Proc. Nutr. Soc. (1974), 33, 191

PROCEEDINGS OF THE NUTRITION SOCIETY

The Two Hundred and Sixty-seventh Scientific Meeting was held at the University of Surrey, Guildford GU2 5XH, on 19 April 1974

SYMPOSIUM ON 'INTERACTION OF DRUGS AND NUTRITION'

Nutrition, age and drug metabolism

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A wide variety of substances enter the body, either by accident or design, that have no nutrient value, and may therefore be considered 'foreign substances', anutrients or xenobiotics. These substances are removed from the body after conversion to water-soluble metabolites by a group of non-specific, drug-metabolizing enzymes that are located predominantly, though not exclusively, in liver microsomes (Brodie, Gillette & La Du, 1958). The enzymatic process takes place in two stages (Williams, 1967). The first stage is asynthetic and includes N-dealkylation, deamination, hydroxylation, oxidation and reduction. These processes result in the formation of a polar compound, or, in the instance of an already polar compound, an increase in polarity. The compounds formed pass into the second, or synthetic, phase of the process in which conjugates are formed with glucuronic acid, sulphate or glycine. The conjugates are then excreted in the bile or urine. The enzymes responsible for the phase 1 reactions are NADPH- and O_2-dependent, and the reactions involve a microsomal haemoprotein, cytochrome *P*-450 (Omura & Sato, 1964). Hepatic cytochrome *P*-450 and its linked mono-oxygenase system also catalyse the hydroxylation of a number of endogenous compounds such as steroid hormones (Conney & Klutch, 1963) and fatty acids (Das, Orrenius & Ernster, 1968).

The activity of hepatic microsomal drug-metabolizing enzymes is affected by a variety of factors: age, sex, pregnancy, nutrition, disease and the pre-administration of foreign compounds (Parke, 1968).

The effects of development

The activity of the microsomal drug-metabolizing enzymes is low in the liver of newborn animals. This was first reported in mice and guinea-pigs (Jondorf, Maickel & Brodie, 1959), but has been reported also in the rabbit (Fouts & Adamson, 1959), rat (Kato, Vassanelli, Frontino & Chiesara, 1964) and pig (Short & Davis, 1970). Studies of the development of a number of these enzymes have shown that, regardless of species, they develop in a characteristic manner to reach mature specific activities by 4–6 weeks after birth. In the rat, we (Basu, Dickerson & Parke, 1971*a*) found

that changes in the activity of three enzymes (biphenyl 4-hydroxylase, *p*-nitrobenzoate reductase and 4-methyl-umbelliferone glucuronyl transferase) and the concentration of cytochrome *P*-450 fell into three distinct phases. From birth to 6 d there was little activity. This was followed by a phase of rapid increase for 3–5 weeks after birth, with a subsequent period from 7 to 10 weeks during which the activity fell to the mature value. Similar patterns of development have been reported for the hippuric acid synthesizing system in the rat (Brandt, 1964), microsomal glucuronyl transferase (Flint, Lathe, Ricketts & Silman, 1964) and aspirin hydrolase (Eyring, Crosfeld & Connelly, 1973) in the rabbit, glutathione conjugation in the mouse (Krasner, 1968) and a number of enzymes in the pig (Short & Davis, 1970).

The pattern of development of a second hydroxylase in rat liver, biphenyl 2-hydroxylase, is an exception to the general pattern, for whilst the activity rises during suckling, it falls to a lower level in the adult than at birth (Basu *et al.* 1971*a*).

Sex differences have been reported for the activity of some drug-metabolizing enzymes. Thus, the specific activities of hepatic aminopyrine N-demethylase and of aniline *p*-hydroxylase rise for the first 5 weeks after birth in rats of both sexes, but, after 3 weeks the activity of the former is higher in the male, whereas there is no difference between the sexes in the latter (MacLeod, Renton & Eade, 1972). In this study, and also in those of Short & Davis (1970) and Basu *et al.* (1971*a*) there was a correlation between the activity of some of the drug-metabolizing enzymes and the concentration of cytochrome *P*-450.

