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

By G. D. V. VAN ROSSUM. (*Department of Pharmacology, Temple University School of Medicine, Philadelphia, Pa. 19140, U.S.A.*)

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

By P. W. KENT, P. F. DANIEL and J. T. GALLAGHER. (*Department of Biochemistry, University of Oxford, Oxford OX1 3QU, U.K.*)

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

in organ culture indicate that a high-molecular-weight fraction isolated in the void volume from Sephadex G-200 could be resolved by electrophoresis and chromatography on DEAE-Sephadex into discrete glycoprotein and mucopolysaccharide components, suggesting that, unlike the calf material, the degree of association, if any, is relatively small.

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Effects of Lysolecithin (Lysophosphatidylcholine) and Fatty Acids on Rat Liver Microsomal Glucose 6-Phosphatase

By B. R. CATER and T. HALLINAN. (*Department of Biochemistry, Royal Free Hospital School of Medicine, London WC1N 1BP, U.K.*)

The phospholipase A group of enzymes form fatty acids and lysophosphatides as their products, and these have been frequently implicated as potent non-specific inhibitors of both membrane-bound and 'soluble' enzymes. For this reason many investigators have refrained from using phospholipase A to study biomembranes, preferring instead to use a phospholipase C whose products, phosphoryl-bases and diglycerides, have been shown not to affect several membrane-bound enzyme systems (Duttera, Byrne & Ganoza, 1968; Graham & Wood, 1969). Nevertheless, extensive use has been made of phospholipase A to study the phospholipid-dependence of glucose 6-phosphatase, so to aid interpretation of these studies the effects of its products, fatty acids and lysolecithin (lysophosphatidylcholine), on glucose 6-phosphatase were investigated.

It was found that lysolecithin could cause either activation or inactivation of glucose 6-phosphatase, depending on the temperature and the presence or absence of the substrate, glucose 6-phosphate. Accordingly, if the enzyme is assayed at 37°C in the presence of lysolecithin (0.2–0.6 $\mu\text{mol/mg}$ of protein) then activation is clearly apparent; however, if the enzyme is preincubated with the same concentration of lysolecithin at the same temperature, before assay, then inactivation occurs. However, identical pretreatment with lysolecithin at 5 or 20°C results in the activated state, so clearly a fairly high tem-

perature is required for inactivation. The extent of thermal inactivation also depends on the lysolecithin concentration and the duration of preincubation.

Similarly the fatty acids palmitate, oleate, linoleate and linolenate all inactivate. Inactivation is again heat-dependent and is not apparent in the presence of glucose 6-phosphate except at high fatty acid concentrations (above 0.4 $\mu\text{mol/mg}$ of protein). Unlike the case with lysolecithin, activation was not seen. Linoleate was the most potent inactivator when compared with the other fatty acids tested or with lysolecithin. A synergistic inactivation was evident when both linoleate and lysolecithin were present together. When added before preincubation bovine serum albumin wholly or partially protected the enzyme from both linoleate and lysolecithin inactivation when these compounds were present either individually or together. However, bovine serum albumin was not capable of restoring activity if added after thermal inactivation.

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Fatty Acids from Acylhydrolase Action on Diglycerides Inhibit Phospholipase C-Treated Microsomal Glucose 6-Phosphatase

By B. R. CATER and T. HALLINAN. (*Department of Biochemistry, Royal Free Hospital School of Medicine, London WC1N 1BP, U.K.*)

A biphasic inhibition of glucose 6-phosphatase is revealed when rat liver microsomal fraction is treated with phospholipase C at 5°C (Poulter, Cater & Hallinan, 1971; Cater, Poulter & Hallinan, 1970). First, there is 40–60% loss of glucose 6-phosphatase activity when the enzyme is assayed directly after phospholipase C treatment at 5°C. This lost activity is completely restored by sonicated lecithin (phosphatidylcholine), which also prevents the second phase of inhibition. This occurs when the microsomal fraction is post-incubated at 20 or 37°C after phospholipase C treatment and before assay. Virtually all remaining glucose 6-phosphatase activity is lost regardless of whether EGTA* is added before post-incubation to stop further phospholipase C action. Lecithin does not restore the heat-inactivated glucose 6-phosphatase.

*Abbreviation: EGTA, ethanedioxybis(ethylamine)-NNN'-tetra-acetate.