effects of propranolol are completely sensitive to uncouplers, and are not produced in the absence of an energy source.

Propranolol may exert its effect by binding fixed negatively charged phospholipids of the membrane, as suggested by Seppälä, Wikström & Saris (1969). The increase of the phosphorylation potential with a concomitant extra uptake of salts focuses the interest on the role of fixed charges of the mitochondrial inner membrane in the mechanism of oxidative phosphorylation and ion transport.

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Differences between the Phosphorylation Potentials of Adenosine Triphosphate Inside and Outside the Mitochondria

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Heldt & Pfaff (1969) have shown that the ATP/ADP ratio found in the mitochondria reflects a steady-state equilibrium between these compounds.

In the present investigations the relationship between the ATP/ADP ratios in the mitochondria and in the medium was studied by silicon-layer filtering centrifugation (Pfaff & Klingenberg, 1968). In a typical experiment carried out at 12°C, the ATP/ADP ratios measured during the state of respiratory control in the mitochondria and in the medium were 3.2 and 20 respectively.

By taking into account the inorganic phosphate assayed in both fractions, the phosphorylation potential of the ATP in the medium was calculated to be 2.8 kcal higher than in the mitochondria.

In another experiment oxidative phosphorylation, adenosine triphosphatase and substrate-level phosphorylation were inhibited by the addition of oligomycin and rotenone. When the mitochondria were kept aerobic in the presence of succinate, there was a linear relationship observed between the ATP/ADP ratios fixed in the medium by addition of various concentrations of ADP and ATP, and the resultant ATP/ADP ratios found in the mitochondria. Again, the ATP/ADP ratios assayed in the mitochondria were five- to ten-fold smaller than those in the medium. When uncoupler was also added no such

difference was observed. In this case the ATP/ADP ratio in the mitochondria was found to be equal to the ATP/ADP ratio fixed in the medium.

The differences of ATP/ADP ratios between the mitochondrial and extramitochondrial space observed with aerobic coupled mitochondria are explained by the ADP-specificity of the adenine nucleotide transport into the mitochondria (Pfaff & Klingenberg, 1968). ADP is transported against a potential from the extramitochondrial space into the mitochondria, the energy for this process being provided from the respiratory chain (Klingenberg, Heldt & Pfaff, 1969). Thus a small increase in the ADP concentration outside the mitochondria leads to a large increase of the ADP concentration within the mitochondria. In this way a stimulation of oxidative phosphorylation is obtained by a small rise in the ADP concentration in the cytosol. This mechanism may have an important function in maintaining the ATP concentration in the cytosol constant.

This work was supported by the Deutsche Forschungsgemeinschaft.

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Multiple Effects of Trialkyltin Compounds on Mitochondria

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Trialkyltin compounds have been reported to affect mitochondria in a manner similar to that of oligomycin but accompanied by a weak uncoupling effect (Aldridge, 1958; Sone & Hagihara, 1964). Aldridge & Rose (1969) have used these observations and the finding that trialkyltin compounds are bound to a pair of histidine residues in haemoglobin to elaborate a hypothesis for the mechanism of oxidative phosphorylation.

We have confirmed that at low concentrations (1 μ M) tripropyltin chloride produces an immediate inhibition of ADP-stimulated respiration of rat liver mitochondria, which in a potassium chloride medium is associated with swelling of the mitochondria. In a potassium chloride medium with succinate or β -hydroxybutyrate as substrate Ca^{2+} induced an extremely rapid swelling of the mitochondria when tripropyltin chloride was added. Further investigation showed that low concentrations of tripropyltin chloride (2 μ M) allowed rapid swelling of mitochondria suspended in 0.1 M ammonium chloride or 0.1 M-

sodium chloride. High concentrations (20 μM) 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.

M.S. is grateful to the Medical Research Council for a Research Studentship.

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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 μs 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; $\text{NAD}^\bullet$ (Land & Swallow, 1968). Conversely, irradiation of NADH in the presence of 0.1M- Br^- and N_2O results in the oxidation of NADH into the same $\text{NAD}^\bullet$ 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 $\text{NAD}^\bullet$, 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 $\text{NAD}^\bullet$ to riboflavin has been shown to proceed at the approximately diffusion-controlled rate of 4×10^9 – $5 \times 10^9 \text{ l mol}^{-1} \text{ s}^{-1}$.

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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, Kuylenstierna, Ernster & Bergstrand, 1967), it was decided to study whether the outer membrane of adrenal-cortex mitochondria contained this pigment in addition to NADH-cytochrome b_5 reductase and cytochrome b_5 . 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