sodium chloride. High concentrations (20 μ M) of tripropyltin chloride produced swelling of mitochondria suspended in iso-osmotic sodium succinate, in the absence of added inorganic orthophosphate, even in the presence of inhibitors of phosphate transport. Trimethyltin produces qualitatively similar effects.

Titration of mitochondria with HCl showed that tripropyltin chloride allowed rapid pH equilibration across the mitochondrial membrane. Thus trialkyltins must act either as chloride-proton symporters or chloride-hydroxide antiporters; their chemical properties suggest that the latter is the more likely mechanism.

In a potassium chloride medium trialkyltin compounds will collapse or even reverse the pH gradient across the mitochondrial membrane, but will have no direct effect on the electrical potential difference across the membrane. Under these conditions inhibition of oxidative phosphorylation would be expected on the basis of the chemiosmotic hypothesis (Mitchell, 1968).

However, low concentrations of trialkyltin compounds produce inhibition of oxidative phosphorylation in a medium (0.25M-sucrose-2mM-N-2-hydroxyethylpiperazine-N'-2-ethanesulphonic acid-NaOH, pH 7.4) in which the only anions are those of the substrate. This suggests that trialkyltin compounds may have some other, more direct, inhibitory action.

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One-Electron Reactions of some Mitochondrial Components Studied by Pulse Radiolysis

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Oxidation and reduction of some components of mitochondria have been studied by means of pulse radiolysis. The technique employs very short (1 μ s or less) pulses of high-energy radiation to prepare high concentrations of short-lived intermediates whose u.v.-visible-absorption spectra can be measured and whose reactions can be followed by fast spectrophotometry.

By suitable choice of conditions it is possible to effect exclusively either the one-electron reduction of the oxidized form of a couple or the one-electron

oxidation of the corresponding fully reduced form. Thus irradiation of aqueous NAD⁺ (approx. 0.1mM) in the presence of formate (0.1M) results in the quantitative conversion of the primary products of water radiolysis, H, OH and e⁻_{aq}, into the semi-reduced radical; NAD[•] (Land & Swallow, 1968). Conversely, irradiation of NADH in the presence of 0.1M-Br⁻ and N₂O results in the oxidation of NADH into the same NAD[•] radical. Semi-reduced riboflavin (Land & Swallow, 1969) and semi-reduced ubiquinone may be prepared similarly. Alteration of the pH of the solvent can lead to the identification of the various states of protonation of these radicals. Preliminary pulse-radiolysis experiments have also been carried out on solutions of cytochrome c.

By use of suitable mixtures it is possible to study the movement of single electrons between the different redox components. Thus in a mixture of NAD⁺ (0.2mM) and riboflavin (0.01mM) in aqueous formate (0.1M) all the primary products of water radiolysis are converted initially into NAD[•], but the final radical observed is characteristic of riboflavin. This species is the result of electron transfer from the NAD semiquinone to riboflavin, accompanied by protonation. From the rate of build-up of this riboflavin semiquinone in the presence of buffer (pH 5.7), electron transfer from NAD[•] to riboflavin has been shown to proceed at the approximately diffusion-controlled rate of $4 \times 10^9 - 5 \times 10^9$ l mol⁻¹ s⁻¹.

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Intramitochondrial Distribution of Cytochrome P-450 in Ox Adrenal Cortex

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It has been reported that adrenal-cortex mitochondria contain a considerable amount of cytochrome P-450 (Harding, Wong & Nelson, 1964; Omura, Sato, Cooper, Rosenthal & Estabrook, 1965). Since this pigment is localized in other tissues only in the endoplasmic reticulum, and since the outer mitochondrial membrane has some properties in common with the endoplasmic reticulum membrane (Sottocasa, Kuylenskierna, Ernster & Bergstrand, 1967), it was decided to study whether the outer membrane of adrenal-cortex mitochondria contained this pigment in addition to NADH-cytochrome b₅ reductase and cytochrome b₅. Mitochondria as well as the microsomal fraction were isolated from ox adrenal homogenates. It has been found that in this tissue also microsomes contain glucose 6-phosphatase whose

