The majority of such studies have utilized isolated microsomal fractions, and involved either the addition of exogenous NADPH or the addition of an NADPH-generating system. In the present study, intact tissue sections have been used, and the NADPH has been generated *in situ*, by allowing the glucose 6-phosphate dehydrogenase and 6-phosphogluconate dehydrogenase present in the section to act on their respective substrates. NADPH production was measured by neotetrazolium chloride reduction, the formazan being eluted and measured spectrophotometrically (Altman, 1969a, 1971). The addition of phenazine methosulphate results in an increased production of formazan. This higher activity is identical with that produced in homogenates of the same tissue, and is equivalent to the total hydrogen generated (Altman, 1969b). The hydrogen that reduces the tetrazole in the absence of phenazine methosulphate has been defined as type I hydrogen and probably represents the NADPH used in microsomal reactions; the 'extra' hydrogen, which is only seen in sections in the presence of phenazine methosulphate, has been defined as type II hydrogen and probably represents NADPH that is used biosynthetically (Altman & Chayen, 1970; Altman, 1971; Chayen, Altman & Butcher, 1971). In homogenates no such distinction is possible, since spectrophotometric determination of NADPH production will measure all the NADPH as soon as it is produced (i.e. type I + type II). It is therefore equivalent to the tetrazolium technique in the presence of phenazine methosulphate (Altman, 1969b).

Male Wistar rats (each weighing approx. 200g) were given phenobarbitone (1 mg/ml) in their drinking water and were killed after 4 days (average consumption 35 ml/day). Controls received plain water. Sections (10 μ m) of the liver were incubated, to demonstrate type I and type II hydrogen from the glucose 6-phosphate dehydrogenase and 6-phosphogluconate dehydrogenase reactions. The capacity to oxidize NADH and NADPH was also tested. The activities, expressed as nmol of hydrogen/10 min per mm³, were as follows for sections from phenobarbitone-treated rats (values for controls are given in parentheses): for the glucose 6-phosphate dehydrogenase reaction, type I hydrogen, 24.40 (8.35), type II hydrogen, 21.60 (19.00), total, 46.00 (27.35); for the 6-phosphogluconate dehydrogenase reaction, type I hydrogen 81.00 (34.40), type II hydrogen, 80.00 (93.50), total, 161.00 (127.90); for capacity to oxidize NADH, 85.00 (64.50); for capacity to oxidize NADPH, 70.00 (43.60). Thus, although the total activity of each enzyme was increased after phenobarbitone treatment, analysis of both types of hydrogen shows that only type I was increased. Moreover, Butcher

(1971) has shown that this increase is confined to the centrilobular region.

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The Effect of Phenobarbitone on the Production of Type I Hydrogen from Reduced Nicotinamide-Adenine Phosphate in Different Regions of the Liver Lobule

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The hydrogen from NADPH generated in the cytosol may be oxidized through the microsomal respiratory chain and used in mixed-function oxidases associated with cytochrome P-450 (type I hydrogen) or used in biosynthetic mechanisms (type II hydrogen). The use of thin tissue sections (10 μ m) makes it possible to differentiate between these two types of hydrogen (Altman & Chayen, 1970). Altman (1971) has shown a selective increase in the production of type I hydrogen generated from the dehydrogenation of glucose 6-phosphate or 6-phosphogluconate after administration of phenobarbitone. The elution methods used in that study give a measure of the activity present in the whole section; they do not show how the activity varies in the different parts of the liver lobule. Such selective measurements can be made by using either a scanning and integrating microdensitometer or a double-beam recording microdensitometer. The results obtained with these instruments (relative absorption with the former; the height of the pen deflexion with the latter) have been converted into absolute extinction by reference to standard filters of known density measured under the same conditions.

In the control liver the rate of production of type I hydrogen generated from the dehydrogenation of 6-phosphogluconate was 2.5 times as

great in the centrilobular hepatocytes as in the periportal cells. After the administration of 1mg of phenobarbitone/ml in the drinking water for 4 days, the activity in the periportal region was unaltered whereas that of the centrilobular region was increased to 2.5 times the value found in the corresponding hepatocytes of the control liver.

Brodie *et al.* (1971) have shown that single doses of chlorobenzene and of α - and β -chlorinated naphthalenes produce a massive centrilobular necrosis in the liver within 24h. This effect is markedly potentiated in rats previously treated with phenobarbitone. It would seem of significance that necrosis is produced in that region of the liver lobule with a high rate of production of type I hydrogen, and that it is intensified by phenobarbitone, which increases the rate of this pathway only in that specific region.

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Changes in the Mechanism Regulating Liver Uridine Diphosphate Glucuronyltransferase Activity during Embryonic Life

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UDP-glucuronyltransferase activity towards *o*-aminophenol appears in chick-embryo liver at 7–8 days, remains low, but then increases dramatically on hatching (Dutton & Ko, 1966). UDP-glucuronyltransferase activity develops precociously in simple embryo-liver cultures, being initiated by removal from an embryonic environment rather than by culture conditions or change in cell replication rate (Ko, Dutton & Nemeth, 1967; Skea & Nemeth, 1969). This development of UDP-glucuronyltransferase activity varies directly with protein synthesis from amino acids (Dutton, Hanson & Burchell, 1970; B. Burchell, A. M. Nemeth & G. J. Dutton, unpublished work).

Development of UDP-glucuronyltransferase activity in culture differs with embryo age. For 5-day-embryo liver it rises to a plateau 5–8 times the adult value; for 11-day-embryo liver development ceases at a plateau equal to the adult value

(Skea & Nemeth, 1969). We now report the influence of phenobarbital on this phenomenon.

Phenobarbital increases liver UDP-glucuronyltransferase activity if administered to chickens or added to liver cultures (Dutton, 1970; Dutton *et al.* 1970). With 5.5mm-phenobarbital present during culture of 5-day-embryo liver the UDP-glucuronyltransferase activity reaches the high plateau more rapidly; when phenobarbital is present with 11-day-embryo liver the lower plateau is raised to that same high value displayed by cultured 5-day-embryo liver.

Kinetic studies permit some tentative conclusions. In cultured liver from 5-day embryos phenobarbital increases, by the same factor, the rate of UDP-glucuronyltransferase synthesis from amino acids, *S*, and the rate constant of degradation, *k*; it may therefore be affecting a single control site. In cultures from 11-day embryos phenobarbital increases *S* but has no effect on *k*. In the older embryo regulation of *S* and of *k* therefore appears to have become dissociated (see Berlin & Schimke, 1965).

Moreover, phenobarbital initiates these changes in UDP-glucuronyltransferase activity in 5-day liver only during the first few hours of culture, but in 11-day-embryo liver it increases activity if added at any time during at least 5 days of culture.

Chick-embryo livers cultured at different ages thus exhibit control mechanisms for UDP-glucuronyltransferase activity that are established sequentially long before hatching; the embryonic environment may therefore not inhibit development of this enzyme in the same way throughout embryonic life.

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