INT, but this is overcome by extrapolation of the experimental results to infinite dye concentration. However, on extrapolation of the above results no effect of Ca^{2+} was observed. At $10\mu\text{M}\text{-Ca}^{2+}$ the slopes of reciprocal plots of activity against concentrations of both INT and glycerol 1-phosphate were independent of the invariant substrate. At $1\text{mM}\text{-Ca}^{2+}$, however, slopes of activity against INT varied with concentrations of glycerol 1-phosphate, and plots of activity against glycerol 1-phosphate concentration were not linear.

These results, together with the effect of Ca^{2+} , could be explained if the catalytic reaction was of the single-displacement random-order type (Dalziel, 1957). Thus one pathway would predominate in the presence of excess of INT, and its rate would not be affected by Ca^{2+} ; at lower concentrations of INT two pathways would be available, and Ca^{2+} would favour the alternative pathway by increasing the affinity of the enzyme for glycerol 1-phosphate.

If the Ca^{2+} activation of glycerol 1-phosphate dehydrogenase is of physiological importance, then it must be reconciled with the Ca^{2+} inhibition of mitochondrial NAD-linked isocitrate dehydrogenase. The latter is located in the mitochondrial matrix and should respond to Ca^{2+} changes in this compartment whereas glycerol 1-phosphate dehydrogenase is bound to the inner membrane. It is suggested that the latter enzyme may be positioned on the outer face of the inner membrane so that it can respond to Ca^{2+} concentration changes in the outer mitochondrial compartment, which is probably in equilibrium with the sarcoplasm. Thus the increased sarcoplasmic Ca^{2+} concentration caused by nervous stimulation of muscle would lead to stimulation of muscular contraction and the glycerol 1-phosphate cycle, whereas the isocitrate dehydrogenase would respond to changes of Ca^{2+} concentration in the mitochondrial matrix that will be dependent on the transport mechanism of the inner mitochondrial membrane.

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Two Types of Mitochondria in Heart Muscle from Euthyroid and Hyperthyroid Rats

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Previous reports (Hülsmann, De Jong & Van Tol, 1968; Hülsmann, Meijer, Bethlem & Van Wijngaarden, 1969) indicated that skeletal muscle of rat and man contains two types of mitochondria: one with loosely coupled oxidative phosphorylation

(M_1) and one with tightly coupled oxidative phosphorylation (M_2).

The present paper extends these findings to rat heart. M_1 mitochondria are released with a Potter-Elvehjem homogenizer (clearance 0.003in) in the following isolation medium: KCl (0.1M), tris-HCl buffer, pH 7.4 (0.05M), EDTA (1mM), MgCl_2 (5mM), ATP (1mM) and bovine serum albumin (0.8mg/ml). The M_1 mitochondria, isolated by differential centrifugation between 600 and 5000g, have a relatively high rate of respiration in the presence of added ATP and Mg^{2+} , due to spontaneous adenosine triphosphatase activity, which is largely oligomycin-resistant. This indicates that M_1 mitochondria are not uncoupled. Indeed, in nine manometric experiments with glutamate as hydrogen donor, the average P/O ratio was 2.7, although the average stimulation of respiration (respiratory control index) by the addition of hexokinase and glucose was only 1.9-fold. M_2 mitochondria, released by Nagarse (EC 3.4.4.16) treatment (Chance & Hagihara, 1960) of the 600g sediments, from which the M_1 mitochondria had been extracted, were also isolated by differential centrifugation. They had typical properties of tightly coupled mitochondria: no spontaneous Mg^{2+} -stimulated adenosine triphosphatase, high respiratory control indices and normal P/O ratios when oxidizing glutamate (in nine experiments the average values were 5.1 and 2.8 respectively).

We concluded that M_1 mitochondria had the properties of moderately aged M_2 mitochondria. Phospholipase action has been proposed to contribute to the aging of liver mitochondria, resulting in the unmasking of latent adenosine triphosphatase and loss of respiratory control (Hülsmann, 1958; Hülsmann, Elliott & Slater, 1960; Rossi, Sartorelli, Tato, Baretta & Siliprandi, 1967). Indeed, phospholipase A (EC 3.1.1.4) treatment of M_2 heart mitochondria results in the formation of lipophilic substances, which when added to M_2 mitochondria cause lowering of the respiratory control index.

