PROCEEDINGS OF THE BIOCHEMICAL SOCIETY

The 499th Meeting of the Society was held at the University of Warwick, Coventry, on Thursday and Friday, 13 and 14 November 1969, when the following papers were presented:

COLLOQUIUM ON 'BIOCHEMISTRY OF MITOCHONDRIA'

Topography of Mitochondrial Membranes in Relation to Metabolism

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Structural changes in mitochondria in relation to metabolism have been studied indirectly by means of light-scattering and apparent extinction changes and directly at various levels of resolution by light and electron microscopy. The techniques most widely applied in the study of mitochondrial structure by electron microscopy are thin sectioning (for review see, e.g., Parsons, 1965) and negative staining (Horne & Whittaker, 1962; Whittaker, 1963; Munn, 1968). The former technique can be applied equally to mitochondria fixed *in situ* or isolated, the latter technique is limited almost entirely to the examination of isolated mitochondria.

The shape and size of mitochondria and the numbers and organization of their cristae are related to the tissues in which they occur (Palade, 1952); mammalian liver, kidney and heart, blowfly flight-muscle and *Tetrahymena* mitochondria are representative of the main types encountered. The mitochondria in any one tissue may show changes in their structure (and enzymic properties) in response to alterations in physiological conditions, and their appearance also depends on the method of preparation of the tissue for electron microscopy.

The structure of isolated mitochondria depends on (a) the tissue from which they are isolated, (b) the nature of the isolation and/or suspension media, (c) their metabolic state and (d) their treatment in preparation for examination in the electron microscope.

Rodent liver mitochondria in 0.25M-sucrose fixed with glutaraldehyde and/or osmium tetroxide appear substantially altered from those fixed *in situ*; the peripheral and intracristal spaces are enlarged and the matrix space is decreased. An appearance more closely resembling that seen in mitochondria fixed *in situ* can be induced by alterations in the metabolic steady states of the mitochondria or by treatment with uncoupling agents (Hackenbrock, 1966, 1968; Weinbach, Garbus & Sheffield, 1967; Deamer, Utsumi

& Packer, 1967). D. E. Green and his colleagues have described similar phenomena in ox heart mitochondria and they have postulated an interrelated series of configurational states determined by conformational changes in the subunits of the inner membranes to account for their various appearances in the electron microscope (Penniston, Harris, Asai & Green, 1968; Harris, Penniston, Asai & Green, 1968; Green, Asai, Harris & Penniston, 1968).

The subunit theory of the composition of membranes as developed by Green & Perdue (1966) arose from the observation that the inner membranes are studded with particulate components (Fernández-Morán, 1962, 1963) attached by a stalk (Fernández-Morán, 1963; Parsons, 1963; Stoeckenius, 1963; Smith, 1963) to a base-piece (Smith, 1963), the latter forming part of the membrane continuum. The reported dimensions of the particles range from 40 to 150 Å (Fernández-Morán, 1962, 1963; Chance & Parsons, 1963; Parsons, 1963, 1965; Smith, 1963) and they occur in all mitochondria except those from neonatal rabbit brown adipose tissue (Lindberg, DePierre, Rylander & Afzelius, 1967), and even in this case Guillory & Racker (1968) have seen them on sonicated fragments. The particles (headpieces) have been identified with an adenosine triphosphatase [coupling factor 1 (Racker & Horstmann, 1967)], the stalks with an oligomycin-sensitivity-conferring protein (MacLennan & Asai, 1968) and at least some of the base-pieces with one or other of the complexes of the electron-transfer chain (Green & Tzagoloff, 1966). Certain other enzymes are located exclusively on the inner membrane [e.g. succinate dehydrogenase and ferrochelatase (Jones & Jones, 1968)], others [e.g. monoamine oxidase (Sottocasa, Kuylenstierna, Ernster & Bergstrand, 1967) and palmitate-CoA ligase (Norum, Farstad & Bremer, 1966)] are restricted to the outer membrane. A number of enzymes, notably many of the citric acid-cycle enzymes (for review see Greville, 1969), are in solution but held in compartments within the inner membrane or between the inner and outer membranes. The large changes in the arrangement of the inner membrane associated with changes in metabolic steady states described for rodent liver and ox heart mitochondria result in large changes in the sizes of

these compartments and, in the case of the peripheral and intracrystal 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

By E. CARAFOLI. (*Institute of General Pathology, University of Modena, Modena, Italy*)

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 & Azzzone (1967), which is thought to reflect the reaction of Ca^{2+} with mitochondrial phospholipids. Recent results of Scarpa & Azzzone (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