The Effect of Nutrition and Hormonal Status on Cytochrome P-450 and its Induction

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Many of the substrates of the hepatic microsomal cytochrome P-450 enzyme system are powerful inducers of the enzyme. Pretreatment of animals with these substrates leads to an increase in overall enzyme activity associated with increases in microsomal protein, cytochrome P-450 and NADPH–cytochrome P-450 reductase activity. We have investigated some of the factors that are responsible for the maintenance of normal enzyme activity and for the control of the response of the enzyme system to inducers.

Apart from genetic and differentiational factors, enzyme activity and inducibility appear to be influenced principally by hormonal status and by environmental factors.

The substances known to act as inducers have few common features (Conney, 1967). An attractive hypothesis is that the activity of inducing substances is mediated through a common endogenous inducer, itself responsible for the maintenance of normal activities of the enzyme.

Conney (1967) suggested that a balance between male and female sex hormones is important in determining the activity of the enzyme system. Mature male rats have higher activities towards certain substrates than do females; the activity can be decreased in males by castration or administration of oestrogens, and increased in females by giving testosterone (Kato & Gillette, 1965; Quinn, Axelrod & Brodie, 1958; Conney, 1967). In male rats we found that castration and adrenalectomy had similar effects on microsomal enzymes; aminopyrine demethylation is decreased and hexobarbital sleeping time is increased, but aniline hydroxylase activity and cytochrome P-450 are unaffected. These effects can be reversed in the adrenalectomized rat by giving cortisone and in the castrated rat by giving testosterone. The effects of each operation were not additive in male rats subjected to adrenalectomy and castration at the same time.

When we gave phenobarbitone to adrenalectomized, castrated or adrenalectomized and castrated rats, there was no difference in cytochrome P-450 concentration between these three groups and phenobarbitone-pretreated controls. Aminopyrine demethylation was likewise unaffected, although in the adrenalectomized animals aniline hydroxylase activity was a little lower. In all cases phenobarbitone was given in a maximally stimulating dose for 7 days (Marshall & McLean, 1969a);

at least 7 days elapsed between the operations and the killing of the animals, which allows considerable depletion of steroid hormone concentrations. We conclude that the induction of cytochrome P-450 and enzyme activity is not mediated through or dependent on the steroid hormones, although hormone balance is involved in the maintenance of normal enzyme activity towards some substrates (e.g. aminopyrine and ethylmorphine) in the non-induced animal.

We think that the most important influences in determining the expression of the genetic potential to synthesize microsomal hydroxylating enzymes come from the chemical environment. This term includes the diet and all the foreign compounds present in foodstuffs, the atmosphere etc.

Male rats fed on a chow diet (M.R.C. diet 41B) have cytochrome P-450 concentrations of 20nmoles/g. of liver. After 8 days of phenobarbitone administration, given as a 0·1% solution in the drinking water, this rises to 120nmoles/g. of liver. In rats fed on a semi-synthetic purified diet containing 15% casein, however, the basal concentration is 14nmoles/g. of liver and rises to only 60nmoles/g. after treatment with phenobarbitone. When rats are fed on a purified protein-deficient diet (3% casein) basal cytochrome P-450 concentrations are 6nmoles/g. of liver and rise to only 30nmoles/g. after treatment with phenobarbitone (Marshall & McLean, 1969b).

Two separate effects are apparent here, one due to the purified diet and one to protein deficiency. The 15%-casein diet is nutritionally adequate, allowing growth at normal rates, suggesting that the chow diet contains some factor, not an essential nutrient, that allows phenobarbitone to induce to a far greater extent than is possible in animals fed on the purified diet. This factor exerts a permissive effect; although not itself a powerful inducer, it facilitates the induction by phenobarbitone. Experiments on fractionation of the chow diet have shown that ether extracts possess some of the permissive activity. We find that neither methyl linoleate nor some of the terpenoids known to be inducers (Parke & Rahman, 1969) have permissive activity. However, oxidized cholesterol, which has some inducing activity (Brown, Miller & Miller, 1954), has a considerable permissive effect. Aged olive oil and cod-liver oil, although having high peroxide numbers, showed little or no permissive activity. The permissive factor could be involved in the control mechanism that regulates induction, or might be a component of the lipid membrane with which cytochrome P-450 is associated.

