detailed interpretation is complicated by the close proximity of the absorption bands of the sulphonamide and protein chromophores.

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## 2-Phenylisatogen as an electron acceptor for mitochondrial NADH dehydrogenase

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Previous investigations have shown that 2-phenylisatogen, a potentially useful anti-mycoplasma agent (Bond, 1969), inhibited ADP-stimulated succinate oxidation and uncoupler stimulated ATPase in tightly coupled rat liver mitochondria at a concentration of 12·5 nmol/mg mitochondrial protein (Sweetman, Green & Hooper, 1971). We have proposed that the site of action of 2-phenylisatogen is similar to that of the antibiotics oligomycin and aurovertin, which have been shown to inhibit the mitochondrial energy-transfer system (Roberton, Holloway & others, 1968; Lee & Ernster, 1968). We now wish to report a second action of 2-phenylisatogen on rat liver mitochondria obtained at higher concentrations with NADH as substrate.

Spectrophotometric determination of NADH oxidation at 340 nm showed that there was a forty-fold stimulation of NADH oxidation in the presence of $8{\cdot}3 \times 10^{-5}$ M 2-phenylisatogen. The stimulated respiration was not inhibited by respiratory chain inhibitors such as rotenone, sodium amylobarbitone, antimycin A and potassium cyanide. The reaction was inhibited by *p*-chloromercuribenzoate. This inhibitor specificity suggests that 2-phenylisatogen interacts with the NADH dehydrogenase system of the mitochondria at site 2, according to the scheme put forward by Ruzicka & Crane (1970). These workers have shown that quinones of the menadione type are reduced to their quinol forms by the respiratory chain-linked NADH dehydrogenase in the presence of NADH. If 2-phenylisatogen was being reduced by a similar mechanism then a possible reduction product would be 2-phenylindolone (see Bunney, 1970). We have obtained preliminary evidence for this possibility by our detection of 2-phenylindolone using a combination of extraction and thin-layer chromatographic techniques, after incubation of 2-phenylisatogen with mitochondria in the presence of NADH. When mitochondria were incubated with 2-phenylisatogen in the absence of NADH no 2-phenylindolone was detected.

Bunney (1970) has shown that 2-phenylisatogen reacts with 1,4-dihydrobenzylnicotinamide, a model compound for NADH, to produce 2-phenyl indolone and 2,2′-diphenyl-2,2′-diindoxyl.

We propose that 2-phenylisatogen interacts with the NADH dehydrogenase system of rat liver mitochondria, in a manner analagous to that found with quinones, to form the reduced compound 2-phenylindolone.

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