minimizes the depression of activity due to lead (de Barriero, 1969). Conversely, although GSH is an essential cofactor for δ -aminolaevulinic acid 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 acid 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

initiated by an acid protease as in cartilages (Ali *et al.*, 1967; Morrison, 1970; Dingle *et al.*, 1971), possibly cathepsin D. Extracts of autopsy specimens of the disc, from which almost all of the proteoglycans had been removed by precipitation with protamine sulphate (Dziewiatkowski *et al.*, 1968), contained significant activity against acid-denatured haemoglobin at pH 3.6. Supernatants obtained by using cetylpyridinium chloride or cetyltrimethylammonium bromide had much lower activities. At 37°C and pH 5 a protamine sulphate supernatant rapidly lowered the viscosity of the light fraction (PPL fraction) of purified disc proteoglycans, which contained a large proportion of keratan sulphate as well as chondroitin sulphate (unlike most cartilage proteoglycans). Incubation for 1.5 h resulted in complete disappearance of the excluded peak found for untreated PPL fraction in gel filtration on Sepharose 2B (Pharmacia Ltd.) and an increase in retarded polyanions, shown by monitoring turbidimetrically with rivanol (6,9-diamino-2-ethoxyacridine lactate) (Nemeth-Csoka, 1961). Preheating the protamine supernatant at 100°C for 15 min diminished but did not entirely eliminate its effects on the viscosity and gel-filtration pattern of PPL fraction.

Autolysis at pH 4–5 of slices of nucleus pulposus for 24 h at 37°C appeared to induce breakdown of the proteoglycans, as judged by gel filtration, but unequivocal evidence that the total quantity released from the tissue was increased was not obtained. Possibly partial autolysis had occurred before autopsy. A more definite mobilization of a proteoglycan fraction that resisted extraction with cold iso-osmotic saline from tissue slices before autolysis was demonstrated.

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Cation-Dependence of Phosphatase Activity in Bull Spermatozoa

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Active transport of alkali-metal cations in spermatozoa has been inferred both from studies on intracellular ionic concentrations (Quinn & White, 1967) and by the demonstration of a $\text{Na}^+ + \text{K}^+$ -stimulated ouabain-sensitive adenosine triphosphatase activity in whole sperm and in extracts (Uesugi & Yamazoe, 1966; Quinn & White, 1968). A metabolically dependent binding of ouabain to bull sperm has also been demonstrated (O'Donnell & Ellory, 1970). The present experiments investigated the ion-dependence of bull sperm nucleotide phosphatase activity in more detail.

Fresh washed bull spermatozoa suspended in an iso-osmotic sucrose medium buffered at pH 7.5 with 10 mM-tris showed an adenosine triphosphatase activity of $1.2 \pm 0.1 \mu\text{mol}$ of P_i/h per 10^8 cells at 37°C. Addition of MgCl_2 (1.5 mM) increased the activity to $3.2 \pm 0.3 \mu\text{mol}$ of P_i/h per 10^8 cells, and the addition of NaCl (165 mM) further stimulated the rate to $5.9 \pm 0.2 \mu\text{mol}$ of P_i/h per 10^8 cells. Rapid freezing and thawing of fresh washed cells gave a supernatant, after mild centrifugation, that had an activity of $3.2 \pm 0.2 \mu\text{mol}$ of P_i/h per mg of protein under the same conditions, and which was also stimulated by Mg^{2+} and further stimulated by Na^+ , or by $\text{Na}^+ + \text{K}^+$. Neither the whole cells nor the extract adenosine triphosphatase activity was inhibited by 0.1 mM-ouabain.

Both cells and extract hydrolysed ITP and CTP as well as ATP in the presence of Mg^{2+} , though further activation by alkali-metal cation was confined, in the extract, to the hydrolysis of ATP. Ca^{2+} could substitute for Mg^{2+} , and ADP but not AMP was also an effective substrate. *p*-Nitrophenyl phosphate was not hydrolysed. Whole sperm hydrolysed ATP more rapidly at alkaline pH, but the extract, in the presence of Mg^{2+} , Na^+ and K^+ , showed an optimum at pH 7.4. Whole cells and extract also differed in the efficacy with which other members of the alkali-metal cation series enhanced the Mg^{2+} -dependent adenosine triphosphatase; for the extract Na^+ was most effective and Cs^+ least so, whereas K^+ stimulated the cell activity most and Li^+ least (Li^+ was also least effective in maintaining the motility of intact cells when other alkali-metal cations were substituted for K^+ in an artificial incubation medium).

Unlike previous results, therefore, the present experiments did not detect any ouabain sensitivity in the Mg^{2+} -dependent adenosine triphosphatase activity of sperm that was stimulated by Na^+ or by $\text{Na}^+ + \text{K}^+$. The measured phosphatase activities were