showed increase though none as great as with dropropizine. Enquiry elicited no adverse side-effects.

In the third phase, 11 of the selected volunteers were used in a double-blind comparison of a single dose of 90 mg dropropizine with placebo. The method was as in phase two. Results showed that dropropizine raised the threshold of sensitivity of 8 subjects by a greater amount than the placebo while in 2 cases the placebo effect slightly exceeded that of dropropizine. One subject showed no change in threshold. Again there were no adverse effects.

These results emphasize the importance of the psychological factor in antitussive assessment in humans, but the method was found to give consistent results for individual volunteers with remarkable constancy of the threshold baseline on repeated measurement. This would therefore seem to offer a simple, non-hazardous method of comparing antitussive potencies.

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Extrinsic circular dichroism resulting from the interaction of sulphonamides with plasma albumin

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The effects of protein binding of sulphonamides on their duration of action and their metabolism are well known (Anton & Boyle, 1964). The mechanism of binding is imperfectly understood (see, e.g., Jardetzky & Wade-Jardetzky, 1965). The use of circular dichroism (CD) in investigating the interactions of drugs with proteins has been indicated by Chignell (1970).

Strong extrinsic CD bands were generated between 260 and 320 nm when solutions of crystalline bovine serum albumin (BSA, $2{\cdot}5 \times 10^{-5}$M, pH 7·4) interacted with a number of optically inactive sulphonamides (5×10^{-5}M) having the general structure $H_2N\text{-}C_6H_4\text{-}SO_2NHR$. These included the highly lipid-soluble, strongly-bound compounds: sulphasomizole, sulphadimethoxine and sulphamethoxypyridazine. Sulphanilamide, sulphadiazine, sulphamerazine, sulphapyridine, sulphasomidine and sulphathiazole generated only weak effects or none at all. Similar, though not identical, results were obtained with human serum albumin.

Difference CD spectra of mixtures of BSA ($2{\cdot}5 \times 10^{-5} - 1{\cdot}0 \times 10^{-3}$ M) and sulphasomizole (molar ratio sulphonamide/protein 0·2–5·0) showed positive and negative peaks at 290 and 260 nm respectively. Graphs of difference ellipticity [$\Delta\psi_\lambda = \psi_\lambda$ (protein + sulphonamide) $- \psi_\lambda$ (protein)] against the number (r) of sulphonamide molecules bound per protein molecule, up to r = 2·6 (determined by equilibrium dialysis) fell into two linear regions with a discontinuity at r = 1. Interaction with the first binding site (Class I) is thus qualitatively different from binding to the second and third sites (Class II). The derived molecular extrinsic CD spectrum of sulphasomizole bound to Class I sites ($K_{assoc.} = 5 \times 10^4$ litre mol^{-1}, approx.) had peaks at 295 nm (molecular ellipticity = $[\theta]_{max} = +5{\cdot}6 \times 10^4$ deg cm^2 dmol^{-1}; dissymmetry factor = $g = [\theta]_{max}/3{,}300\epsilon = +1{\cdot}54 \times 10^{-3}$) and 260 nm($[\theta]_{max} = -5{\cdot}6 \times 10^4$ deg cm^2 dmol^{-1}; $g = -1{\cdot}13 \times 10^{-3}$). The positive peak is due to the heterocyclic ring and the negative peak is probably due to the *p*-aminobenzenesulphonic acid moiety. The results indicate that sulphasomizole is bound to Class I sites in such a way that both chromophores are subjected to asymmetric perturbations of similar magnitude by the protein. Sulphonamide bound to Class II sites ($K_{assoc.} = 10^3$ litre mol^{-1}, approx.) appears to have an extrinsic CD spectrum of lower magnitude ($\lambda_{max} = 275$ nm; $[\theta]_{max} = -1{\cdot}8 \times 10^{-4}$ deg cm^2 dmol^{-1}). The peak does not coincide with a peak in the absorption spectrum of the drug but may be due to perturbation of the tertiary structure of the protein. Determination of CD spectra between 200 and 250 nm indicated that none of the sulphonamides studied affected significantly the protein secondary structure.

Similar results were obtained with sulphadimethoxine or sulphamethoxypyridazine but

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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