The second nutritional factor, which may well be of importance in man, is protein deficiency. Diets generally deficient in protein (3% casein) or deficient in specific amino acids (e.g. 15% zein) rapidly

lead to decreased cytochrome P-450 concentrations and low enzyme activity. Response to phenobarbitone (Marshall & McLean, 1969a) and DDT (McLean & McLean, 1966) is greatly decreased. However, some enzymes are increased under these circumstances (Szepesi & Freedland, 1968). This suggests that, in conditions of dietary protein deficiency, the control of protein synthesis is altered to divert the flow of available amino acids into those proteins essential for survival.

When rats are maintained on phenobarbitone and are changed from a low-protein diet to a high-protein diet, a curious phenomenon is observed. In the first 48 hr. after the change of diet there is an enormous increase in liver weight and liver protein. The synthesis of serum albumin increases rapidly also (Kirsch, Frith, Black & Hoffenburg, 1968). However, there is no increase in cytochrome P-450 or associated enzyme activities until about 96 hr. after the change in diet. This again suggests that

there is a system of priorities that switches the flow of amino acids in response to nutritional status.

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STEROID BIOCHEMISTRY GROUP MEETING (HELD IN CONJUNCTION WITH THE SOCIETY FOR ENDOCRINOLOGY) ON 'HORMONAL ACTION AT THE CELLULAR LEVEL'

Oestrogen–Receptor Interaction in Target Tissues

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As first suggested by their striking affinity for oestradiol, both *in vivo* and *in vitro*, oestrogen-responsive tissues, such as uterus, vagina, anterior pituitary and certain mammary tumors, contain unique amounts of specific oestrogen-binding components, which have been called 'oestrogen receptors' or 'oestrophiles'. Strong but reversible association of hormone with receptor, without chemical transformation of the steroid molecule, appears to be a primary step in the uterotrophic process and can be specifically blocked, both *in vivo* and *in vitro*, by such oestrogen inhibitors as nafoxidine (Upjohn 11100), Parke–Davis CI-628 or clomiphene. The interaction of oestradiol with target tissues *in vivo* involves two distinct phenomena: uptake, which is not saturable even with hyperphysiological amounts of administered hormone, and retention, which becomes saturated as the dose exceeds the physiological value.

Centrifugal fractionation experiments, confirmed by radioautographic studies, demonstrate two sites of oestrogen binding in uterine cells. Most of the hormone resides in the nuclei, from which it can be extracted by 0·3 M-KCl as a macromolecular complex sedimenting at about 5 s. A smaller but

significant amount of hormone is bound to a macromolecular substance of apparent extranuclear origin, present in considerable excess in the high-speed supernatant fraction, which shows a sedimentation coefficient of about 8 s. The 8 s oestradiol–receptor complex forms directly on addition of oestradiol to uterine cytosol, but the 5 s complex is not produced by treatment of either nuclei or nuclear extract with oestradiol in the absence of the cytosol. Both receptor substances are proteins containing thiol groups vital to complex-formation. The 8 s oestradiol–receptor complex shows a marked tendency to form large aggregates with other proteins of the cytosol and also to form a well-defined complex with ribonuclease. In 0·3 M-KCl, the 8 s protein dissociates reversibly into 4 s subunits; if Ca^{2+} is also present, the 4 s subunit does not revert to the 8 s form or to larger aggregates when salt is removed. The Ca^{2+}-stabilized 4 s unit, which represents the oestradiol-binding moiety of the 8 s protein, has been purified about 5000-fold by salt precipitation, gel filtration and ion-exchange chromatography.

A variety of experimental evidence suggests that the interaction of oestradiol with uterine tissue involves a two-step mechanism in which the hormone associates first with the 8 s protein, causing it to interact with the nucleus by a temperature-dependent process that consumes 8 s receptor. Whether this phenomenon involves specific transfer of the hormone to some pre-existing nuclear