specific activity is eight- to ten-fold lower than that in liver microsomes. This enzyme can be utilized therefore as a marker for microsomal contamination in mitochondria. Rotenone-insensitive NADH-cytochrome *c* reductase has been found to be present in both mitochondrial and microsomal fractions at approximately the same specific activity, under conditions where glucose 6-phosphatase specific activity in mitochondria was at least 20-fold lower than in microsomes. Mitochondria could oxidize α -oxoglutarate, succinate, pyruvate, malate, citrate and isocitrate and could not oxidize β -hydroxybutyrate. Glutamate was oxidized only very slowly. P/O ratios obtained with these substrates were very close to those reported in the literature for this tissue (Cammer & Estabrook, 1967). Respiratory control ratios were as high as 5.

By swelling in hypo-osmotic medium, osmotically shrinking and sonication of mitochondria followed by continuous density-gradient centrifugation it was possible to separate the outer from the inner mitochondrial membranes. As enzyme markers of the two membranes, rotenone-insensitive NADH-cytochrome *c* reductase and succinate-cytochrome *c* reductase were followed respectively. The distribution pattern of cytochrome *P*-450 in the gradient follows essentially that of succinate-cytochrome *c* reductase, indicating that the pigment is localized in the inner rather than in the outer mitochondrial membrane. Similar results have been obtained by subfractionating mitochondria by means of differential rather than isopycnic centrifugation.

These findings open the possibility that hydroxylations taking place inside the mitochondrion make use of intramitochondrial NADPH, under the control of energy-conservation mechanisms of the respiratory chain, via the energy-linked transhydrogenase. In the endoplasmic reticulum, on the other hand, hydroxylation of steroids makes use of NADPH produced in the cytoplasm by the pentose phosphate pathway under the control of adrenocorticotrophin. The inner mitochondrial membrane would provide a barrier between the two control mechanisms.

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The Long-Wavelength Absorption Band of Cytochrome *c* Oxidase

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The broad absorption band of oxidized cytochrome *c* oxidase centred at 830nm, first noticed by Griffiths & Wharton (1961) and since identified with cupric copper in the enzyme by Wharton & Tzagoloff (1964), has been used by a number of workers (Gibson & Greenwood, 1965; Van Gelder & Slater, 1966) to investigate the role of copper. In all the investigations to date little attention has been paid to the state of the protein, and we have therefore been concerned to look at conditions that affect the 830nm band and to try to relate these to protein structure through the use of circular dichroism and ultracentrifugal analysis.

Pig heart cytochrome *c* oxidase as prepared by Yonetani's (1960) method yields a material of *s* value 10.5S at 20°C. It possesses a broad absorption band at 830nm that is optically active. The circular dichroism has been measured throughout the visible region and found to be in agreement with the measurements made by Urry & Van Gelder (1968) for the Soret band. When treated with cyanide at alkaline pH (Wharton & Tzagoloff, 1964) to remove copper, it was found that the structural integrity of the oxidase was completely abolished. Not only was the 830nm band bleached, but also the dichroism throughout the range 500–830nm.

Further investigation showed that alkaline pH alone caused a diminution of the *s* value from 10.5S to 5.8S, indicating that the prepared material had split into two. For complete transition from 10.5S to 5.8S material a pH of at least 10 is required.

Dialysis of the 5.8S material back to neutrality leads to partial non-specific reassociation to form aggregates of various *s* values. In particular a 16.2S unit, presumably a trimeric form, may be discerned. The 5.8S material may be separated from the aggregated protein by centrifugation at 27000g for 30min. It retains the 830nm band and dichroism in this region both at pH7.4 and pH10.4. There are however, significant changes in the electron-spin-resonance spectrum of the copper and in the dichroic spectrum of the haem as compared with the native material. Treatment of the 10.5S or 5.8S preparation at neutral pH with a high concentration of cyanide (approx. 0.5M) leaves the 830nm band and the ultracentrifuge behaviour unmodified.

This information suggests: (i) that as the subunits are dispersed by treatment with alkali and reassociate non-specifically, it is possible that electrostatic forces are the major factors contributing to the aggregated subunits' stability; (ii) that internal changes occur