The Effect of Respiratory Inhibitors and Oligomycin on Sodium Ion Transport and Adenine Nucleotides in Rat Liver Slices

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The active transport of Na^+ and K^+ by liver slices from adult rats is almost completely inhibited by anoxia (Flink, Hastings & Lowry, 1950), cyanide and 2,4-dinitrophenol (Elshove & van Rossum, 1963), but only partially by oligomycin (van Rossum, 1964, 1966). The effects of various concentrations of cyanide, Amytal and oligomycin have now been studied (i) to determine the minimal ATP content and rate of respiration consistent with full transport activity and (ii) to re-examine the partial inhibition of transport by oligomycin.

Ion-transport activity was measured as the net extrusion of Na^+ by slices incubated at 38°C , after preincubation at 1°C . Respiration was measured manometrically. Slices were taken before and after incubation at 38°C and analysed for Na^+ and adenine nucleotides.

After 70 min at 38°C control slices contained 6.2 ± 0.4 mmol of ATP/kg of protein (mean $\pm$ S.E.M., 25 determinations); the ATP/ADP concentration ratio was 3.6. These quantities showed a linear relation to the respiration when slices were treated with cyanide or Amytal, falling to 0.75 ± 0.05 mmol of ATP/kg of protein (mean $\pm$ S.E.M., 25 determinations) and an ATP/ADP concentration ratio 0.35 when respiration was maximally inhibited. In contrast, the net extrusion of Na^+ during 70 min, and the maximal rate of transport, were not affected unless respiration was inhibited by more than 50%; the ATP content was then 2.0–2.5 mmol/kg of protein and the ATP/ADP concentration ratio was 0.5. Ion transport is thus quite resistant to inhibition of energy metabolism in adult liver.

The slopes of the lines relating ATP content to inhibition of respiration were similar for oligomycin and for cyanide, but the minimal ATP content remained higher in the presence of oligomycin [2.10 ± 0.30 mmol/kg of protein (mean $\pm$ S.E.M., 15 determinations)]. Na^+ extrusion was again inhibited only when the ATP concentration fell below 2.5 mmol/kg of protein; but the inhibition was not more than 30%, which was consistent with the relatively high concentration of ATP remaining. Ouabain (1 mM) did not affect the ATP content. These results suggest: (i) that oligomycin was able effectively to inhibit oxidative phosphorylation in the slices; (ii) that the persisting synthesis of ATP (presumably by substrate-level phosphorylations) was adequate to support the oligomycin-resistant transport of Na^+ ; (iii) that the partial inhibition of

transport by oligomycin can be adequately accounted for by the inhibition of oxidative phosphorylation.

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Metabolism of Respiratory Tissue: Secretion of Mucosubstances by Organ Cultures of Mammalian Trachea

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The involvement of respiratory mucosubstances in a number of pathological conditions, such as bronchitis and cystic fibrosis, prompted our present studies into the metabolic activity of tracheal tissue.

Earlier work on organ culture of calf trachea (Kent, Molineux, Haegle & Stevenson, 1969) indicated that the tissue secreted high-molecular-weight protein-polysaccharide complexes, the principal secretory product, complex A, having molecular weight 2.5×10^5 . Improved culture conditions (medium 199, CO_2 -bicarbonate buffer) have clearly established that this complex is the major biosynthetic component; [^{35}S]sulphate, D-[^{14}C]glucosamine and DL-[G- ^3H]valine serve as metabolic precursors of the complex, which is isolated in the void volume after fractionation of the spent culture medium on Sephadex G-200 or Sepharose 6B. Electrophoresis on cellulose acetate showed the presence of a single polyanionic component; analytic ultracentrifugation gave s^0 3.8S (0.2M-KCl-0.2M-phosphate buffer, pH 7.0). Analysis by g.l.c. of the monosaccharide residues indicated that N-acetylgalactosamine, N-acetylglucosamine, glucuronic acid, galactose and sialic acid were the main constituents, together with smaller amounts of fucose, mannose and xylose. In the light of current knowledge of mucosubstance structure (Spiro, 1970; Brimacombe & Webber, 1964), the presence of both sialic acid and uronic acid in the same macromolecule strongly suggests an aggregate of glycoprotein and mucopolysaccharide moieties. The i.r. spectrum of papain-degraded material was characteristic of that of chondroitin 4-sulphate (Lloyd, Dodgson, Price & Rose, 1961).

Studies on the secretory activity of rabbit trachea