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.

Boardman, N. K., Francki, R. I. B. & Wildman, S. G. (1966). *J. molec. Biol.* **17**, 470.

Ellis, R. J. (1963). *Nature, Lond.*, **200**, 596.

Ellis, R. J. & MacDonald, I. R. (1967). *Plant Physiol.* **42**, 1297.

Hanson, J. B. & Krueger, W. A. (1966). *Nature, Lond.*, **211**, 1322.

Kirk, J. T. O. & Tilney-Bassett, R. A. (1967). *The Plastids*. London and San Francisco: W. H. Freeman and Co.

Rendi, R. & Ochoa, S. (1962). *J. biol. Chem.* **237**, 3711.

Sagan, L. (1967). *J. theoret. Biol.* **14**, 225.

Spencer, D. & Wildman, S. G. (1964). *Biochemistry*, **3**, 954.

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-