The active components of the ribosomal fraction were further separated from extraneous material by sucrose-density-gradient centrifugation of the mitochondrial supernatant. By this procedure, a particulate enzyme preparation was obtained, partially separated from ribosomes, of sedimentation coefficient approx. 80s. This preparation was five to ten times as active as the ribosomal preparation (50 μ moles of UTP incorporated/mg. of protein/min.), and no polymerase activity could be detected in similar fractions from uninfected cells. Analysis of the RNA product of the particulate polymerase reactions revealed the presence of several high-molecular-weight RNA species of sedimentation coefficient ranging from 6s to 18s.

Analyses of the protein and nucleic acid components of the particulate enzyme preparation will be reported.

Stereospecificity of Chloramphenicol Inhibition of Chloroplast Ribosomes

By R. J. ELLIS. (*Department of Botany, University of Aberdeen*)

Chloroplasts contain ribosomes that resemble bacterial ribosomes in their sedimentation behaviour, the size of their RNA components, the ability to use *N*-formylmethionine as the initiating amino acid in protein synthesis, and in the inhibition of amino acid incorporation by chloramphenicol (Kirk & Tilney-Bassett, 1967). These similarities have led to a revival of interest in the hypothesis that chloroplasts have evolved from symbiotic prokaryotes (Sagan, 1967). By contrast, cytoplasmic ribosomes from plants are larger than chloroplast ribosomes, and are insensitive to chloramphenicol (e.g. Boardman, Francki & Wildman, 1966; Ellis & MacDonald, 1967).

The inhibition by chloramphenicol of amino acid incorporation by bacterial ribosomes is highly specific for the *D*-threo isomer (Rendi & Ochoa, 1962), but a similar stereospecificity has not been reported in chloroplast ribosomes. This point is important to establish because other processes in plants, such as ion uptake (Ellis, 1963) and oxidative phosphorylation (Hanson & Krueger, 1966), are inhibited by several isomers of chloramphenicol including the *D*-threo isomer.

Expanding leaves from young plants of *Nicotiana tabacum* L. were surface-sterilized and deribbed. The laminae were sliced in Honda medium by the hand-chopping procedure of Spencer & Wildman (1964) to minimize breakage of the chloroplasts. The chloroplasts were sedimented at 1500g for 10 min. and resuspended in Honda medium. The postmitochondrial supernatant was dialysed briefly

before use as a source of cytoplasmic ribosomes. All glassware and media were sterilized.

Intact chloroplasts incorporated 50–100 μ moles of [14 C]leucine/mg. of chlorophyll into protein when supplemented with ATP and GTP; the incorporation was inhibited 80% by 2 μ g. of ribonuclease/ml., and bacterial contamination was less than 100 colonies/assay tube. The inhibition of incorporation by chloramphenicol was found to be stereospecific: the *D*-threo isomer inhibited about 80% at 1 mM, but the *L*-threo and *D*-erythro isomers were inactive. Cycloheximide, which inhibits incorporation by animal and plant cytoplasmic ribosomes but not by bacterial ribosomes, did not affect leucine incorporation at 100 μ g./ml. Similar results were obtained with chloroplasts disrupted by Triton X-100, indicating that the negative effects were not due to a permeability barrier. Incorporation by cytoplasmic ribosomes was not affected by any of the chloramphenicol isomers. Thus chloroplast and bacterial ribosomes show the same stereospecificity of inhibition by chloramphenicol.

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Nuclear Binding of Aldosterone in Toad Bladder

By K. G. M. M. ALBERTI and G. W. G. SHARP. (*Departments of Medicine and Pharmacology, Harvard Medical School, and Massachusetts General Hospital, Boston, Mass., U.S.A.*)

Aldosterone stimulates sodium transport in the bladder of the toad, *Bufo marinus*. Previous studies on the binding of this hormone to the toad bladder have shown that at least two sets of binding sites exist in the tissue (Sharp, Komack & Leaf, 1964), and that aldosterone is bound to physiologically active sites in the nuclei of the mucosal epithelial cells (Ausiello & Sharp, 1968). The present study was undertaken to elucidate the nature and function of this binding.

Half-bladders were incubated in Ringer's solution containing 0.01–0.2 μ M-[3 H]-aldosterone for 1–2 hr. Mucosal cells, crude nuclei or purified nuclei, prepared by sucrose-gradient-centrifugation tech-

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

By R. J. ELLIS. (*Department of Botany, University of Aberdeen*)

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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