these compartments and, in the case of the peripheral and intracristal spaces, in the extent to which they are interconnected. The changes in the configuration of the inner membrane must be accompanied by alterations in the interaction of its subunits (whether it be cause or effect) and it is to be expected that this is accompanied by alterations in its properties. Thus Pollak & Munn (1969) ascribe the altered behaviour of rat liver mitochondria on sucrose density gradients associated with the induction of steady state (State 4) to alterations in the permeability of the inner membrane to sucrose.

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Calcium Ion Transport in Mitochondria

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Isolated mitochondria can bind Ca^{2+} according to three different processes. The first is the classical energy-linked uptake (Lehninger, Carafoli & Rossi, 1967), which requires coupled respiration or the hydrolysis of added ATP. When energy is provided by the respiratory chain, the process is inhibited by respiratory-chain inhibitors but not by oligomycin. When the process is energized by ATP, it is sensitive to oligomycin but not to respiratory-chain inhibitors. Both processes are sensitive to uncouplers. As for the mechanism, Ca^{2+} is supposed to discharge the 'energy pressure' maintained in mitochondria by respiration or ATP hydrolysis. According to the three most popular theories of energy coupling, the 'energy pressure' is thought to be a high-energy compound, or an electrochemical gradient, or an energy-rich conformational state of the mitochondrial membrane. In the absence of permanent anions, isolated mitochondria bind a maximum of about 100nmol of Ca^{2+} /mg of protein in the energy-linked process (Rossi & Lehninger, 1964). The affinity of the system for Ca^{2+} is rather high: recent measurements of the apparent K_m have yielded values of about 10^{-6}M (E. Carafoli & A. Azzi, unpublished work).

The second and the third process occur in the strict absence of respiration or ATP hydrolysis, and have been termed 'low-affinity' and 'high-affinity' binding (Reynafarje & Lehninger, 1969). The former is probably identical with the metabolism-independent binding described by Rossi, Azzi & Azzone (1967), which is thought to reflect the reaction of Ca^{2+} with mitochondrial phospholipids. Recent results of Scarpa & Azzone (1969) on phospholipid extraction and restoration indicate that phosphatidylethanolamine is responsible for this type of Ca^{2+} binding. As expected, the binding is inhibited by the local anaesthetic Butacaine, which displaces Ca^{2+} from phospholipids (Scarpa & Azzi, 1968). The maximum amount of Ca^{2+} bound in the process is about 50nmol/mg of mitochondrial protein. The affinity is rather low: the apparent K_m is about 10^{-4}M (Rossi *et al.* 1967; Reynafarje & Lehninger, 1969).

The high-affinity binding of Ca^{2+} (Reynafarje & Lehninger, 1969) also occurs in the absence of metabolism. It involves a small number of sites (0.5–5/mg of protein) having a very high affinity for Ca^{2+} (apparent K_m 10^{-6} – 10^{-7}M). Despite the absence of metabolism, it is strongly inhibited by uncouplers, a fact that suggests its implication in the energy-linked translocation of Ca^{2+} . Reynafarje & Lehninger (1969) and Lehninger & Carafoli (1969) have concluded that the high-affinity sites probably represent carrier molecules specific for Ca^{2+} in the mitochondrial membrane. The phenomenon of 'superstoichiometric

metry' between Ca^{2+} and oxygen (Carafoli, Rossi, Gamble & Lehninger, 1967) and the occurrence of Ca^{2+} -H⁺ rebounds non-stoichiometric with electron transport (Carafoli, Gamble & Lehninger, 1966) had indeed already indicated that under certain conditions Ca^{2+} could enter and leave mitochondria via a specific carrier independent of electron transport and oxidative phosphorylation. By analogy with other carriers, the Ca^{2+} carrier should have specificity for Ca^{2+} , high affinity for it and saturability; it should operate independently from the respiratory chain and move Ca^{2+} in either direction. No Ca^{2+} should be expected to be transported by the carrier against a gradient without expenditure of energy. The results of Reynafarje & Lehninger (1969) have indeed verified most of these requirements. On the basis of experiments on the effect of La^{3+} on the initial rate of energy-linked Ca^{2+} uptake, Mela (1968) and Mela & Chance (1969) have also postulated the existence of a Ca^{2+} carrier in the mitochondrial membrane and have concluded that La^{3+} is a specific inhibitor of the carrier. Since a carrier functions in either direction, a specific inhibitor would be expected to block both the influx and the efflux of Ca^{2+} . Very recent experiments (E. Carafoli, unpublished work) have, however, shown that La^{3+} does not prevent the discharge of Ca^{2+} from mitochondria; rather it promotes it. Thus the mechanism of the effect of La^{3+} is still unclear.

Since it has been shown that the carriers for succinate and isocitrate are present only in some mitochondrial types (Chappell, 1968), a survey has been carried out of the high-affinity binding capacity in different mitochondria (Lehninger & Carafoli, 1969; E. Carafoli & A. L. Lehninger, unpublished work). Energy-linked and metabolism-independent Ca^{2+} transport have also been compared to clarify the relationships among the three types of binding and to check whether energy-linked Ca^{2+} transport is a universal attribute of mitochondria. It has been found that energy-linked transport occurs in all mitochondria studied except yeast: however, in those from blowfly muscle it is mandatorily linked to the transport of phosphate. Thus in blowfly mitochondria the uptake of Ca^{2+} probably follows the primary uptake of phosphate anion. Low-affinity binding is absent from blowfly muscle mitochondria; phospholipid analyses have revealed that they are very rich in phosphatidylethanolamine, a finding that is hard to reconcile with the conclusion of Scarpa & Azzone (1969) on the role of phosphatidylethanolamine in the metabolism-independent binding. High-affinity binding is present only in liver, kidney, brain and adrenal-cortex mitochondria, and normally absent from all the other tested. However, under special conditions it can be demonstrated also in rabbit heart mitochondria.

The comparative observations on high-affinity binding, as reflecting a hypothetical Ca^{2+} carrier, are

consistent with the results on other permeases, which are present only in certain mitochondrial types: when present, the Ca^{2+} carrier is thus presumably genetically determined. It is also clear that energy-linked Ca^{2+} uptake is not a universal property of mitochondria, but possibly only of those from mammalian tissues. Indeed, Ca^{2+} does not stimulate respiration in plant mitochondria (Bonner & Pressman, 1965). The observations on blowfly muscle mitochondria recall those by Hanson & Miller (1967) on the primary transport of inorganic phosphate in maize mitochondria. The apparently settled question of what is transported first, Ca^{2+} or inorganic phosphate, is thus still open, at least for some mitochondrial types.

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Mitochondrial and Microbial Anion-Transporting Systems

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The existence of seven anion-transporting systems located in the inner membrane of liver mitochondria has been postulated (see Chappell, 1968; Greville, 1969). These carriers deal with the following compounds or classes of compounds: phosphate (or arsenate), dicarboxylate anions (e.g. malate, succinate), 2-oxoglutarate, tricarboxylate anions (isocitrate, citrate, *cis*-aconitate), glutamate, aspartate and adenine nucleotides. Not all mitochondria possess these transporting systems. Thus of the carriers mentioned blowfly mitochondria appear to have only