J. Pharm. Pharmac. 1971, **23**, 131

LETTERS TO THE EDITOR

NOR_2Chlorpromazine sulphoxide, a "pink spot" produced *in vivo* and *in vitro* from chlorpromazine

Friedhoff & Van Winkle (1962) examining the urine of patients with schizophrenia, reported a substance characterized by its R_F values in different chromatographic systems and by a positive reaction with ninhydrin and thereafter a pink colour with Ehrlich reagent ("pink spot"). The authors claimed this substance to be 3,4-dimethoxyphenylethylamine (DMPE). Closs, Wad & Ose (1967) thought that the "pink spot" was not DMPE but rather the main metabolite of chlorpromazine (CP) excreted in human urine, 2-chloro-1,5-oxide-10-(3-aminopropyl)phenothiazine (NOR_2CPSO) (Fishman & Goldenberg, 1960).

In this report we present the final analysis of "pink spot" and also an *in vitro* experiment with rabbit liver which transforms CP to "pink spot".

"Pink spot" was isolated (Friedhoff & Van Winkle, 1962) from the urine of a patient who received chlorpromazine daily. The substance was fractionated on thin layers of silica gel using acetone–1-butanol–ammonia (25% aqueous) (70:5:1), extracted with alkali (NaOH at pH 10) and this solution was then further extracted with chloroform. The extract was evaporated to dryness and the residue purified chromatographically on thin-layer silica gel plates using chloroform–2-propanol–acetic acid–water (45:35:20:5). Fifteen μg of the isolated substance gave a distinct ninhydrin-positive spot with the same R_F value as DMPE on paper in 1-butanol–acetic acid–water (4:1:1). But it did not give any fluorescence after treatment with the highly specific fluorescence method of Bell & Somerville (1966) in which as little as 0·03 μg DMPE can be detected, and, unlike DMPE, it became pink with Ehrlich reagent without previous treatment with ninhydrin.

Comparison of the infrared spectra of CP and "pink spot" suggests that the "pink spot" is a sulphoxide and a primary amine derivative of CP. That it is a sulphoxide derivative is also supported by the ultraviolet spectra, the curves for "pink spot" and of CP oxidized with H_2O_2 (Kofoed, Korczak-Fabierkiewicz & Lucas, 1966) being almost identical. There was no similarity between the ultraviolet spectrum of "pink spot" and that of CP. "Pink spot" behaved like a sulphoxide derivative of a phenothiazine when tested with the reagent described by Forrest & Forrest (1960).

No hydroxyl groups could be detected by the characteristic colour reaction described by Huang (1967), and Beckett & Hewick (1967) for hydroxylated derivatives of CP.

The presence of a non-substituted amino-group was confirmed using dansyl chloride, which reacts with hydroxy- and amino-groups (Gray & Hartly, 1963); this gave a fluorescent substance with "pink spot".

Since the reaction with ninhydrin is not specific for primary amino-groups the substance was treated with nitrite, after which the positive ninhydrin reaction disappeared; this is characteristic for primary amines. "Pink spot" cannot be a secondary amine since there was no reaction with the nitroprusside-acetaldehyde reagent of Sweeley & Horning (1957). Finally, the presence of a chlorine atom in the molecule was demonstrated by Beilstein's test. It therefore seems likely that the "pink spot" is identical with NOR_2CPSO.

We confirmed that incubation of CP with a suitable fortified rat liver homogenate did not result in NOR_2CPSO formation (Robinson, 1966; Beckett & Hewick, 1967). According to Gaudette (1956) rabbit liver has the most suitable demethylation enzymes among animal species.

Liver (6 g) from a male rabbit was homogenized in 30 ml of 0·25M sucrose, centrifuged at 25 000 *g* for 15 min at 2° and the supernatant withdrawn and centrifuged at 110 000 *g* for 60 min at 2°. The supernatant was decanted and the sediment dissolved in 6 ml 0·1M phosphate buffer pH 7·4. The reaction medium was prepared according to Hook & Smith (1967) replacing tris buffer with 0·1M phosphate buffer, pH = 7·4. One ml portions of microsome suspension, total volume 3·5 ml, were mixed in 50 ml beakers with 1 mg of CP or CPSO. The solutions were incubated at 35° for 1·5 h in a shaking incubator. The incubation mixture was deproteinized with 1 ml M trichloroacetic acid and centrifuged. The supernatant was made alkaline (pH 10) and extracted with chloroform. The extract was evaporated to dryness, the residue was dissolved in ethanol and applied on thin-layer silica gel plates with NOR_2CPSO, CP and CPSO as standards. A solvent system of acetone–1-butanol–ammonia (25% aqueous) (70:5:1) was used. The chromatogram was visualized first with 0·2% ninhydrin in acetone. Both substrates, CP and CPSO, were transformed to a small extent to NOR_2CPSO. The purple-coloured spots had the same R_F (0·53) as standard NOR_2CPSO. NOR_2CPSO was produced in greater yield when CPSO was used as substrate. The purple colour changed to pink when sprayed with 50% H_2SO_4, which also produced colours with the non-primary amine metabolites of CP formed *in vitro*.

We wish to thank Dr. E. Ose, Neevengården sykehus, Bergen, for the urine samples and for the diagnoses, Mr. O. E. Fjellbirkeland for the liver samples and R. Scheline, Ph.D., Dept of Pharmacology, Bergen, for advice and criticism during the study.

University of Bergen,
School of Medicine,
Department of Clinical Biochemistry,
Haukeland sykehus,
Bergen, Norway.

NILS WAD
KARL CLOSS

October 27, 1970

REFERENCES

BECKETT, A. H. & HEWICK, D. S. (1967). *J. Pharm. Pharmac.*, **19**, 134–136.
BELL, C. E. & SOMERVILLE, A. R. (1966). *Biochem. J.*, **98**, 1c.
CLOSS, K., WAD, N. & OSE, E. (1967). *Nature, Lond.*, **214**, 483.
FISHMAN, V. & GOLDENBERG, H. (1960). *Proc. Soc. exp. Biol. Med.*, **104**, 99–103.
FORREST, I. S. & FORREST, F. M. (1960). *Clin. Chem.*, **6**, 11–15.
FRIEDHOFF, A. J. & VAN WINKLE, E. (1962). *Nature, Lond.*, **194**, 897–898.
GAUDETTE, L. E. (1956). Ph.D. Thesis, Georgetown Univ., Washington, D.C. cit: WILLIAMS, R. T. 1959. *Detoxication Mechanisms*, 2nd edn., p. 723. London: Chapman & Hall Ltd.
GRAY, W. R. & HARTLY, B. S. (1963). *Biochem. J.*, **89**, 59.
HOOK, G. E. R. & SMITH, J. N. (1967). *Ibid.*, **102**, 504.
HUANG, C. L. (1967). *Int. J. Neuropharmac.*, **6**, 1–13.
KOFOED, J., KORCZAK-FABIERKIEWIEZ, C. & LUCAS, G. H. (1966). *Nature, Lond.*, **211**, 147–150.
ROBINSON, A. E. (1966). *J. Pharm. Pharmac.*, **18**, 19–32.
SWEELEY, C. O. & HORNING, E. C. (1957). *J. Am. chem. Soc.*, **79**, 2620.