The activities of microsomal drug-metabolizing enzymes can be induced by pretreating the animals with certain drugs (Conney, 1967). Thus, in the rabbit the activities of enzymes metabolizing hexobarbital, aminopyrine and *p*-nitrobenzoic acid are induced by the injection of phenobarbital at birth (Fouts & Hart, 1965). Injection of phenobarbital into pregnant rabbits during the last week of gestation induces the activity of the enzymes in the liver of the foetus (Hart, Adamson, Dixon & Fouts, 1962; Rane, Berggren, Yaffe & Ericsson, 1973). This mode of induction of drug-metabolizing enzymes is used in the human to minimize the risk of neonatal hyperbilirubinaemia due to a deficiency of glucuronyl transferase in the liver of the newborn baby (Trolle, 1968) though there is the possibility that hyperbilirubinaemia actually inhibits glucuronidation.

The response of the liver to inducers like phenobarbital decreases with age, and is higher in liver undergoing regeneration after partial hepatectomy than in the intact adult organ (Cramer, Miller & Miller, 1960; Chiesara, Clementi, Conti & Meldolesi, 1967; Kato & Takanaka, 1968; Mueller, Reichenbach & Klinger, 1971; Basu *et al.* 1971*a*). Induction results in liver enlargement by cell multiplication in the actively growing organ, and by cell enlargement in the mature animal (Basu, 1971; Paulini, Beneke & Kulka, 1971).

The regulation of the development of drug-metabolizing enzymes

Enzyme activity may be induced in the liver of the suckling rat by traces of 'foreign substances' in food. Phenobarbital passes from lactating rats to their pups

and induces an increase in the concentration of cytochrome *P*-450 and in the rates of metabolism of other drugs (Darby, 1971). A wholly synthetic maternal diet has no effect, however, on the developmental pattern of the enzymes in the livers of the pups (Basu *et al.* 1971*a*). In the semi-mature male rat corticosterone induces a considerable growth of the liver, and an even greater increase in the amount of biphenyl 4-hydroxylase (Basu *et al.* 1971*b*). Circulating levels of this hormone are high in the rat immediately after birth and fall with age (Holt & Oliver, 1968) but the role of the hormone in development has not been elucidated. There is some evidence (Wilson, 1968, 1970, 1972) that growth hormone represses the development of the enzymes.

Studies by Feuer & Liscio (1969, 1970) suggest that the hormonal metabolism of the mother, and in particular the metabolism of progesterone (Kardish & Feuer, 1972), may inhibit enzyme development in the foetus. Species differences in the development of steroid metabolism may account for the fact that drug-metabolizing activity has been found in the early human foetus (Pelkonen, 1973), but only in the near-term rat foetus. In man, the foetal adrenal cortex has a 'foetal zone' which develops during gestation and involutes after birth. This zone converts progesterone to a wide variety of hydroxylated steroids (Villee, 1973) most of which circulate in the sulphurylated form. There is some evidence that there is a sharp rise in blood cortisol levels just prior to parturition in several species (Murphy & Diez d'Aux, 1972) and this may play a role in enzyme induction.

Carcinogenic polycyclic hydrocarbons induce hepatic and non-hepatic microsomal enzymes in growing animals, although the susceptibility differs in its timing in different species. Thus, in the rat the administration of benzo(α)pyrene during pregnancy induces the corresponding hydrolase activity in all foetuses only from the 15th day of gestation onwards (Schlede & Merker, 1972), whereas in man the activity of benzo(α)pyrene hydrolase can be detected in the livers of foetuses from smokers at 11 or 13 weeks gestation (Juchau, 1971).

Effect of ageing on drug metabolism

There have been comparatively few studies of drug metabolism in old age. Studies on hospital geriatric patients (O'Malley, Crooks, Duke & Stevenson, 1971) have shown that the half-lives of antipyrine and phenybutazone were longer in patients over 60 years of age. Walker, Rahim & Parke (1973) found that the liver weight relative to body-weight at 440 d of age in the rat was below the neonatal value. Whereas there was little change after maturity to 440 d in the concentrations of hepatic microsomal protein and cytochromes *P*-450 and b_5, the relative activities of biphenyl 4-hydroxylase and ethylmorphine N-demethylase fell steadily, and the former was below neonatal values by 440 d. The extent of induction of the cytochromes and enzymes, whether by drugs (Kato & Takanaka, 1968) or ethoxyquin (Walker *et al.* 1973) also falls with age. A fall with age in the capacity to metabolize drugs could be a factor contributing to the increased incidence of drug reactions in the elderly (Hurwitz, 1969). The fact that the relative activities of some enzymes

may be below neonatal levels in some geriatric patients might precipitate a toxic response to certain drugs such as that reported for chloramphenicol in newborn infants (Weiss, Glazko & Weston, 1960).

