The Biochemical Mode of Action of the Fasciolicides Nitroxynil, Hexachlorophane and Oxyclozanide

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There is little information on the biochemical mode of action of compounds for controlling *Fasciola hepatica* L. The chemicals used can be divided into two groups: (*a*) carbon tetrachloride and other compounds containing the trichloromethyl group; (*b*) substituted phenols and salicylanilides. We have investigated the latter group, taking nitroxynil (4-hydroxy-3-iodo-5-nitrobenzonitrile), hexachlorophane [2,2′-methylenebis-(3,4,6-trichlorophenol)] and oxyclozanide (3,3′,5,5′,6-pentachloro-2′-hydroxysalicylanilide) as structurally representative and commercially significant examples.

It is well known that substituted phenols and salicylanilides are uncouplers of oxidative phosphorylation, and symptoms of uncoupling of the host's metabolism have been shown for several fasciolicides of this group. However, the obvious hypothesis that the fasciolicidal effect is due to uncoupling does not seem to have been suggested. This communication gives our evidence that the uncoupling hypothesis is correct.

Using an oxygen electrode with rat liver mitochondria we found the following minimum uncoupling concentrations: nitroxynil, 27–33 μM; hexachlorophane, 0.6–0.8 μM; oxyclozanide, 0.3–0.4 μM.

Although the adult fluke is thought to live anaerobically in the bile duct it will take up oxygen from air. Using Warburg flasks with glucose as a food source we found that the compounds stimulated oxygen uptake by some 20–40% over the previous untreated rate at the following concentrations: nitroxynil, 10–100 μM, hexachlorophane, 1–10 μM; oxyclozanide, 0.1–1 μM. There is therefore an approximate correlation between the concentration causing stimulation of oxygen uptake and the minimum uncoupling concentration. After the stimulation the oxygen uptake decreases to a rate much lower than that of control flukes, which received solvent only, presumably owing to the progressive death of the flukes treated with fasciolicide.

A measure of the effect of the three compounds *in vitro* on fluke was gained by keeping flukes in Tyrode's solution plus bovine serum and examining the animals 24h after treatment. The following concentrations were required to cause apparent death (i.e. lack of visible movement): nitroxynil, 170–350 μM; hexachlorophane, 3.5–7 μM; oxyclozanide, 3.5–7 μM. This result is complicated by the presence of serum, which might bind the compounds to a differing extent, but there is a reasonable correlation with the minimum uncoupling concentrations of the fasciolicides, suggesting that death is indeed due to uncoupling.

The Effect of Organic Anions on the Biliary Excretion of Tri[^{14}C]methyl-(3-hydroxyphenyl)ammonium *O*-Glucuronide

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In previous experiments differences between the biliary excretion of tri[^{14}C]methyl-(3-hydroxyphenyl)ammonium iodide in normal Wistar animals and in Gunn strain of jaundiced rats were observed (Calvey, Somani & Wright, 1970). Both strains of animals mainly excreted the quaternary amine as a *O*-glucuronide. In the normal rat small amounts of this metabolite (equivalent to 3% of the dose in 4h) were eliminated in bile; in contrast homozygous Gunn rats excreted large amounts of the glucuronide in bile (26% of the dose in 4h). It was suggested that the diminished excretion in normal animals was dependent on competition between bilirubin glucuronide and tri[^{14}C]methyl-(3-hydroxyphenyl)-ammonium *O*-glucuronide for a common excretory pathway in the liver cell. In the homozygous Gunn rat competition for transport did not occur, so that large amounts of the exogenous glucuronide were transferred from liver to bile.

In the present experiments the effect of [^{3}H]-bilirubin and [^{3}H]bilirubin glucuronide on the biliary excretion of tri[^{14}C]methyl-(3-hydroxyphenyl)ammonium *O*-glucuronide was studied in normal Wistar animals and in homozygous Gunn rats. In all experiments the quaternary amine was administered as the iodide salt (2.0 μmol/kg body wt.). ^{3}H and ^{14}C radioactivity was simultaneously assayed in samples of bile by liquid-scintillation spectrometry.

In normal Wistar animals [^{3}H]bilirubin or [^{3}H]-bilirubin glucuronide (1.0 μmol/kg body wt.) decreased the biliary excretion of tri[^{14}C]methyl-(3-hydroxyphenyl)ammonium *O*-glucuronide by 0–20%; a similar decrease was induced by other organic anions (e.g. sulphobromophthalein, phenolsulphonphthalein and dehydrocholate). Different results were obtained in the homozygous Gunn rat. [^{3}H]Bilirubin (1.0 μmol/kg body wt.) has no effect on the biliary elimination of the ^{14}C-labelled quaternary glucuronide; in contrast [^{3}H]bilirubin glucuronide (1.0 μmol/kg body wt.) decreased its excretion by 80–90%, and a similar but less marked effect was induced by sulphobromophthalein and phenolsulphonphthalein.