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Plasma protein binding of chlorpromazine

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More than 90% of the plasma content of chlorpromazine over a concentration range from 0·008 to 15·1 μg/ml was bound to human plasma protein. Binding was affected by the pH of the aqueous medium; with few exceptions the higher values were obtained at the higher pH values. Binding was highest in some of the plasma samples from humans, and successively lower in plasma from dogs, rabbits and rats. Binding of chlorpromazine after administration of the drug to psychiatric patients, and after *in vitro* addition of the drug to plasma, was reversible. Variation in binding in plasma from different humans was marked; the amount bound varied from 91·0 to 99·0%. Thus the variation in the amount free was from 1·0–9·0%.

In assessing the significance of concentrations of drugs in plasma, it is essential to consider binding of drugs to plasma protein (Brodie, 1966; Meyer & Guttman, 1968). Studies of chlorpromazine distribution in animals (Salzman & Brodie, 1956) have included the observation of high binding to plasma protein, but the phenomenon appears not to have been studied extensively. This report concerns the binding of chlorpromazine to plasma proteins as a function of chlorpromazine concentration, pH, species and reversibility; these studies contribute towards the understanding of chlorpromazine concentrations in the plasma of psychiatric patients (Curry, 1968a).

EXPERIMENTAL

Materials

Samples of chlorpromazine hydrochloride were obtained from Smith Kline and French Laboratories. Radioactive chlorpromazine (^{35}S) was purchased from Radiochemical Centre, Amersham. At the commencement of the experiments this material had a specific activity of 12·7 mCi/mmol and in all experiments allowance was made for decline in specific activity.

Human plasma was obtained from six psychiatric patients and from pooled sources (blood-bank supplies). The psychiatric patients had received chlorpromazine for at least one month previously, in twice daily dosage (range of dose 100–600 mg/day). These patients were suffering from schizophrenia, but no other known disease, and had received no drugs other than chlorpromazine during the previous month. In animal studies, plasma was obtained from an inbred population of healthy Sprague Dawley rats, from an inbred population of healthy albino rabbits, and from three healthy pedigree beagles. All plasma was obtained from citrated blood.

Determination of protein binding

Samples (2 ml) of plasma containing chlorpromazine were placed in sections of previously moistened (soaked overnight in a sample of the solvent to be used) Visking dialysis tubing (10 mm wide when flat) knotted at each end. An air bubble was

included to aid mixing. The sack containing the plasma was placed in a stoppered test tube containing 10 ml of 0·1M phosphate buffer (pH 7·4) (or other suitable solutions in studies of influence of pH). The tube was agitated gently at room temperature until equilibrium was achieved and the material was assayed in the final solutions inside and outside the dialysis membrane. Preliminary experiments, with plasma, demonstrated that equilibration was achieved within 48 h of the commencement of the experiment.

Assay of chlorpromazine

In experiments in which non-radioactive drug was used, chlorpromazine was assayed by gas chromatography (Curry, 1968b). The content of the solutions of radioactive chlorpromazine was determined by direct sampling of suitable volumes of the solution into liquid scintillation vials. The liquid scintillation fluid used was prepared from BBOT (2,5-bis-(5-t-butylbenzoxazoyl)thiophene), 0·4%, naphthalene, 0·8% and methylcellosolve, 40%, in toluene. Standard quench corrections were made. In all assays of chlorpromazine, both by liquid scintillation spectrometry and by gas chromatography, fresh standard solutions were used, and concentrations were determined in terms of chlorpromazine hydrochloride.

RESULTS

Over a wide range of concentrations for chlorpromazine in samples of mixed human plasma, determinations of protein binding varied from 91·8–97·0% of the plasma content of drug (Table 1). Binding varied only slightly within the therapeutic concentration range (0·01–1 μg/ml). There was a tendency to a lower binding value at the highest drug concentration, indicating the possibility of saturation, but it appeared that immense quantities of the drug would be required to occupy all its potential binding sites on plasma protein.

Table 1. *Plasma protein binding of chlorpromazine at various concentrations*, Plasma, from a single bottle of mixed human material, containing radioactive chlorpromazine at a number of concentrations, was dialysed as described in the Experimental section. Percentage binding was measured from the ratio of radioactivity in dialysate and plasma

Concentration of chlorpromazine in plasma after dialysis (μg/ml)	% Bound to protein (mean with range on 3–4 determinations in parentheses)
0·008	94·9 (93·3–97·0)
0·019	94·8 (93·9–94·9)
0·039	93·7 (91·8–95·4)
0·08	94·5 (93·6–95·9)
0·21	95·7 (93·0–97·0)
0·4	94·8 (94·5–95·2)
0·8	95·5 (94·9–96·3)
15·1	92·0 (91·3–93·6)

Determinations of protein binding of chlorpromazine added to human plasma were affected by the pH of the fluid into which dialysis was made (Table 2). With the unexplained exception of pH 4, binding was higher at higher pH values, and

Table 2. *Plasma protein binding of chlorpromazine at various pH values.* Samples from a solution of radioactive chlorpromazine at a concentration of 20 μg/ml were dialysed against aqueous solutions of various pH value, as described in the Experimental section. Percentage binding was measured from the ratio of radioactivity in dialysate and plasma

pH	Solvent	% Bound to protein (mean, with range on 3 determinations in parentheses)
1·7	0·05N HCl	67·0 (66·0–68·0)
4	0·1M Phthalate buffer	89·0 (89·0–90·0)
4·7	0·1M Acetate buffer	81·0 (80·0–81·6)
7	0·1M Phosphate buffer	88·5 (87·0–89·0)
7·4	0·1M Phosphate buffer	91·0 (90·7–92·1)
10	0·1M Carbonate buffer	98·9 (98·8–99·1)
12·5	0·05N NaOH	98·2 (97·8–98·4)

lower at lower pH values. Even when the drug was in solutions of pH 1·7 a large amount was bound.

