minimizes the depression of activity due to lead (de Barriero, 1969). Conversely, although GSH is an essential cofactor for δ -aminolaevulinic dehydratase, excessive concentrations of GSH lower the activity of the enzyme (Moore *et al.*, 1971). Ethanol oxidation substantially alters the redox potential of the cell and, in doing so, alters the [GSH]/[GSSG] ratio in the cell. It is therefore suggested that, although lead depresses GSH concentrations and thus lowers δ -aminolaevulinic dehydratase activity, and ethanol raises GSH concentrations and lowers the activity, these two in conjunction act in opposite directions and lead to an effective maintenance of activity.

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The Increased Anti-Tumour Activity of Chlorambucil after Pretreatment with Phenobarbitone

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Differences in the utilization of chlorambucil (4-[4-bis-(2-chloroethyl)aminophenyl]butyric acid) after its uptake by tumour cells *in vitro* have been demonstrated (Harrap & Hill, 1970): cells of an alkylating-agent-resistant strain of the Yoshida ascites sarcoma are capable of hydrolysing the chloroethyl groups and modifying the aromatic ring of the drug more rapidly than are alkylating-agent-sensitive cells. Phenobarbitone, which is known to induce a number of microsomal drug-metabolizing enzymes (Kuntzman, 1969), was used in the present studies. Rats bearing the drug-sensitive strain of the Yoshida sarcoma were treated with phenobarbitone 12h before the administration of chlorambucil ($\frac{1}{2}$ LD₅₀ 8mg/kg body wt.). This combination resulted in a greater tumour-cell kill than was observed in tumour-bearing animals treated with chlorambucil alone, but was not more toxic to peripheral leucocytes. However, the growth rate of the drug-resistant tumour, maintained by routine passage in rats treated with an alkylating agent, was unaffected by this combination of phenobarbitone and chlorambucil.

These differing responses to phenobarbitone pretreatment were also observed in the two Yoshida cell lines growing in suspension culture. In addition, with a line of L5178Y cells sensitive to alkylating agents it has been shown, by assay of colony-forming

ability, that phenobarbitone pretreatment increases the tumour-cell-killing effect of chlorambucil three- to four-fold. 3-Methylcholanthrene and benz[*a*]anthracene, hydrocarbons that are also capable of inducing drug-metabolizing enzymes (Nebert & Gelboin, 1968), have similarly been shown to enhance the toxic effects of chlorambucil in these drug-sensitive cells.

The administration of phenobarbitone to experimental animals is known to enhance the overall rate of hepatic protein synthesis (Conney, 1967). Exposure of L5178Y and drug-sensitive Yoshida cells in culture to phenobarbitone resulted in a doubling of their rates of protein synthesis. This effect was not observed in drug-resistant Yoshida cells.

Although chlorambucil is generally thought to be a direct-acting alkylating agent (Ross, 1962), these results suggest that metabolic activation may be involved in the anti-tumour activity of this compound in drug-sensitive cells. Some possible implications of these findings and of the lack of response of alkylating-agent-resistant cells to enzyme inducers will be discussed.

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Lysosomal Enzymes and Degradation of the Proteoglycans of the Human Intervertebral Disc

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Previous work has indicated (Lyons *et al.*, 1964, 1966; Davidson & Woodhall, 1959) that the proteoglycans in prolapsed discs are degraded compared with those in normal tissue, and a similar change occurs on aging. Lysosomal enzymes may be involved, but their presence in the disc has received little attention. A particulate fraction obtained by differential centrifugation from homogenates in 0.25M-sucrose contained several forms of acid phosphatase (Osborn, 1971) that were released by treatments known to disrupt lysosomes. Acid protease and at least three exoglycosidases (Happey *et al.*, 1971) were present, but hyaluronidase was not detected in the particulate fraction or in proteoglycan-free supernatants prepared from concentrated homogenates of nucleus pulposus.

The degradation of the proteoglycans is likely to be