These studies were considered to support the view that tri[^{14}C]methyl-(3-hydroxyphenyl)-ammonium *O*-glucuronide is normally transferred from liver cell to bile in competition with bilirubin glucuronide, and that the enhanced excretion of the quaternary metabolite in the homozygous Gunn rat is due to the absence of conjugated bilirubin from the liver cell.

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Biochemical Effects of the Hypoglycaemic Compound Diphenyleneiodonium in Rat Liver Mitochondria: Inhibition of Adenosine Triphosphate Synthesis

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Diphenyleneiodonium nitrate is a very active hypoglycaemic compound (Stewart & Hanley, 1969). We therefore investigated its effects on some reactions in rat liver mitochondria, since several unrelated hypoglycaemic compounds impair gluconeogenesis secondarily to inhibition of energy metabolism (see Senior & Sherratt, 1968; Sherratt, 1969).

Diphenyleneiodonium (10–30μM) strongly inhibits State 3 respiration (with succinate as substrate) stimulated by ADP or 2,4-dinitrophenol in a standard medium containing 130mM-Cl^-. Since diphenyleneiodonium catalyses a Cl^-–OH^-exchange across the inner mitochondrial membrane (Holland & Sherratt, 1971) two Cl^--free media were also used: one in which glycerol 2-phosphate^{2-} was substituted for Cl^- (based on Aldridge, 1957) and which gave similar respiratory rates and ADP/O ratios (with succinate as substrate) as in standard medium; and 0.3M-sucrose buffered with 2mM-*N*-2-hydroxyethylpiperazine-*N*-2-ethanesulphonate (cf. Stockdale, Dawson & Selwyn, 1970). In these Cl^--free media diphenyleneiodonium had little effect on respiration stimulated by ADP or by 2,4-dinitrophenol.

The effects of trialkyltin compounds on mitochondria resemble those of diphenyleneiodonium in that they inhibit State 3 respiration stimulated by ADP or 2,4-dinitrophenol in Cl^--containing medium (Aldridge, 1958) and they catalyse a Cl^-–OH^- exchange across the inner mitochondrial membrane (Selwyn, Dawson, Stockdale & Gains, 1970). By contrast they inhibit State 3 respiration stimulated by ADP but not by 2,4-dinitrophenol in a Cl^--free sucrose medium (Stockdale *et al.* 1970) or in glycerol 2-phosphate-containing medium. Stockdale *et al.* (1970) suggested that inhibition of respiration stimulated by ADP is due to an oligomycin-like effect of the trialkyltin compounds and that by contrast their inhibition of 2,4-dinitrophenol-stimulated respiration in Cl^--containing media is indirectly dependent on the Cl^-–OH^- exchange. Our results suggest that diphenyleneiodonium does not have a strong oligomycin-like effect and that its inhibition of State 3 respiration in Cl^--containing media depends on its ability to catalyse a Cl^-–OH^- exchange. Indeed, in Cl^--containing media 10μM-diphenyleneiodonium inhibits the 2,4-dinitrophenol-stimulated mitochondrial adenosine triphosphatase by only 20% while completely suppressing the State 4 to State 3 transition. These results also suggest that diphenyleneiodonium may not sufficiently impair the synthesis of ATP *in vivo* to account for its hypoglycaemic effects, since the intracellular Cl^- concentration is very low.

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Biochemical Effects of the Hypoglycaemic Compound Diphenyleneiodonium in Rat Liver Mitochondria: Anion–Hydroxyl Ion Exchange

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The possibility that diphenyleneiodonium blocks State 3 respiration (Holland & Sherratt, 1971) by inhibiting entry of substrate anions into mitochondria was investigated by using the technique of P_i-induced swelling of mitochondria suspended in ammonium succinate or malate solutions buffered with 5mM-tris–HCl (Chappell, 1968). No inhibition was found, but it was noticed that some swelling was caused by diphenyleneiodonium and that this was dependent on the Cl^- present. Rapid and ex-