4. J. J. SCHILDKRAUT, A. WINOKUR, P. R. DRASKOCZY and J. H. HENSLE, *Am. J. Psychiat.* **127,** 1032 (1971).
5. J. GLOWINSKI and L. L. IVERSEN, *J. Neurochem.* **13,** 655 (1966).
6. A. H. ANTON and D. F. SAYRE, *J. Pharmac. exp. Ther.* **138,** 360 (1962).
7. S. H. SNYDER, J. AXELROD and M. ZWEIG, *Biochem. Pharmac.* **14,** 831 (1965).
8. A. CARLSSON and B. WALDECK, *Acta physiol. Scand.* **44,** 293 (1958).
9. A. DAHLSTROM and K. FUXE, *Acta physiol. scand.* **62,** suppl. 232 (1964).
10. S. ROFFLER-TARLOV and J. J. SCHILDKRAUT, *Fedn Proc.* **30,** 381 (1971).
11. H. S. ALPERS and H. E. HIMWICH, *J. Pharmac. exp. Ther.* **180,** 531 (1972).
12. L. MANARA, M. J. SESTINI, S. ALGERI and S. GARATTINI, *J. Pharm. Pharmac.* **18,** 194 (1966).
13. A. PHILIPPU, H. BECKE and A. BURGER, *Eur. J. Pharmac.* **6,** 96 (1969).
14. W. D. REID, F. J. E. STEFANO, S. KURZEPA and B. B. BRODIE, *Science, N.Y.* **164,** 437 (1969).
15. F. H. LEITZ, *J. Pharmac. exp. Ther.* **173,** 152 (1970).
16. M. J. STEINBERG and C. B. SMITH, *J. Pharmac. exp. Ther.* **173,** 176 (1970).
17. F. H. LEITZ and F. J. E. STEFANO, *Biochem. Pharmac.* **19,** 1797 (1970).
18. A. J. MANDELL, D. S. SEGAL, R. T. KUCZENSKI and S. KNAPP, in *The Chemistry of Mood, Motivation and Memory* (Ed. J. L. MCGAUGH), p. 105. Plenum Press, New York (1972).

Biochemical Pharmacology, Vol. 21, pp. 2926–2928. Pergamon Press, 1973. Printed in Great Britain.

Interaction of metronidazole with human and bovine plasma albumin

(*Received* 14 *March* 1973; *accepted* 9 *May* 1973.)

METRONIDAZOLE [1-(β-hydroxyl ethyl)-2-methyl-5-nitromidazole] has been shown to have tri. chromonacidal activity.[1] Clinical trials[2] have confirmed that this drug is of great value in humans-Although metronidazole is usually well absorbed after oral administration, some patients fail to respond to treatment and this poor response may be due to a low systemic concentration of the drug. Whether this is due to relatively poor absorption from the gastrointestinal tract[3] or to rapid metabolism of the drug[4] is open to question. In view of the fact that protein-binding affects pharmacokinetic properties of drugs;[5] we examined the thermodynamics of the binding of this compound to serum albumins.

Metronidazole was the generous gift of May & Baker (Dagenham, Essex). Human serum albumin (HSA), Sigma Chemical Co., U.S.A., and bovine serum albumin (BSA), British Drug Houses, Ltd, Pool, U.K. were used as supplied by the manufacturers. We considered it unnecessary to purify further the crystalline albumins. All solutions were prepared in 0·067 M sodium phosphate buffer, pH 7·4 (I = 0·170). The albumin solutions were made at a concentration of 0·4 mg/ml. A 200 μM solution of the drug was made and diluted as required.

The uv spectrum of metronidazole was recorded with a Perkin–Elmer (Model 137 UV) spectrophotometer. We recorded only one maximum value. This measurement is the longest wavelength, in the 260–400 nm region, at which a peak absorption occurs for the compound. A knowledge of this value was necessary and useful in our binding experiments.

