrrolase activity as measured *in vitro* (Green & Curzon, 1970). Also, tryptophan pyrrolase activities and plasma tryptophan concentrations are not correlated significantly (Ross *et al.*, 1972). The particularly high tryptophan pyrrolase activity obtained after treatment with α -methyltryptophan is, however, associated with markedly decreased tryptophan concentrations in both plasma and brain (Sourkes *et al.*, 1970).

Another possibility is that increased tryptophan pyrrolase activity resulting from increased tryptophan intake can prevent the large increases of plasma tryptophan concentrations that might otherwise occur. It is noteworthy that, although Fernstrom & Wurtman (1971) found that injection of tryptophan at low dosage increased the rat plasma tryptophan concentration, when Moir (1971) gradually infused large amounts of tryptophan into dogs the initial large increases in plasma tryptophan concentration were not maintained during continued infusion.

The problem of whether altered tryptophan pyrrolase activity can by diverting tryptophan or by other means decrease the turnover of tryptophan on the 5-hydroxytryptamine pathway is of pathological interest (Curzon, 1969), as there is some evidence of both functional impairment of brain 5-hydroxytryptamine synthesis and altered diurnal variation of cortisol concentrations in depressive illness. When tryptophan pyrrolase activity is increased by corticoids, α -methyltryptophan or immobilization for 5-6h, then the rat brain 5-hydroxytryptamine content decreases (Curzon & Green, 1968; Green & Curzon, 1968; Scapagnini *et al.*, 1969; Sourkes *et al.*, 1970). With α -methyltryptophan, as plasma and brain tryptophan concentrations fall considerably the diversion of tryptophan due to increased tryptophan pyrrolase activity is a reasonable explanation for the fall in 5-hydroxytryptamine concentration. With corticoids and immobilization, although increased tryptophan pyrrolase activity precedes de-

creased brain 5-hydroxytryptamine concentrations, the latter cannot be explained by decreased brain tryptophan concentrations. One possibility is that tryptophan metabolites produced by the action of tryptophan pyrrolase may interfere with 5-hydroxytryptamine biosynthesis (Green & Curzon, 1970). An especially complex situation occurs during immobilization or other stress situations, as brain 5-hydroxytryptamine biosynthesis can also be increased because of increased brain tryptophan concentrations, which occur even in the absence of the adrenal and without change of total plasma tryptophan concentration (Curzon *et al.*, 1972).

The decrease in brain 5-hydroxytryptamine concentration on cortisol treatment or immobilization is not found after chronic treatment or prolonged immobilization. This finding and the general observation that brain 5-hydroxytryptamine turnover is altered more readily than its concentration both suggest homeostasis of 5-hydroxytryptamine biosynthesis. A homeostatic role for increased tryptophan hydroxylase activity caused by increased adrenocortical secretion is suggested by the fall in the activity of this enzyme in rat brain after adrenalectomy and its restoration by corticosterone (Azmitia & McEwen, 1969). However, when adrenalectomized rats were either immobilized for 3h or deprived of food for 24h then brain 5-hydroxytryptamine concentrations were maintained even though breakdown increased, as indicated by the raised brain concentration of the 5-hydroxytryptamine metabolite 5-hydroxyindol-3-ylacetate. This suggests that corticoid-mediated increased synthesis of tryptophan hydroxylase is not required for 5-hydroxytryptamine homeostasis and therefore that increased conversion of tryptophan by existing tryptophan hydroxylase molecules may be of greater physiological importance.

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The Effects of Drugs on the Activities of 5-Aminolaevulinate Synthetase and other Enzymes in the Pathway of Haem Biosynthesis

By F. DE MATTEIS (*Biochemical Mechanisms Section, Medical Research Council Toxicology Unit, Carshalton, Surrey, U.K.*)

