(Walters & Taylor, 1964). The present communication describes analyses of head-space gases produced from anaerobic incubations of nitrite (2.9 mM) and minced porcine skeletal muscle at pH 6.0 at 37°C in the presence of the antibiotic chloramphenicol to restrict bacterial involvement in the experiments. Analyses were performed by using medium-resolution mass spectrometry and g.l.c. and employed nitrite labelled with the stable isotope ^{15}N . With $\text{Na}^{14}\text{NO}_2$ major peaks were obtained in the mass spectrum at m/e values 28, 30 and 44; when $\text{Na}^{15}\text{NO}_2$ was employed the only new peaks observed were at m/e 31 and 46. This indicates the formation of NO and N_2O respectively. NO production increased linearly with nitrite concentration up to 14 mM, and the output of N_2O , although much less than that of NO, also increased.

Peaks were also observed at m/e values 26 and 27 and were considered to be part of the fragmentation pattern of ethylene, the molecular ion of which ($m/e = 28$) would have been obscured by background $^{14}\text{N}_2$. Confirmation of the formation of C_2H_4 has been provided by g.l.c. and high-resolution mass spectrometry. C_2H_4 production increased with nitrite concentration up to 50 mM, and, as the bacterial count of the muscle with nitrite present was very low, the formation of C_2H_4 was apparently not associated with bacterial action. CO_2 has also been detected (at $m/e = 44$) as a product. A markedly decreased output of NO and C_2H_4 that was observed in the presence of the respiratory-chain inhibitor Amytal (Ernster *et al.*, 1963) suggested that gas evolution was associated with the respiratory oxidation-reduction systems of the tissue. This view is supported by the earlier observation that NO production was enhanced by the inclusion of the electron donor reduced Methylene Blue (Walters & Taylor, 1964).

The reduction of nitrite to NO involves a redox potential change of +0.99 V (Charlot *et al.*, 1958), close to that for the reduction of O_2 . The combination of two nitrogen atoms as in N_2O could proceed through the labile dimerization of any nitroxyl (NOH) formed, followed by elimination of H_2O from the resultant hyponitrous acid. N_2O may also be formed from the action of nitrous acid with quaternary ammonium compounds and tertiary amines (Fiddler *et al.*, 1972) and with NADH (Evans & McAuliffe, 1956) or thiol groups (Möhler & Ebert, 1971); C_2H_4 is possibly produced by loss of HNO_2 from ethyl nitrite, which has been found in meat products preserved with nitrite.

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A Microsomal Catechol O-Methyltransferase from Rat Liver

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A microsomal enzyme capable of O-methylating catechols differs from cytoplasmic catechol O-methyltransferase in its response to 3,4-benzopyrene and to cold stress (Inscoc *et al.*, 1965). The two enzymes also have different pH optima.

We have studied the properties of catechol O-methyltransferase in rat liver microsomal preparations and in the fraction obtained on solubilization of the microsomal membrane. Microsomal preparations were obtained by differential centrifugation of rat liver homogenates in iso-osmotic KCl, and the washed microsomal pellets were finally suspended either in 0.2 M- Na_2HPO_4 adjusted to pH 6.5 with 0.2 M- KH_2PO_4 or in iso-osmotic KCl. Alternatively rat liver microsomal preparations were obtained by the technique described by Dallner (1963) and finally suspended either in 0.2 M- Na_2HPO_4 adjusted to pH 6.5 with 0.2 M- KH_2PO_4 or in 0.25 M-sucrose.

The microsomal fractions were incubated at 37°C with L-adrenaline bitartrate (640 μM) and S-adenosyl-[Me- ^{14}C]methionine (75 μM) and the reaction was terminated by the addition of borate buffer, pH 10.0. The [Me- ^{14}C]methylcatechol formed in the reaction was extracted from the incubation mixture with toluene-pentanol (3:2, v/v) and its radioactivity measured by liquid-scintillation counting.

Initial observations of microsomal catechol O-methyltransferase show that the pH optimum is about 6.5 whereas that of the cytoplasmic enzyme is approx. 7.9. The microsomal enzyme is not released from the membranes in the presence of saturated NaCl, which suggests that catechol O-methyltransferase is not ionically bound to the microsomal membrane. Optimal solubilization was

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).

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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.

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