When chlorpromazine was added to plasma from rats, rabbits and dogs, protein binding of the drug varied from 89·1–95·7% of the plasma content (Table 3). Higher binding than this was recorded in some, but not all, samples of human plasma; large differences in the binding capacity of various batches of human plasma were noted. Triplicate determinations of binding in one sample of plasma never varied by more than 1·85% from the mean; determinations in samples of plasma from six patients varied from 91·0–99·0%.

Table 3. *Plasma protein binding of chlorpromazine in three species.* Plasma from three species was dialysed as in the Experimental section after addition of radioactive chlorpromazine. The percentage binding of chlorpromazine was measured from the ratio of radioactivity in plasma and dialysate samples

Species	Concentration of chlorpromazine after dialysis (μg/ml)	% Bound to protein (mean, with range on 3 determinations in parentheses)
Rat	0·061	89·4 (89·1–89·9)
Rabbit	0·076	94·1 (93·8–94·5)
Dog	0·082	95·7 (94·6–97·4)

The reversibility of binding of chlorpromazine to plasma protein was examined in three ways. First, a solution of chlorpromazine was dialysed as described under Experimental; after determination of the percent bound, the plasma was re-dialysed twice, to give a total of three determinations of protein binding; the three values determined were 95·2, 95·9 and 95·9%. Second, in a pair of experiments, binding was studied by adding chlorpromazine to the buffer solution or to plasma; after equilibration, values of binding were: 94·4% when the drug had transferred from the outside of the membrane to the inside; and 94·7% when the drug had transferred from the inside of the membrane to the outside. Third, trace quantities of radioactive chlorpromazine were added to two samples of plasma obtained from patients

treated with chlorpromazine. The plasma was dialysed as described in the Experimental section. Percentage binding, measured from the ratio of chlorpromazine assays by gas chromatography in dialysate and plasma, and from the ratio of radioactivity in dialysate and plasma, was respectively 97·7 and 98·1% for a chlorpromazine plasma concentration after dialysis of 0·90 μg/ml, and 98·9 and 99·0% for a concentration of 0·64%. All records were from single assays. Thus the results of binding were similar by the two methods.

The remote possibility that dialysed radioactivity from chlorpromazine was a metabolite, decomposition product or other compound different from chlorpromazine was considered in a specificity experiment (Table 4). Authentic chlorpromazine, and radioactivity in plasma and a dialysate, were shown to distribute similarly between n-heptane containing 1·5% isoamyl alcohol and aqueous solutions of various pH values. These materials contrasted in this respect with known metabolites and decomposition products of chlorpromazine (Curry, 1968b).

Table 4. *Specificity checks for chlorpromazine binding.* The radioactivity in a typical pair of dialysate and plasma fractions after a protein binding experiment was totally extracted into n-heptane containing 1·5% isoamyl alcohol from alkaline solutions. The organic layer was separated, and the radioactivity extractable into aqueous solutions of various pH values was determined. Standard data were similarly obtained for a solution of an authentic sample of chlorpromazine. Each figure is a single determination

	% Of material in organic layer extracted into aqueous layers		
pH of aqueous solution	Authentic sample	Plasma	Dialysate
1·7	100	100	100
3·5	85	90	83
4·2	62	53	66
5·0	30	33	40
5·5	1	3	4
6·3	0	0	0

DISCUSSION

Chlorpromazine is highly bound to plasma protein. Even at concentrations approaching 20 μg/ml, 90% of the plasma content of the drug was bound. This resulted in only a slight change in percent binding over the therapeutic range of drug concentration. However, a tendency to saturation was demonstrated, in keeping with established theory concerning the reversibility of binding and the possibility of saturation of binding sites on plasma proteins. The reversibility of the binding was further shown by the ease of removal of drug by dialysis, and by the fact that drug molecules added to plasma equilibrated rapidly between plasma protein and plasma water.

Results of studies of pH-dependency of binding indicated that it is most probably the non-ionized form of the drug that is bound to protein. However, even at pH 1·7, much binding occurred, adding to the evidence that the association constant for binding of the unionized form of the drug to protein is relatively high.

The maximum analytical error recorded in the present studies was ±1·85 in percent bound. In the three pedigree species, binding was significantly greater in the order dogs>rabbits>rats, and the differences were significantly different from the analytical error. The highest values of all were obtained from some of the human samples, but the range in man was wide. It is difficult, in view of this range, to draw any conclusions concerning the relation between binding capacity and species.

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