It has been known for some time that certain drugs can alter the regulation of haem biosynthesis in the liver of the experimental animal and cause a condition of hepatic porphyria, characterized by the accumulation of the intermediates of the pathway that are the porphyrins and their precursors, 5-aminolaevulinate and porphobilinogen (reviewed by Tschudy & Bonkowsky, 1972). A very important achievement in the elucidation of the mechanism of induction of porphyria by drugs was the finding by Granick & Urata (1963) that the activity of liver 5-aminolaevulinate synthetase was markedly increased in experimental porphyria caused by 3,5-diethoxycarbonyl-1,4-dihydrocollidine, a finding later extended to hepatic porphyrias caused by 2-allyl-2-isopropylacetamide (Marver *et al.*, 1966) and griseofulvin (Nakao *et al.*, 1967). This enzyme appears to be the rate-limiting step in the overall pathway and also the site where haem, the end product, exercises a feedback control (reviewed by Granick & Sassa, 1971). The activities of the two subsequent enzymes in the pathway, 5-aminolaevulinate dehydratase and porphobilinogen deaminase, have also been reported to be increased in the liver of animals rendered porphyric with drugs (Gibson *et al.*, 1955; De Matteis & Gibbs, 1972; Miyagi *et al.*, 1971; Strand *et al.*, 1970); these increases are, however, relatively small and may be secondary to the stimulation of the synthetase and to the consequent accumulation of 5-aminolaevulinate and porphobilinogen in the liver (cf. Onisawa & Labbe, 1962).

The mechanism by which drugs stimulate the activity of liver 5-aminolaevulinate synthetase is not yet known. Since the enzyme is subject to feedback control by haem, a likely possibility is that the drugs increase the activity of the enzyme by interfering in

some way with this feedback control. A clear distinction must be made between drugs that increase markedly the activity of the enzyme and induce porphyria and drugs that cause only a small stimulation of the enzyme without a significant accumulation of porphyrins in the liver. Examples of drugs of the first group are the porphyrogenic compounds 2-allyl-2-isopropylacetamide, 3,5-diethoxycarbonyl-1,4-dihydrocollidine and griseofulvin. A transient decline in the concentration of cytochrome P-450 and haem is observed in the liver microsomal fraction after administration of any of these drugs to the experimental animal, coincidental with the rise in activity of 5-aminolaevulinate synthetase. This suggests that the porphyrogenic drugs may stimulate markedly the activity of the enzyme by lowering the concentration of haem in the liver, thereby diminishing the normal feedback control.

With 2-allyl-2-isopropylacetamide the loss of liver haem is due to increased destruction and conversion into certain unidentified green pigments (De Matteis, 1971; Meyer & Marver, 1971). The allyl group in the molecule of the drug appears to be important for both destruction of liver haem and stimulation of 5-aminolaevulinate synthetase activity, possibly after conversion, by way of drug metabolism, into a reactive derivative (Abbritti & De Matteis, 1971-72). Although the microsomal fraction is the main site of haem destruction, a loss of radioactivity is also observed from rapidly labelled haem in the cytosol and in the crude mitochondrial fraction of the liver homogenate of treated animals: this may be relevant to the finding (Hayashi *et al.*, 1969) that a stimulation of the synthetase is observed after the administration of 2-allyl-2-isopropylacetamide in the cytosol as well as in the mitochondria.

With 3,5-diethoxycarbonyl-1,4-dihydrocollidine there is probably some degree of liver haem destruction, but inhibition of liver haem synthesis may also be important in causing the loss of liver haem and the stimulation of 5-aminolaevulinate synthetase activity. A rapid inhibition of the chelatase activity is caused by the drug in liver mitochondria, before any increase in the activity of the synthetase becomes apparent (De Matteis & Gibbs, 1972); mice, which are more sensitive than rats to the stimulation of the synthetase by this drug, are also more sensitive to the inhibition of the chelatase (F. De Matteis, G. Abbritti & A. Gibbs, unpublished work). Inhibition of haem synthesis may also be important in the stimulation of 5-aminolaevulinate synthetase by griseofulvin. Lockhead *et al.* (1967) reported an inhibition of the incorporation *in vivo* of ^{59}Fe into liver haem in mice made porphyric by feeding with griseofulvin for 2 weeks. I have now found that griseofulvin causes an early inhibition of the mitochondrial chelatase, before a marked rise in the activity of the synthetase becomes apparent; also, the 2'- β -hydroxyethyl thio-