to Pacha, 1969) were compared after appropriate time intervals by the *t*-test. Only at 1 h after administration, did suspension (I) give different (higher) blood concentrations than did the syrup. To obtain information on the absorption of thioridazine oral preparations in man, a single dose, 3-way cross-over study was carried out with six male adults. The preparations used were the syrup, suspension (I), and suspension (II) which contained 2·25% micronized thioridazine base and pharmaceutical formulating agents (Patent application no: 118–3076). The three preparations were given in a latin square order, with three weeks between successive administrations for elimination of thioridazine. Blood samples were taken at appropriate time intervals. Differences in thioridazine blood concentrations between subjects after the same preparation were greater than differences in a single subject after the three preparations. A modified *t*-test (Moroney, 1953) was therefore used to compare the differences in individuals after the three preparations. There was no significant difference between the blood concentrations after suspension (I) and suspension (II), but both these were consistently higher ($P < 0{\cdot}01$) than those after the syrup. Adjuvants added to suspension (II) had not reduced absorption. Thioridazine is a base and would be expected to be well-absorbed from the intestine and poorly absorbed from the stomach. The free base in suspension (I) and (II) will dissolve in the stomach. If solution is rapid and neither syrup nor suspension formulation affects absorption, the drug should be equally well absorbed from syrup and suspension. The superior absorption found for the suspensions could be due either to an ingredient included in both suspensions (but not the syrup) that increased thioridazine absorption, or to an ingredient in the syrup that reduced thioridazine absorption.

REFERENCES

MELLINGER, T. J. (1965). *Am. J. Psychiatr.*, **121**, 1119–22.
MORONEY, M. J. (1953). *Facts from Figures*, 2nd edn, p. 228. Harmondsworth: Penguin.
PACHA, W. L. (1969). *Experientia*, **25**, 103.
WAGNER, J. G. (1968). *Drug Intelligence*, **2**, 30–34.

* Present address: Department of Pharmacy, Heriot-Watt University, Edinburgh, U.K.

Human volunteer studies of the antitussive activity of dropropizine

K. CARTWRIGHT* AND J. L. PATERSON†
**The Boots Co., Nottingham, †Department of Pharmacology, University of Strathclyde, Glasgow, C.1, U.K.*

The production of cough and its assessment is possible with reasonable accuracy in laboratory animals but it is much more difficult in human subjects. A series of experiments was designed to induce cough in order to measure the effects of antitussives. The purpose was the verification of the antitussive property of 1-(2,3-dihydroxypropyl)-4-phenylpiperazine (dropropizine). In this study cough was induced by inhalation of citric acid aerosol using a modification of the method of Bickerman, German, Cohen & Itkin (1957).

In the first phase of the experiment, healthy volunteers were screened for response to citric acid inhalation using concentrations from 2% to 16%. The apparatus consisted of Rybar inhalers attached to a constant air supply of 14 p.s.i. so that each would deliver 0·175 ml/min of aqueous solution in atomized form. Initial screening was of 25 volunteers of ages ranging from 19 to 48 years and including both smokers and non-smokers. Of these, 14 were selected as positive cough responders on the basis of inability to tolerate a given threshold concentration of citric acid aerosol for 30 s or following 30 s inhalation to cough persistently for at least 30 s. The selected volunteers were rechecked to establish constancy of tussigenic threshold.

In the second phase, 9 of the selected volunteers were used in a double-blind study comparing a single dose of 20 mg codeine phosphate with a single dose of 60 mg dropropizine. After verification of the sensitivity threshold to citric acid inhalation for each volunteer, the first capsule was administered and the threshold redetermined 1½ h later. 4 h after ingestion of the first capsule, the second capsule was given and a further redetermination of the threshold made after 1½ h. The selection of medication was radomized and the subjects were unaware of the concentrations of citric acid that they were inhaling. Results showed that after dropropizine 6 subjects showed an increased threshold of response while after codeine phosphate 7

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.

REFERENCES

BICKERMAN, H. A., GERMAN, E., COHEN, B. N. & ITKIN, S. E. (1957). *Am. J. med. Sci.*, **234**, 191–205.
BICKERMAN, H. A. (1962). *Clin. Pharmac. Ther.*, **5**, 353–368.
NICOLIS, F. B. & PASQUARIELLO, G. (1962). *J. Pharmac. exp. Ther.*, **136**, 183–189.

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

G. C. WOOD AND SHEENA STEWART
Department of Pharmaceutical Chemistry, University of Strathclyde, Glasgow, C.1, U.K.

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