Nutrition and drug metabolism

The relationship of nutrition to the toxicity of drugs and other anutrients has been the subject of considerable research effort in recent years. The present review will be restricted to the relationship of nutrition to drug metabolism in the developing and ageing animal. The subject has been reviewed in more detail elsewhere (Basu & Dickerson, 1974).

The first 3 weeks of postnatal life in the rat is usually considered to be one of the most vulnerable periods of life. Undernutrition during this period, however, results in no significant change in the activity, per g liver weight, of a number of drug-metabolizing enzymes (Basu *et al.* 1973). The total activity of the enzymes is reduced owing to the lower weight of the livers. Phenobarbital produces a greater percentage increase in activity in the liver of the undernourished animals, and this is in agreement with the results of induction being greater in juvenile than in adult rats (Kato & Takanaka, 1968; Basu *et al.* 1971*a*). The reason for this increased induction is not clear. It could be that there is an increased availability of enzyme sites in either chronologically or physiologically younger animals, or that within the limitations imposed by the liver size, the synthesis of the microsomal enzymes has a high priority.

Kato, Oshima & Tomizawa (1968) were possibly the first to demonstrate in young animals that the toxicity of a number of drugs such as strychnine, pentobarbital and zoxazolamine varied inversely with the protein content of the diet. Marshall & McLean (1969) showed that giving a diet with 30 g casein/kg to adult male rats for 14 d reduced the level of cytochrome *P*-450 in the liver, and that this did not return to a normal concentration after induction with phenobarbital.

Adaptive response of drug-metabolizing enzymes to a low-protein diet. Giving a diet with 70 g protein/kg to weanling male rats for 14 and 28 d results in an increase in the specific activities of biphenyl 4-hydroxylase, *p*-nitrobenzoate reductase and 4-umbelliferone glucuronyl transferase (Dickerson, Basu & Parke, 1971*a*). In the instance of biphenyl 4-hydroxylase, the total activity of enzyme per liver was not changed by the low-protein diet, but was reduced by giving the same amount of protein with less energy. This was in contrast to *p*-nitrobenzoate reductase, which was reduced on a low-protein diet but was independent of the energy intake. The low-protein diet did not affect the animal's ability to metabolize biphenyl and excrete it as hydroxybiphenyl in the urine (Basu, 1971). There was no evidence of a conformational change in biphenyl 4-hydroxylase in these studies (Basu *et al.* 1971*b*). The raised specific activity was, however, associated with elevated concentrations of plasma corticosterone, and evidence to support the possible involvement of steroids was obtained from experiments in which corticosterone was given to

normal immature rats. This induced considerable liver enlargement but an even greater increase in the activity of biphenyl 4-hydroxylase. Further support can be derived from an experiment in which increased metabolism of barbiturates occurred in stressed animals only when the pituitary–adrenal axis was intact (Driever, Bousquet & Miya, 1966).

A low-protein diet and reduced toxicity. A low-protein diet reduces the toxicity of carbon tetrachloride in young male rats (McLean & McLean, 1966). The reason for this is not clear, but it is probable that the production of a toxic metabolite is depressed and that antioxidants are involved (Cawthorne, Palmer & Green, 1973). Similarly, a low-protein diet protects young rats from the toxic effects of dimethyl nitrosamine (McLean & Verschuuren, 1969) but the mechanism is not known.

Effects of other nutrients. Giving a diet containing 600 g sucrose/kg to weanling rats reduced the specific activity and the total activity of biphenyl 4-hydroxylase in the liver when compared with a diet containing a similar concentration of starch (Dickerson *et al.* 1971*b*). A similar effect is obtained in young, but not mature, rats given 100 g sucrose/kg diet (Basu, 1971). Bender, Damji & Ismail (1973) found that in adult rats a diet containing sucrose (700 g/kg) reduced the sleeping time due to administration of pentobarbitone.

The activity of drug-metabolizing enzymes is depressed by diets deficient in calcium, zinc, magnesium and ascorbic acid and increased by diets supplemented with α-tocopherol (see Basu & Dickerson, 1974).

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Printed in Great Britain