lower values of sedimentation. Yeast mitoribosomes were found to be more susceptible to dissociation into subunits than were cytoribosomes.

The RNA components of mitoribosomes were isolated and separated by centrifugation in the sucrose density gradient. The sedimentation diagram showed two major peaks at 21S and 16S and a minor one at 12.5S, in contrast with RNA from cytoribosome (24S and 16S). Whereas RNA from cytoribosomes is totally stable when kept at or under 0°C, RNA from mitoribosomes was found to be unstable even at -20°C.

Biogenesis of Phospholipids in Liver Mitochondria

By F. B. JUNGALWALA and R. M. C. DAWSON.
(*Department of Biochemistry, Agricultural Research Council Institute of Animal Physiology, Babraham, Cambridge, U.K.*)

Recent work with [³²P]phosphate and CDP-[Me-¹⁴C]choline as precursors has indicated that the limited incorporation of radioactivity into the nitrogen-containing phospholipids and phosphatidyl-inositol of rat liver mitochondria can be largely accounted for by the microsomal contamination present (McMurray & Dawson, 1969). On the other hand, phosphatidic acid can be synthesized intramitochondrially. Although recent reports have indicated that mitochondria have an energy-dependent system for incorporating [Me-¹⁴C]choline into their phospholipids (Kaiser & Bygrave, 1968; Bygrave & Kaiser, 1968; Kaiser, 1969), we have now found that the labelling of mitochondrial phospholipids from this precursor was minimal compared with that in microsomes provided that an incubation medium was used that adequately supported phosphatidylcholine synthesis. In the presence of a suitable medium and a cell supernatant fraction, a plot of choline incorporation into the lipids against the concentration of microsomes added to a constant amount of mitochondria extrapolated to the origin if allowance was made for microsomal contamination of the mitochondria by NADPH-cytochrome c reductase assays. Although recent evidence has suggested that some of this marker enzyme may be associated with the outer membranes of mitochondria (Brunner & Bygrave, 1969), the present results suggest that mitochondria possess a very low if not negligible capacity for forming phosphatidylcholine.

A rapid exchange of phospholipids when isolated microsomes and mitochondria are incubated together has been recently demonstrated independently in a number of laboratories (Wirtz & Zilversmit, 1968; McMurray & Dawson, 1969; Akiyama & Sakagami, 1969). It seems likely therefore that the appreciable

turnover of mitochondrial phospholipids observed in the liver *in vivo* can be explained most satisfactorily by assuming a transfer of newly synthesized phospholipid molecules from the endoplasmic reticulum. In an attempt to obtain direct evidence for such a transfer in intact cells we have performed experiments with isolated liver cells that were pulse-labelled with various phospholipid precursors. To obtain satisfactory phospholipid synthesis in such cells it was found necessary to add a certain proportion of a supernatant fraction obtained by homogenizing liver in sucrose. After pulse labelling with [³²P]phosphate, [Me-¹⁴C]choline, [2-¹⁴C]ethanolamine or [U-¹⁴C]-inositol, incubation of the cells in the absence of precursor caused the specific radioactivity of the individual labelled phospholipids to fall in the microsomes and rise or remain static in the mitochondria. These observations are therefore consistent with the postulate that mitochondrial phospholipids are largely formed in the endoplasmic reticulum.

Akiyama, M. & Sakagami, T. (1969). *Biochim. biophys. Acta*, **187**, 105.

Brunner, G. & Bygrave, F. L. (1969). *Eur. J. Biochem.* **8**, 530.

Bygrave, F. L. & Kaiser, W. (1968). *Eur. J. Biochem.* **4**, 578.

Kaiser, W. (1969). *Eur. J. Biochem.* **8**, 120.

Kaiser, W. & Bygrave, F. L. (1968). *Eur. J. Biochem.* **4**, 582.

McMurray, W. C. & Dawson, R. M. C. (1969). *Biochem. J.* **112**, 91.

Wirtz, K. W. A. & Zilversmit, D. B. (1968). *J. biol. Chem.* **243**, 3596.

Lipid-Protein Interactions in the Structure of Mitochondrial Membranes

By G. LENAZ, A. M. SECHI, G. PARENTI-CASTELLI and L. MASOTTI (introduced by D. E. GRIFFITHS). (*Istituto di Chimica Biologica, University of Bologna, Bologna, Italy*)

There are new lines of thought suggesting a complex interdigitation of proteins and phospholipids in the structure of the mitochondrial (and other) membranes (Green & Tzagoloff, 1966; Benson, 1966), in contrast with the unit membrane model (Robertson, 1959). Preliminary work in our laboratory has indicated the existence of hydrophobic interactions in the binding of phospholipid micelles to lipid-depleted mitochondria (Lenaz, Sechi, Masotti & Parenti-Castelli, 1969). Experiments reported here clearly demonstrate the hydrophobic character of the interaction.

The binding of phospholipids to lipid-depleted mitochondria is inhibited by increasing concentrations of lyotropic agents (Von Hippel & Schleich,

1969), which induce disorder in the structure of water. The curve of phospholipid binding at increasing ionic strengths has a diphasic character with an initial increase that is independent of the nature of the salt employed, and a subsequent decrease that is higher with the more strongly lyotropic salts ($\text{NaSCN} > \text{NaCl}$).

