the preferred electron donor (K_m 16 μ M compared with 2 mM for NADH). The enzyme was not inhibited by CO, cyanide or SKF 525-A (2-diethylaminoethyl 2,2-diphenylvalerate hydrochloride), nor by chelating agents. No metal ion requirement could be demonstrated, and Co^{2+} , Ca^{2+} and Zn^{2+} were inhibitory. The enzyme was rather unstable (half-life in the crude extract 20 min at 35°C) and a sixfold purification is the best so far obtained. Such preparations are free of cyanide-sensitive secondary-amine oxidase activity (Eady *et al.*, 1971) and trimethylamine *N*-oxide demethylase activity (Large, 1971), and have an absorption spectrum suggesting the presence of flavin. The enzyme had optimum pH 7.7 and was stable at 0°C for 30 min at any pH value in the range 4–9. The reaction product was identified as trimethylamine *N*-oxide by its identical mobility with the authentic material in t.l.c., by its identical behaviour on ion-exchange chromatography (Blau, 1961), and by showing that it was converted into formaldehyde by (a) SO_2 (Mitchell & Ziegler, 1969) and (b) partially purified preparations of trimethylamine *N*-oxide demethylase (Large, 1971; Myers & Zatman, 1971). The stoichiometry of the reaction agrees with the equation:

It differs from the secondary-amine mono-oxygenase present in the same extracts (Eady *et al.*, 1971) by its lack of sensitivity to CO and cyanide, and by its specificity for NADPH. We have also found it present in trimethylamine-grown *Hyphomicrobium vulgare* NQ.

No evidence for a phenazine methosulphate-linked trimethylamine dehydrogenase has been found in *Ps. aminovorans*. Since other bacteria that can grow on trimethylamine contain such an enzyme (Colby & Zatman, 1971), it appears that a diversity of pathways for amine oxidation occur in bacteria (Large, 1972).

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Resistance of Alkylated Deoxyribonucleic Acid to Nuclease Attack

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We have investigated the ability of some deoxyribonucleases to degrade methylated DNA in connexion with studies on carcinogenesis, first for their potential as analytical tools and secondly for the possible significance of these enzymic reactions in the metabolism of DNA methylated *in vivo* by carcinogens. In view of the suggested importance of DNA synthesis to the carcinogenic process (Craddock, 1971; Stewart & Magee, 1971), enzymes known to exhibit increased activity during DNA replication in partially synchronized systems (Brody & Balis, 1959; O'Connor, 1971) are of special interest.

In these initial studies, calf thymus DNA was hypermethylated by using dimethyl sulphate (Michelson & Pochon, 1966) and the amount of methylation was determined by ion-exchange chromatography of acid hydrolysates of the DNA. Endonuclease and exonuclease activity was measured by using acid-soluble nucleotide assays, and paper chromatography was used to verify the exonuclease assays.

The endonucleases studied were pancreatic deoxyribonuclease I, an alkaline deoxyribonuclease from rat liver (O'Connor, 1969), and hog spleen acid deoxyribonuclease (Bernardi, 1966); the exonucleases were from snake venom (*Crotalus adamanteus*) and from hog spleen (Bernardi & Bernardi, 1968). The deoxyribonucleases from pancreas and from rat liver showed only slightly lower activity with hypermethylated DNA compared with normal DNA, and the venom exonuclease readily digested methylated DNA to mononucleotides, indicating the usefulness of these enzymes for the base analysis of chemically methylated DNA.

On the other hand the activity of the spleen acid deoxyribonuclease with methylated DNA was greatly diminished. This effect was obtained with commercial samples and the enzyme purified as described by Bernardi (1966). Results from initial reaction rates obtained with purified enzyme and DNA methylated to various extents suggested that the enzyme would be virtually inactive when about half of the guanine residues were converted into 7-methylguanine.

The preferred substrate for the spleen exonuclease is the 3'-oligodeoxyribonucleotide produced from DNA by the action of the spleen deoxyribonuclease. However, when hypermethylated DNA oligonucleotides were prepared, either by digestion of methylated DNA or by methylation of the oligonucleotides from a digest of normal DNA, and subjected to

spleen exonuclease attack, only a limited amount of hydrolysis took place.