The method of equilibrium dialysis which was employed to observe albumin binding has been described by Bassir and Bababunmi.[6] This method permits easy determination of protein interaction with drugs.[7] In the present studies, 10 cm lengths (3 cm dia.) or 15 cm lengths (1·5 cm, dia.) of Visking cellophane tubing (Scientific Instrument Centre Ltd, London, U.K.) were used. They were cleaned by rinsing in a shaking bath (Gallenkamp, U.K.) of de-ionized water for 72 hr and were then stored in the 0·067 M sodium phosphate buffer at 4°. The tubing was washed thoroughly with the buffer before use. Optical measurements of the drug were made at 318 nm with a Carl Zeiss Model PMQ II spectrophotometer, using rectangular quartz spectrophotometer cells having a 1 cm light path.

The standard method for the measurement of free and bound drug was as follows: 8·0 ml of albumin solution was placed inside the bag and dialysed against 15·0 ml of medium containing metronidazole in a 50 ml borosilicate AG glass tube and covered with cotton wool. Four different concentrations (25, 50, 100 and 150 μM) of the drug were used while the concentration of the protein was kept constant. For each concentration of the drug four tubes were placed and rocked at 150 cycles/min for 18 hr in the water bath which was regulated either at 15 ± 1° or 25 ± 1°. At 18 hr equilibrium

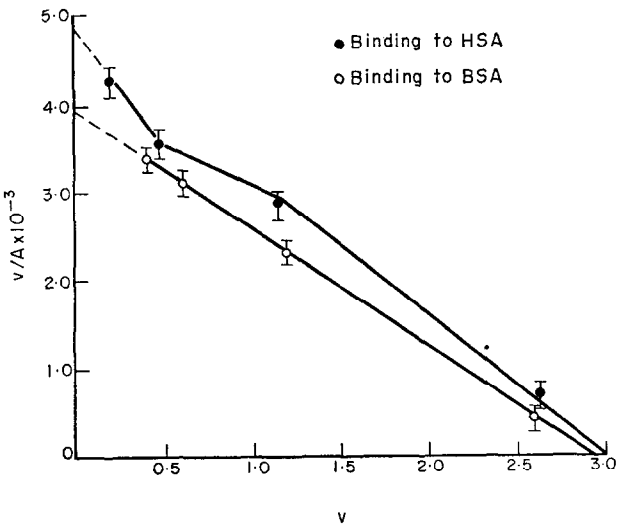


FIG. 1. Scatchard profiles of the interactions of metronidazole with BSA and HSA. Four different concentrations of the drug were used, while the concentration of the protein was kept constant. Each point is the mean of four determinations. Assuming the law of mass action, Scatchard equation may be written as $V/A = K(n - v)$ where V is the molar ratio of bound drug to albumin; A is the molar concentration of the free drug outside the dialysis tubing, at equilibrium; K is the average apparent association constant for the binding at each site of the drug and albumin; and n is the average maximal number of binding sites for the drug present on the albumin molecule which is evaluated from the V-intercept.

had occurred under our experimental conditions. Control experiments, with bags which contained only buffer, demonstrated that the buffer solution had no observable effect on binding. The amount of albumin-bound drug was then calculated by subtracting the free and unbound drug concentration from the initial concentration. Binding constants were analyzed using the method of Scatchard *et al.*[8]

The Scatchard profiles shown in Fig. 1 are the average of four observations. Because Foster[9] has reported that HSA stays in the α-helical form at pH 7·4 we have assumed that the albumins were not denatured under our conditions. At 25° there was no significant difference ($P > 0{\cdot}05$) in the number of binding sites of metronidazole to the albumins. The profiles obtained at 15° were essentially the same. The drug had an averaged number of 2·9 on the BSA whilst it averaged 3·0 on the HSA. The apparent association constants (K) were, however, quite different ($K = 1{\cdot}4 \times 10^{-3}$ for BSA; $K = 1{\cdot}63 \times 10^{-3}$ for HSA). When the binding studies were repeated the 15°, we were able to calculate the enthalpy (ΔH) from the free energy (ΔF) according to the second law of thermodynamics. In this respect, the $\Delta F°$ and ΔH for the binding of the drug to BSA were − 3·95 kcal/mole and 4·1 kcal/mole respectively whilst for HSA the values were − 4·9 kcal/mole and 3·1 kcal/mole respectively. According to Bezkorovainy,[10] these values suggest hydrophobic interactions between the drug and albumin.

