niques, were ultrasonically treated and high-speed supernatants prepared. Sephadex G-25 column chromatography was used to demonstrate macromolecular binding of aldosterone in the supernatants. Binding material was present in all fractions. After incubation with $0.1 \mu\text{M}$ - $[^3\text{H}]$ p-aldosterone, 3.5×10^{-14} – 2.8×10^{-13} moles of aldosterone/100 μg . of DNA was bound to material obtained from the crude nuclear fraction; with $0.01 \mu\text{M}$ - $[^3\text{H}]$ -D-aldosterone, 8.5×10^{-15} – 5.9×10^{-14} moles of aldosterone/100 μg . of DNA was bound. Of the crude nuclear binding, 40–70% was displaced with excess of deoxycorticosterone in the incubation medium and 80% was displaced with excess of non-radioactive aldosterone.

From the time constants for the breakdown of the bound material it appears that at least three sets of binding sites are present in the nuclei. At 0° , 40–60% of the bound material dissociated in 2 hr.; 10–40% of the binding was stable and still present

after 24 hr., and about 20% of the material had a half life of about 4 hr.

Further fractionation of the bound material with Agarose column chromatography failed to show the highly labile component, but showed two complexes, one stable and of high molecular weight and the other that broke down slowly with time and had a molecular weight in the range 100 000–150 000. Neither of these fractions was displaceable with deoxycorticosterone, all displacement being reflected in the highly labile fraction.

It is suggested that the highly labile fraction that is displaceable by deoxycorticosterone is responsible for the stimulation of sodium transport. The roles of the remaining two fractions have yet to be elucidated.

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DEMONSTRATION

The Use of *N*-Ethylmaleimide in Stabilizing and Measuring Inorganic Sulphur Compounds

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A serious problem encountered in the study of the metabolism of sulphur compounds is the extreme reactivity and instability of many of the intermediates, especially the inorganic ones (Postgate, 1963). The reaction between thiol groups and *N*-ethylmaleimide (NEM) has been widely used to stabilize organic thiol compounds in both free and protein-bound forms (Benesch, Benesch, Gutcho & Laufer, 1956; Boyer, 1959). However, NEM reacts not only with organic thiols but also with sulphite, thiosulphate and sulphide (Gregory, 1955; Ellis, 1964) and the compounds formed can be separated by paper chromatography (Margolis & Mandl, 1958). The potential usefulness of NEM for studying sulphur metabolism is thus greater than is generally realized (Ellis, 1966).

The demonstration will illustrate the following.

(1) The kinetics of the reaction between NEM and the inorganic sulphur compounds. These reactions can be followed by measuring the loss of extinction at $300\text{m}\mu$. Sulphite and sulphide react more slowly than organic thiols, but the reactions approach completion in 5 min. at pH 7.4. The reaction with sulphite is equimolar.

(2) A rapid assay for sulphite reductase. Inorganic sulphide gives a red solution when it is incubated with an excess of NEM at pH 8.0 and then made alkaline with Na_2CO_3 . Inorganic sulphite gives no colour under these conditions. The activity of

sulphite reductase (hydrogen sulphide–NADP⁺ oxidoreductase, EC 1.8.1.2) can thus be measured without removing the sulphide from the assay tube. An extinction of 1.0 at $520\text{m}\mu$ in a 1 cm. cell is given by 260 μmoles of sulphide (Ellis, 1964).

(3) The trapping of intermediates in sulphate reduction by intact cells of *Escherichia coli*. Thick suspensions of cells are exposed to carrier-free $[^35\text{S}]$ sulphate and samples removed at intervals into ethanol containing NEM. The supernatant is run on Whatman 3MM paper in two dimensions and radioautographs of the chromatograms are prepared. Radioactive areas corresponding to sulphide, sulphite, cysteine, glutathione and methionine can be identified. Control radioautographs prepared from extracts made in ethanol alone show only methionine. The time-course of sulphate reduction by intact cells has been studied by this technique (Ellis, 1966). The use of $[^{14}\text{C}]$ NEM has enabled measurements to be made of the internal pool size of cysteine in *E. coli* cells grown on various sulphur sources (Wheldrake, 1967).

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