In thyrotoxicosis the yields of M_1 mitochondria from the hypertrophic hearts are increased. The average yield of M_1 mitochondrial protein from the ventricles of an euthyroid rat heart is 5.6mg (ten animals) and that from a hyperthyroid rat heart 12.0mg (eight animals, with the same average body weight as the controls, namely 240g). If in thyrotoxicosis the amount of M_1 mitochondria in other tissue also increases a simple explanation for the increased basal metabolic rate can be given.

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The Relationship between Mitochondrial Structural Changes and Respiration Rate in Intact Mucosal Cells

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We have shown in our earlier work that slices of rat jejunal mucosa will respire at a high rate when they are incubated with an amino acid mixture (Jasper & Bronk, 1968). Electron micrographs of mucosal tissue show that under these conditions a high proportion of the mitochondria become condensed. This change in structure involves a shift in volume from the inner compartment to the outer compartment and a close association of dense matrix material with the inner membrane. Although these mitochondrial structural changes are associated with high rates of mucosal respiration they do not occur in the presence of the uncoupler 2,4-dinitrophenol.

Recently we have been investigating in more detail the extent to which these mitochondrial structural changes are linked to a rapid rate of mucosal respiration. Several lines of evidence have made it questionable that mitochondrial condensation is obligatory for oxidative phosphorylation. Thus the presence of glucose in the incubation medium is more effective in preventing the changes in mitochondrial structure than in decreasing cell respiration. Also, when mucosal tissue was obtained from animals in different thyroid states, the same type of condensation was observed over a wide range of respiratory rates.

Even more important evidence was obtained by following the time-course of the development of mitochondrial condensation. Polarographic recordings showed that the maximum respiration rate was reached after 15–20 s of incubation. Also, electron micrographs revealed that the characteristic changes in mucosal cytoplasm that accompanied incubation in the amino acid mixture were well advanced after an incubation period of 30 s. By contrast the mitochondrial structure was not condensed after 30 s, and only after an incubation period of about 3 min could the fully condensed structure be observed. In our view these results indicate that the development of the condensed mitochondrial structure lags behind the

initiation of a rapid rate of electron transport. This contrasts with the view expressed by Harris, Penniston, Asai & Green (1968) and others that mitochondrial structural changes are required for the process of energy conservation. From our observations it would appear that, although these changes are a consequence of a high rate of coupled oxidative phosphorylation, they are not essential for the process.

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Factors Affecting the Production of Ketone Bodies during Fatty Acid Oxidation by Isolated Rat Liver Mitochondria

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Isolated mammalian liver mitochondria, unlike those of heart and muscle, can oxidize added fatty acids by two pathways. One pathway leads to the complete oxidation of the fatty acids to carbon dioxide and water. The end products of the other pathway are the ketone bodies. The initial formation of acetyl-CoA during β -oxidation is common to both pathways.

A study has been made of the factors that may determine the distribution of acetyl-CoA between the two pathways during fatty acid oxidation by isolated rat liver mitochondria. No intermediates of the tricarboxylic acid cycle were added to the reaction medium. Oxygen uptake was measured manometrically. The production of ketone bodies was measured by determining the amounts of acetoacetate and β -hydroxybutyrate in the deproteinized reaction medium by the enzymic method of Williamson, Mellanby & Krebs (1962). If it is assumed that no intermediates accumulate, the relative contribution of the two pathways can be calculated from these results.

It was found that two factors, which had hitherto not been recognized, may in certain conditions greatly influence the relative importance of the two pathways of fatty acid breakdown. These factors are the concentration of inorganic phosphate and the presence of bovine serum albumin.

When 1 mM-DL-palmitoylcarnitine was oxidized in the presence of 0.1 mM-2,4-dinitrophenol and in the absence of added inorganic phosphate, 24% of the

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