These results suggest that chemically methylated regions of DNA are not readily hydrolysed by the acid nucleases of lysosomal origin (Beaufay *et al.*, 1959) and that they may be afforded some protection from nuclease attack *in vivo*. Since acid deoxyribonuclease activity has been found in nuclei during active growth periods (Lesca, 1968), these findings may be of significance in the metabolism of chemically methylated DNA.

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Inhibition by Ascorbic Acid of the Sodium-plus-Potassium Ion-Stimulated Adenosine Triphosphatase in Rat Brain

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The $\text{Na}^+ + \text{K}^+$ -stimulated ouabain-sensitive adenosine triphosphatase activity of a homogenate (10%, w/v) of rat cerebral cortex prepared in 0.32M-sucrose containing 10mM-tris-HCl buffer, pH7.4, and 0.5mM-EDTA is found exclusively in the particles obtained by centrifugation at $6 \times 10^4 g_{av}$ for 90 min. The enzyme activity was determined by resuspending the washed particles in buffered sucrose so that the protein concentration was 1–2mg/ml and an appropriate sample was taken for assay (Wheeler & Whittam, 1970). Under these conditions the activity of the enzyme was $13.0 \mu\text{mol}$ of P_i/h per mg of protein. However, if the particles were resuspended in the supernatant obtained by centrifuging a brain homogenate (10%, w/v) prepared in buffered sucrose, the activity of the enzyme was $3.8 \mu\text{mol}$ of P_i/h per mg of protein. The addition of 0.5mM-EDTA abolished this inhibition. These results suggest that rat brain contains an inhibitor of $\text{Na}^+ + \text{K}^+$ -stimulated adenosine triphosphatase whose effects are antagonized by 0.5mM-EDTA.

The inhibitory activity of brain supernatant is apparently not due to metal ions because it was destroyed by strong acid and mild alkaline hydrolysis and by treatment with activated charcoal. It remained after boiling for 10 min at pH7.4, and after centrifugation to remove the precipitated protein the material was applied to a column of DEAE-Sephadex A-25 equilibrated with 25mM-tris-HCl buffer, pH7.4. The column was eluted with a linear NaCl concentration gradient, and inhibitory activity was eluted in a fraction that contained a single peak of material absorbing at 267 nm at pH7.4. This fraction did not contain nucleotides (Bendich, 1957) and contained only traces of organic phosphorus. In 0.01M-HCl the absorption maximum shifted to 245 nm and in 0.01M-NaOH the spectrum slowly disappeared. Ascorbic acid has similar spectroscopic properties, and it was found that the ascorbic acid content of the active fraction measured by the method of Roe & Kuether (1943) agreed well with that calculated from the spectroscopic data. The ascorbic acid content of rat brain is about $2 \mu\text{mol/g}$ wet wt. (Rajalakshmi & Patel, 1968). Ascorbic acid added to brain supernatant co-chromatographed on the DEAE-Sephadex A-25 column with the inhibitory factor.

Finally, 0.5mM-L-ascorbic acid inhibited $\text{Na}^+ + \text{K}^+$ -stimulated adenosine triphosphatase by 70% and this inhibition was abolished by 0.5mM-EDTA.

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The Metabolism of some Anionic Surface-Active Compounds in the Rat

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Previous work in these laboratories on the metabolism of alkyl sulphate esters (Denner *et al.*, 1969) has shown that dodecyl sulphate is degraded in the liver of the rat by ω -oxidation and subsequent β -oxidation. The end product, butyric acid 4-sulphate, is eliminated in the urine.

This study has been extended to include the potassium salts of decyl sulphate, octadecyl sulphate and undecyl sulphate.

Free-ranging male and female rats received intraperitoneal injections of ^{35}S -labelled decyl sulphate, octadecyl sulphate and undecyl sulphate (1 mg/200 g body wt.), and urine and faeces were collected over