Garten and Wosilait[5] suggested that amino acids (with non-polar side chains) are capable of interacting with the hydrophobic moieties of molecules whose structures are similar to that of metronidazole. However, we found that the binding of the drug to albumin was not accompanied by any measurable quantity of entropy. This zero quantity of entropy is characteristic of the formation of a hydrogen-bonded ring.[10] These observations, based on thermodynamic considerations, suggest that the binding of metronidazole to serum albumin involves the formation of both hydrogen and hydrophobic bonds.

If the binding of metronidazole to albumins involves both hydrogen and hydrophobic bonds and at three sites, we suggest that the drug binds very strongly and depending on the dose, only a relatively small amount might be available in plasma to achieve a therapeutic effect. It is therefore probable that the low systemic concentration of the drug in circulation[3,4] is due to its high affinity for plasma albumins.

Acknowledgement—The authors wish to thank Dr. G. O. Ogunmola for some technical assistance and Mr. F. H. J. Rowley of May & Baker (West Africa) Ltd.

Departments of Pharmacology and Biochemistry,
Faculty of Medicine,
University of Ibadan,
Ibadan,
Nigeria

OLUSEGUN A. BAMGBOSE
ENITAN A. BABABUNMI

REFERENCES

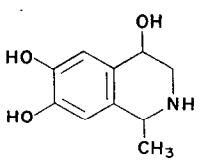
1. C. COSAR and L. JULOU, *Annls Inst. Pasteur, Paris* **96,** 238, (1959).
2. P. DUREL, V. ROIRON, A. SIBOULET and L. J. BONEL, *Br. J. vener. Dis.* **36,** 21 (1960).
3. P. O. KANE, J. A. MCFADZEN and S. SQUIRES, *Br. J. vener. Dis.* **37,** 276 (1961).
4. J. E. STAINBAUGH, L. G. FEO and R. W. MANTHEI, *J. Pharmac. exp. Ther.* **161,** 373 (1968).
5. S. GARTEN and W. D. WOSILAIT, *Biochem. Pharmac.* **20,** 1661 (1971).
6. O. BASSIR and E. A. BABABUNMI, *Biochem. Pharmac.* **22,** 132 (1973).
7. J. A. GORDON and J. R. WARREN, *J. biol. Chem.* **243,** 5663 (1968).
8. G. S. SCATCHARD, J. S. COLEMAN and A. L. SHEW, *J. Am. chem. Soc.* **79,** 12, (1957).
9. J. F. FOSTER, *The Plasma Proteins* (Ed. F. W. PUTMAN) p. 179. Academic Press, New York (1960).
10. A. BEZKOROVAINY, *Biochemistry* **2,** 10, (1963).

Biochemical Pharmacology, Vol. 22, pp. 2928–2931. Pergamon Press, 1973. Printed in Great Britain.

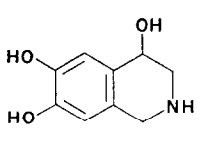
Tetrahydroisoquinolines derived from noradrenaline–aldehyde condensations—Pyrogallol-sensitive *O*-methylation in rat homogenates*

(*Received* 24 *February* 1973; *accepted* 4 *May* 1973)

THE CATECHOL *O*-methyltransferase (COMT) pathway is a well established metabolic route for endogenous catecholamines (CAs).[1] With rat brain and liver homogenates, we have examined *O*-methylation in the presence and absence of the COMT inhibitor, pyrogallol, of tetrahydroisoquinoline (TIQ) alkaloids (Fig. 1) which are cyclized derivatives of noradrenaline (NA) and acetaldehyde (AcH) or formaldehyde (HCHO). It has been suggested that TIQ alkaloids may form in neuronal and chromaffin cells during alcohol metabolism and assume a physiological role in the development of alcoholism.[2–4] *O*-methylation of some TIQs structurally related to dopamine (DA) has been recently observed.[5–7] The substrate specificity of COMT has now been extended to include the TIQ alkaloids having a β-hydroxylated CA skeleton.



TIQ I



TIQ II

FIG. 1. 4,6,7-Trihydroxy-tetrahydroisoquinoline derivatives of noradrenaline.

* This work was supported by funds from the U.S. Public Health Service (NIMH-MH 19153), the State of Illinois (MH 114-11-RD) and a Loyola University General Research Support grant.