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

48 h. In each case most of the injected radioactivity (over 60%) was recovered in the urine as ester sulphate. Similar results were obtained after oral administration of octadecyl [^{35}S]sulphate and after oral and intravenous administration of undecyl [^{35}S]sulphate.

After intravenous administration of decyl [^{35}S]sulphate (1 mg/200 g body wt.) to wholly anaesthetized rats with bile-duct and ureter cannulae 54–62% of the injected radioactivity was excreted in the urine as ester sulphate over 6 h; less than 2% appeared in the bile. In similar experiments with octadecyl [^{35}S]sulphate (0.1 mg/animal) approx. 73% of the radioactivity was recovered in the urine as ester sulphate; again less than 2% appeared in the bile.

Chromatography and electrophoresis of urine samples obtained from all animals receiving decyl [^{35}S]sulphate or octadecyl [^{35}S]sulphate showed one major radioactive component. This was identified as butyric acid 4-[^{35}S]sulphate.

When cannulated animals were injected with undecyl [^{35}S]sulphate (1 mg/200 g body wt.) 30% of the administered radioactivity appeared as ester sulphate in the urine, whereas at a lower dose (0.5 mg/200 g body wt.) 48% was excreted. At both dosages the amount of ^{35}S -labelled ester in bile obtained from male rats was low (approx. 2%), whereas bile from female rats contained between 6 and 14% of the injected radioactivity. The ester sulphate appearing in urine is associated with three metabolites, none of which can be identified with the parent ester. The chromatographic and electrophoretic properties of these metabolites suggest that they are short-chain sulphate esters structurally related to butyric acid 4-sulphate.

Whole-body radioautographic studies pinpoint the liver as the site of metabolism of all three parent esters.

Presumably the even-numbered alkyl sulphate esters studied are degraded by the mechanism proposed for the breakdown of dodecyl sulphate. The similar properties of butyric acid 4-sulphate and the metabolites derived from undecyl sulphate suggest that this odd-numbered alkyl sulphate ester might also be degraded by a similar mechanism.

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Metabolism of [^{14}C]Phenacetin in Various Species

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The metabolic fate of phenacetin in various species is being investigated (a) to obtain information on interspecies variation in its metabolism and (b) to attempt to find an animal species that metabolizes the drug in a manner analogous to that reported to occur in certain human beings having a defective ability to de-ethylate phenacetin (Shahidi, 1968).

In this communication preliminary findings on the metabolism and excretion of [^{14}C]phenacetin in five species including man are reported. Animals were dosed orally with [^{14}C]phenacetin (100 mg/kg; 5 μCi) dissolved in aq. 70% (v/v) propane-1,2-diol, and the urine, faeces and expired air were collected for up to 4 days. Two male human subjects took a 500 mg oral dose of [^{14}C]phenacetin (5 μCi). The metabolites in the urine were identified and determined by quantitative radiochromatogram scanning and in some cases by reverse isotope dilution.

The species examined excreted 64–88% of an oral dose of [^{14}C]phenacetin in the urine in 3 days, the highest recoveries being obtained for the rabbit and guinea pig and the lowest for the ferret and rat. Faecal excretion varied from the low value of 0.2% found for the guinea pig to 4% found for the two human subjects and the rabbit. The excretion of radioactive CO_2 in the expired air, which could be regarded as a measure of the extent of deacetylation of the drug, accounted for 3–8% of the dose in the guinea pig, rabbit and two human subjects, but was higher in both the ferret (13%) and the rat (16%).

The major metabolite in the urine in all the species was conjugated *N*-acetyl-*p*-aminophenol, which accounted for 36–87% of the dose, the highest value being for the guinea pig and the lowest for the ferret. There were marked species variations in the pattern of conjugation of the *N*-acetyl-*p*-aminophenol, since the main conjugate in the urine in the rabbit, guinea pig and ferret was *N*-acetyl-*p*-aminophenyl glucuronide, whereas in the rat it was *N*-acetyl-*p*-aminophenyl sulphate. The two human subjects excreted slightly more of the glucuronide conjugate (38% of dose) than the sulphate (27%). In addition to these metabolites the urine contained small amounts of unchanged phenacetin (0.1–0.5% of dose), free 2-hydroxyphenacetin (0.1–0.3%) and free *N*-acetyl-*p*-aminophenol (0.9–3.2%), and also an unidentified metabolite that accounted for 0.4–4% of the dose in the rat, guinea pig and two human subjects and more (7–10%) in the rabbit and ferret.