radioactive peak was most readily obtained when Nonidet (NP 40) or BRIJ 58 was used to disrupt the mitochondria after pulse-labelling, but similar ribosome-like particles were also obtained by use of sodium deoxycholate or Triton X-100. Mitochondria disrupted in very low Mg^{2+} concentrations showed radioactivity to about 50S. The results are entirely consistent with the assumption that animal mitochondria contain mitoribosomes sedimenting at less than 80S. Experiments in which radioactive mitoribosomes were mixed with unlabelled *E. coli* ribosomes and sedimented in a sucrose density gradient containing 10mM- Mg^{2+} showed that the two peaks (radioactivity and extinction) were close together whereas *E. coli* ribosomes were readily separable under these conditions from rat liver cytoribosomes and Krebs-cell cytoribosomes. These results reinforce the suggestion that in protein synthesis mitochondria resemble bacteria much more closely than they do the eukaryotic cytoplasmic ribosomes.

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Factors Affecting Protein Synthesis in Yeast Mitochondria

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Mitochondria isolated essentially free of micro-organisms from protoplast lysates of *Saccharomyces carlsbergensis* have been found to incorporate [^{14}C]leucine into protein during incubation *in vitro* (Grivell, 1967). Incorporation takes place mainly into an RNA-rich fraction, possibly located within the inner mitochondrial membrane, and is sensitive to chloramphenicol and erythromycin, but resistant to cycloheximide and ribonuclease. Activity requires K^+ , Mg^{2+} and phosphate and has a broad optimum at pH 6.7; Li^+ and Na^+ are inhibitory. Response to Mg^{2+} is dependent on the phosphate concentrations and the adenine nucleotide present. The system requires ATP, which may be exogenous or generated by respiratory phosphorylation in the presence of oxidizable substrate and ADP; the phosphoenolpyruvate-pyruvate kinase ATP-regenerating system is fully effective only when succinate is present. Experiments with oligomycin suggest that there is not an obligatory participation of the hypothetical high-energy intermediates of oxidative phosphorylation.

Transport of [^{14}C]leucine has also been measured by a centrifugation-filtration technique; there is approximately twofold concentration of amino acid in a process that appears to be complete within 20s of addition of mitochondria to the incubation medium and that is not markedly affected by the addition of oxidizable substrate, ATP, oligomycin or antimycin. It is concluded that there is not a simple relationship between the ability of the mitochondrion to accumulate amino acids and to synthesize protein.

Direct measurement of the changes occurring in the concentrations of mitochondrial amino acids during incubation reveal discrepancies between radioactive and chemical methods of estimation of concentration ratios; equilibration between [^{14}C]leucine and the endogenous pool is not complete even after 30min. Although the size of the endogenous leucine pool does not increase during incubation, there is an approximately threefold increase in exogenous leucine. The difficulties of assessing true rates of protein synthesis in a system where the amino acid pool is not homogeneous and increases during incubation are to be stressed.

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Ribosome Particles in Yeast Mitochondria

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Evidence for the existence of ribosomes within mitochondria from yeast cells (*Candida utilis*, C.B.S. 1516) has been obtained from examination of electron micrographs of isolated mitochondria, and also from the characterization of the mitoribosomes after their isolation from mitochondria.

Electron micrographs of thin sections of yeast mitochondria stained with uranyl acetate and lead citrate showed rows of particles with diameters of 180 Å attached along the matrix side of the inner membrane and of the cristae. Free mitoribosomes were obtained by disruption of mitochondria with deoxycholate and purified by centrifugation on a linear 5–20% (w/v) sucrose density gradient in 5mM- $MgCl_2$ –50mM-KCl–10mM-tris–HCl buffer, pH 7.6. The sedimentation profile of mitoribosomes obtained under the above conditions is characterized by a major peak at 53–55S and a smaller one at 76–78S. Increasing the $MgCl_2$ concentration in the sucrose density gradient above 20mM results in an increase of the 76–78S peak with a slight shift to

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

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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.

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Lipid-Protein Interactions in the Structure of Mitochondrial Membranes

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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,