Additionally, advantage was taken of the convenient fractionation of hypoglycins A (one amino and one carboxyl group free) and B (one amino and two carboxyl groups free), on a strongly basic anion-exchange resin by dilute acetic acid; this was based on the work of Hirs, Moore & Stein (1954).

This work was supported by a grant from the Wellcome Trust.

Department of Biochemistry,
University of the West Indies,
Kingston 7, Jamacia.

E. A. KEAN

January 18, 1974

REFERENCES

ABRAHAMS, D. E. & KEAN, E. A. (1969). *West Indian medl J.*, **18**, 147–151.
ELLINGTON, E. V., HASSALL, C. H., PLIMMER, J. R. & SEAFORTH, C. E. (1959). *J. chem. Soc.*, 80–85.
HASSALL, C. H. & RYLE, K. (1955). *Biochem. J.*, **60**, 334–339.
HIRS, C. W. H., MOORE, S. & STEIN, W. H. (1954). *J. Am. Chem. Soc.*, **76**, 6063–6065.
MILLINGTON, D. S. & SHEPPARD, R. C. (1968). *Phytochem.*, **7**, 1027–1029.
WEST, M. E. (1968). *J. Pharm. Pharmac.*, **20**, 974–975.
WOODS, K. R. & WANG, K-T. (1967). *Biochim. Biophys. Acta*, **133**, 369–370.

The binding of chlorpromazine to human serum albumin

The binding of chlorpromazine to human serum albumin (HSA) is a well established property (Curry, 1970). The kinetics of binding have been investigated by Jähnchen, Krieglstein & Kuschinsky (1969) using the techniques of equilibrium dialysis and gel filtration. They reported the following results: number of binding sites (n) = 30, affinity constant (k) = $2{\cdot}1 \times 10^4$ litre mol^{-1} (22°), pH 7·4. Nambu & Nagai (1972), however, using the same techniques reported: number of binding sites (n) = 2, affinity constant (k) = $7{\cdot}81 \times 10^3$ litre mol^{-1} (10°), pH 7·4. In order to resolve this apparent contradiction, we reinvestigated the kinetics of binding of chlorpromazine to HSA using the techniques of equilibrium dialysis and spectrofluorimetric quenching titration (Chignell, 1972). The results were evaluated using two different mathematical treatments, the Scatchard method (Scatchard, 1949) and the double log method (Thompson & Klotz, 1971).

Human serum albumin (HSA) was a lyophilized preparation for transfusion and a gift of the Manchester Blood Bank. Albumin solutions in Sorensen phosphate buffer (pH 7·4) were prepared immediately before use and assayed spectrophotometrically for albumin. Chlorpromazine hydrochloride (Largactil) was a gift of May and Baker Ltd.

Equilibrium dialysis. Dialysis was carried out using Visking Dialysis tubing (32/32) in a five compartment dialysis cell made to the specifications of Katz & Weissburger (1969). Drug concentrations in the range $2 - 10 \times 10^{-6}$ M and albumin concentrations of $6{\cdot}07 \times 10^{-6}$ or $12{\cdot}14 \times 10^{-6}$ M were placed on one side of the membrane and blank Sorensen phosphate buffer (pH 7·4) on the other. The cells were allowed to come to equilibrium by standing at 28° (± 1°) for 3 days and then the free drug concentration was determined spectrophotometrically using a Unicam SP 500 Series 2 spectrophotometer.

Spectrofluorimetric quenching titration. The titrations were made using a Baird-Atomic Fluorispec SF 100 E spectrofluorimeter. The excitation and emission wavelengths of HSA are 285 and 354 nm respectively and drug concentrations in the range

Table 1. *Binding constants for the binding of chlorpromazine to HSA.*

HSA concn (M)	Scatchard		Thompson & Klotz	
	n	k(litre mol^{-1})	n*	k(litre mol^{-1})*
Equilibrium dialysis				
6.07×10^{-6}	2·3	4.05×10^{5}	2·1	3.46×10^{5}
12.14×10^{-6}	1·7	4.33×10^{5}	1·7	4.21×10^{5}
Spectrofluorimetric quenching titration				
6.8×10^{-6}	1·94	1.9×10^{5}	2·1	1.68×10^{5}
11.88×10^{-6}	1·91	3.3×10^{5}	2·3	2.0×10^{5}

* Average of six calculations.

1–20 $\times$ 10^{-6} M in 1 $\times$ 10^{-6} M stages were titrated against HSA solution (concentration 6·8 $\times$ 10^{-6} and 11·88 $\times$ 10^{-6}M in Sorensen phosphate buffer pH 7·4). Measurements were made at room temperature (20°).

The results indicate that the number of binding sites for chlorpromazine on HSA is 2 which is in agreement with the results of Nambu & Nagai. This is also in line with the results obtained by Ma, Jun & Luzzi (1973) for the binding of tetracyclines to bovine serum albumin. HSA fluorescence is caused by the tryptophan residue in HSA which can be quenched by compounds binding to the HSA (Chignell, 1972). The finding of two binding sites for chlorpromazine by spectrofluorimetric quenching suggests the presence of two hydrophobic binding sites of equal affinity both of which affect the fluorescence of the tryptophan residue. This is feasible since Stryer (1969) has shown that compounds binding up to 60 Å away from the tryptophan can still quench the fluorescence while X-ray crystallography has shown that the size of the phenothiazine ring of chlorpromazine is no more than 10 Å (McDowell, 1969). Binding to sites which do not affect the fluorescence of tryptophan can be ruled out since the results obtained by both techniques are equivalent. The differences in affinity constants obtained by the two methods and by Nambu & Nagai could be due to temperature differences. The Thompson & Klotz method of evaluation gives a more consistent result since many more estimations of n and k can be made instead of the one as in the Scatchard method.

Department of Pharmacy,
University of Manchester,
Manchester M13 9PL, U.K.

D. SHARPLES

March 29, 1974

REFERENCES

CHIGNELL, C. F. (1972). In *Methods in Pharmacology.* Vol. 2 Physical Methods, p. 33, New York: Meredith.

CURRY, S. H. (1970). *J. Pharm. Pharmac.*, **22**, 193–197.

JÄHNCHEN, E., KRIEGLSTEIN, J. & KUSCHINSKY, G. (1969). *Naunyn-Schmiedebergs Arch. Pharmak. exp. Path.*, **263**, 375–386.

KATZ, S. A. & WEISSBURGER, H. E. (1969). *Experientia*, **25**, 672–674.

MA, J. K. H., JUN, H. W. & LUZZI, L. A. (1973). *J. pharm. Sci.*, **62**, 1261–1264.

McDOWELL, J. J. H. (1969). *Acta Crystallogr.*, **B25**, 2175–2181.

NAMBU, N. & NAGAI, T. (1972). *Chem. Pharm. Bull.*, **20**, 2463–2470.

SCATCHARD, G. (1949). *Ann. N.Y. Acad. Sci.*, **51**, 660–672.

STRYER, L. (1969). Cited in *Fluorescence Assay in Biology and Medicine.* Volume 11, p. 272. Editor: Udenfriend, S. New York: Academic Press.

THOMPSON, C. & KLOTZ, I. M. (1971). *Arch. Biochem. Biophys.*, **147**, 178–185.