The restoration of succinoxidase activity by phospholipids has a behaviour that is in line with the above effect. We conclude that there is an initial increase of binding sites for phospholipids at low salt concentrations, and an inhibition of binding randomly on all sites available at higher salt concentrations.

Further, the binding is inhibited by aliphatic alcohols, and the inhibition depends on the chain length of the alcohol.

All of these effects are in line with the hydrophobic character of this binding *in vitro*. Additional evidence demonstrates that the lipid-protein interactions in the mitochondrial membranes are largely hydrophobic *in vivo*. There is, in fact, a precise correlation between the concentration of alcohol necessary to extract phospholipids from mitochondrial membranes and the chain length of the alcohol. Some evidence also suggests the participation of polar interactions in the structure of mitochondrial membranes, and more precisely in the binding of the 'headpieces' to the membrane. Ether extracts a large proportion of phospholipids from sonic particles freed of headpieces, but removes no phospholipid from intact membranes. These experiments, in addition to previous work (Lenaz, Sechi, Parenti-Castelli & Masotti, 1969) showing the availability of phospholipids to lipid reagents at the surface of the mitochondrial membranes, prove the complexity of these membranes beyond the simpler formulations of the unit membrane hypothesis.

- Benson, A. A. (1966). *J. Am. Oil Chem. Soc.* **43**, 265.
 Green, D. E. & Tzagoloff, A. (1966). *J. Lipid Res.* **7**, 587.
 Lenaz, G., Sechi, A. M., Masotti, L. & Parenti-Castelli, G. (1969). *Biochem. biophys. Res. Commun.* **34**, 392.
 Lenaz, G., Sechi, A. M., Parenti-Castelli, G. & Masotti, L. (1969). *Abstr. FEBS 6th Meet., Madrid*, no. 201.
 Robertson, J. D. (1959). *Biochem. Soc. Symp.* **16**, 3.
 Von Hippel, P. H. & Schleich, T. (1969). In *Structure and Stability of Biological Macromolecules*, p. 417. Ed. by Timasheff, S. N. & Fasman, G. D. New York: Marcel Dekker Inc.

Further Similarities between Chloroplast and Bacterial Ribosomes

By R. J. ELLIS. (*Department of Biochemistry, University of Aberdeen, Aberdeen AB9 1AS, U.K.*)

Chloroplasts contain ribosomes that resemble bacterial ribosomes in their sedimentation coefficient (Lyttleton, 1962), the sizes of their RNA components

(Loening & Ingle, 1967), their ability to use *N*-formyl-methionine as the initiating amino acid in protein synthesis (Schwartz, Meyer, Eisenstadt & Brawerman, 1967) and in the inhibition of their amino acid incorporation by chloramphenicol in a stereospecific manner (Ellis, 1969). These similarities have led to a revival of interest in the hypothesis that chloroplasts have evolved from symbiotic prokaryotes (e.g. Sagan, 1967).

Besides chloramphenicol, several other antibiotics inhibit bacterial protein synthesis by binding to various sites on the ribosome (Davies, Anderson & Davis, 1965; Vazquez & Monro, 1967). It follows that the larger the number of such structurally unrelated antibiotics that can be shown to inhibit amino acid incorporation by chloroplast ribosomes but not by cytoplasmic ribosomes, the stronger becomes the argument for the endosymbiont hypothesis, and the harder it becomes to regard the similarities between chloroplast and bacterial ribosomes as fortuitous. This communication reports that the antibiotics spectinomycin, lincomycin and erythromycin inhibit amino acid incorporation by bean and tobacco chloroplast ribosomes but not that by cytoplasmic ribosomes from either plants or animals.

Expanding leaves from young plants of *Nicotiana tabacum* and *Phaseolus vulgaris* were surface-sterilized with hypochlorite. 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 1000g for 10 min and resuspended in low-osmotic-strength buffer to rupture their outer membranes. All glassware and media were sterilized.

Bean chloroplasts incorporated 0.5–1.0 nmol of [^{14}C]leucine/mg of chlorophyll into protein when supplemented with ATP and GTP; tobacco chloroplasts gave about one-tenth of this incorporation. The incorporated radioactivity was almost completely solubilized by 0.2% Triton X-100, indicating that the incorporation was not due to bacteria or nuclei (Parenti & Margulies, 1967). Spectinomycin, lincomycin and erythromycin inhibited leucine incorporation by the chloroplasts, 50% inhibition being given by about 5, 30 and 100 $\mu\text{g/ml}$ respectively with bean. These antibiotics also inhibited the greening of etiolated plants, suggesting that their effects on chloroplast incorporation are not isolation artifacts. Leucine incorporation by cytoplasmic ribosomes from tobacco leaves (Ellis, 1969), red-beetroot discs (Ellis & MacDonald, 1967) and rat liver was not inhibited by any of these antibiotics at 200 $\mu\text{g/ml}$, nor was leucine activation by the 105 000g supernatant from bean chloroplasts.

- Davies, J., Anderson, P. & Davis, B. D. (1965). *Science, N.Y.*, **149**, 1096.
 Ellis, R. J. (1969). *Science, N.Y.*, **158**, 477.