achieved with sodium deoxycholate (3g/l) in 0.2M-Na₂HPO₄ adjusted to pH6.5 with 0.2M-KH₂PO₄. The solubilized enzyme, in the presence of sodium deoxycholate, has a pH optimum of 7.9, which approaches that of the cytoplasmic catechol *O*-methyltransferase.

Under our experimental conditions tyramine, a monohydroxy amine, does not serve as a substrate for the microsomal preparations. The optimal substrate concentrations of L-adrenaline and L-nor-adrenaline were found to be similar for both enzymes.

The total activities of the cytoplasmic and microsomal catechol *O*-methyltransferases in the livers of normal rats were investigated. The relative ratios of specific activities of the two enzymes were found to increase in rats subjected to isolation and in those treated orally with phenobarbitone (23 mg/day).

Dallner, G. (1963) *Acta Pathol. Scand. Suppl.* 166

Inscoc, J. K., Daly, J. & Axelrod, J. (1965) *Biochem. Pharmacol.* 14, 1257-1263

Effects of Ethoxyquin on Hepatic Microsomal Enzymes

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Ethoxyquin (6-ethoxy-1,2-dihydro-2,2,4-trimethylquinoline) is an antioxidant permitted for limited use in foods in the U.K. Claims have been made that ethoxyquin improved survival rates in mice (Harman, 1968; Comfort *et al.*, 1971), and it was postulated that this could have been partly due to calorie restriction or induction of the hepatic enzyme systems (e.g. see Ross, 1969). Paired-feeding studies have been carried out with male Wistar rats and some hepatic enzyme activities measured.

Three groups of rats were caged singly and maintained on a commercial powdered diet containing 2.5% (w/w) antioxidant-free groundnut oil. In the diet of one group ethoxyquin was incorporated at a concentration of 0.5% (w/w). This test group and one control group were fed *ad libitum*, and the third group were paired-fed with an equal amount of food to that consumed by the test group. Food intakes and body weights were monitored daily.

Four animals from each group were killed after 0, 3, 7, 14, 30 and 60 days and liver homogenates were prepared. Microsomal protein (Lowry *et al.*, 1951), cytochrome *P*-450 (Sladek & Mannering, 1966) and cytochrome *b*₅ (Schenkman *et al.*, 1967) concentrations and biphenyl 4-hydroxylase (Creaven *et al.*, 1965) and ethylmorphine *N*-demethylase (Holtzman *et al.*, 1968) activities were determined on these homogenates.

Food intake was decreased in the test group by 6% over the 60-day period relative to controls fed *ad libitum* and growth rate was consequently depressed, but the difference in body weight was not statistically significant by the end of this period.

By the 14th. day ethoxyquin caused a 50% increase in relative liver weight that was maintained thereafter for the whole 60-day period. Concentrations of hepatic microsomal protein, cytochrome *P*-450 and cytochrome *b*₅ were all markedly induced (85-100% by the 14th day), and the cytochrome concentrations remained at this elevated value throughout. Biphenyl 4-hydroxylase activity was increased nearly threefold in test rats by the 7th day and remained at more than twice control values for the remaining period, but there was no evidence for induction or inhibition of ethylmorphine *N*-demethylase. By comparison with paired-fed controls it was seen that the hepatomegaly, increased microsomal protein and elevated enzyme activities were not a consequence of calorie restriction.

Similar experiments carried out with *n*-propyl gallate did not result in any of the effects observed with ethoxyquin, possibly because hydrolysis occurred before absorption and the hydrolysis products would not be expected to be substrates for the microsomal enzymes.

The antioxidant butylated hydroxytoluene (2,6-di-*t*-butyl-4-methylphenol) is also a powerful inducer of hepatic microsomal enzymes (Gilbert & Golberg, 1965) and has increased life-span in mice (Harman, 1968), but these effects have not been reported for *n*-propyl gallate. The prolongevity produced by butylated hydroxytoluene and ethoxyquin may thus depend on their being enzyme inducers, and further work is in progress with other antioxidants to clarify this.

Creaven, P. J., Parke, D. V. & Williams, R. T. (1965) *Biochem. J.* 96, 879

Comfort, A., Youhotsky-Gore, I. & Pathmanathan, K. (1971) *Nature (London)* 229, 254

Gilbert, D. & Golberg, L. (1965) *Food Cosmet. Toxicol.* 3, 417

Harman, D. (1968) *Gerontologist* 8, 13

Holtzman, J. L., Gram, T. E., Gigon, P. L. & Gillette, J. R. (1968) *Biochem. J.* 110, 407

Lowry, O. H., Rosebrough, N. J., Farr, A. L. & Randall, R. J. (1951) *J. Biol. Chem.* 193, 265

Ross, M. H. (1969) *J. Nutr.* 97, 565

Schenkman, J. B., Fray, I., Remmer, H. & Estabrook, R. W. (1967) *Mol. Pharmacol.* 3, 516

Sladek, N. E. & Mannering, G. J. (1966) *Biochem. Biophys. Res. Commun.* 24, 668