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.

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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.

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A New Procedure for the Separation of Mitochondrial Membrane Proteins

By P. J. CURTIS. (*Beatson Institute for Cancer Research, Royal Beatson Memorial Hospital, Glasgow C.3, U.K.*)

Studies on mitochondrial membrane proteins so far have been in aqueous solutions. The hydrophobic nature of the membrane protein suggests that a large part of the protein may exist in the non-polar section of the membrane. Therefore organic solvents might be more suitable and there have been reports of membrane protein dissolved in such solvents (Lowden, Moscarello & Morecki, 1966; Zahler & Wallach, 1967; Green, Haard, Lenaz & Silman, 1968). Preliminary work by Curtis (1969) showed that rat liver mitochondrial membrane protein dissolved completely in acidic chloroform-methanol (2:1, v/v) in the absence of phospholipids. Further use of chloroform-methanol as a solvent for membrane proteins has been investigated.

Exhaustive methylation of Sephadex G-75, G-100 and G-200 (Nyström & Sjövall, 1965) gave products that swelled in chloroform-methanol (2:1, v/v) approximately as much as in water. Chromatography of polystyrene fractions of narrow molecular-weight range in chloroform-methanol (3:1, v/v) showed that separation was according to size. Gel filtration of the mitochondrial membrane protein dissolved in chloroform-methanol (2:1, v/v) was achieved most successfully on methylated Sephadex G-100 equilibrated with chloroform-methanol (2:1, v/v) containing acetic acid (10mm). Polyacrylamide-gel electrophoresis (Neville, 1967) at pH 2.7 in 8M-urea showed that the major component of membrane protein was largely separated from the other components, and further purification was possible by rechromatography on methylated Sephadex G-100.

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Presence and Spatial Localization of Carbonic Anhydrase in Rat Liver Mitochondria

By F. A. HOLTON. (*Department of Physiology, Royal Veterinary College, London N.W.1, U.K.*)

About 2–10% of the carbonic anhydrase activity in homogenates of rat liver, kidney and cerebral cortex is recovered in the mitochondrial fraction (Datta & Shepard, 1959; Karler & Woodbury, 1960). Datta & Shepard (1959) believed these low recoveries indicated that the enzyme was confined to the cytoplasm, but Karler & Woodbury (1960) concluded that carbonic anhydrase was present in mitochondria and was tightly bound to membranes.

We recently reported that choline oxidation in liver mitochondria was accelerated under conditions enabling carbon dioxide to pass into the mitochondria and suggested that carbonic anhydrase in the matrix space accelerated the production of internal H^+ ions, which then facilitated choline entry by ionic exchange (Holton & Tyler, 1969). This hypothesis has now been tested by making electrometric measurements of carbonic anhydrase activity in mitochondria. Reaction mixtures containing carbon dioxide were rapidly stirred in a closed cell at 0.5–2°C. Changes of pH were measured by recording the e.m.f. of a glass electrode-calomel electrode system, with a strip-chart recorder running at 4 in/min. Rapid addition of acid or alkali yielded biphasic traces, which were analysed to yield a first-order velocity constant for the rate of approach to pH equilibrium. Between pH 6 and pH 7 mitochondria in distilled water increased the rate constant for the approach to equilibrium from the alkaline side (CO_2 hydration predominating). At pH 6.4 the rate constant was doubled by 1.2mg of mitochondrial protein/ml. In contrast, mitochondria had no effect on the rate constant for approach to equilibrium from the acid side (HCO_3^- and H_2CO_3 dehydration predominating).

These properties suggest the presence of a membrane-bound enzyme accessible to substrate by a diffusion path traversed more rapidly by CO_2 than by HCO_3^- ion. They are interpreted as showing that liver mitochondria contain carbonic anhydrase located on the matrix side of the inner membrane, which is known to be permeable to CO_2 but impermeable to