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# HYDROPHOBIC AND IONIC INTERACTIONS OF PHENOTHIAZINE DERIVATIVES WITH BOVINE SERUM ALBUMIN

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**Abstract**—The binding of promethazine, trimeprazine, desmethylpromazine, desmethylchlorpromazine and perazine to bovine serum albumin was measured by sephadex gel filtration and equilibrium dialysis. The binding of these drugs to albumin is characterized by the following parameters: percentage ($\beta$) of bound drug, the association constant $K_1$, the apparent binding constant $k^*$, and the free energy $\Delta F°$. In addition, the displacing activity of hydroxyzine and acetylsalicylic acid on the binding of phenothiazines to albumin was examined. The binding of phenothiazine derivatives to bovine serum albumin is correlated with their octanol–water partition coefficients. The results allow the following interpretation of the phenothiazine–albumin complex. Only one of the phenothiazine benzene rings is attached to a hydrophobic area on the albumin molecule, while the basic dimethylamino group is associated with a negatively charged group on the protein surface. The aliphatic side chain obviously does not directly take part in binding. But a methyl group in the aliphatic chain as in the case of promethazine or trimeprazine enhances the binding of the molecule significantly.

THE BINDING of drugs to albumin has been investigated in numerous papers because of its pharmacokinetic significance.[1–3]

But the drug–albumin complex may be considered also as a model for gaining general fundamental insights into drug–protein binding. General rules of protein binding gained from this model could apply at least partially to the drug–receptor complex, provided that the receptor has a protein structure. The determination of albumin binding of several structurally related compounds is a valuable tool for identifying the groups of a drug molecule which are involved in binding, and for characterizing the binding forces concerned with the interaction of drugs with protein.

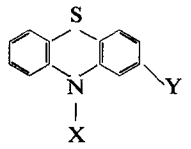
In the present study the binding of several phenothiazine derivatives to bovine serum albumin and the octanol–water partition coefficients of these drugs were examined in order to get further information about the nature of phenothiazine–albumin complex.

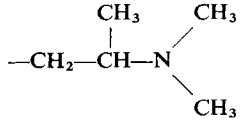
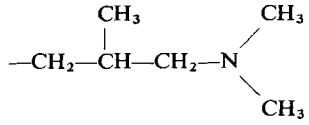
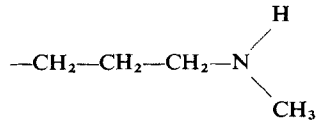
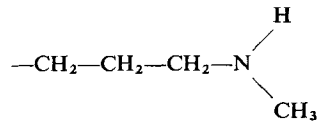
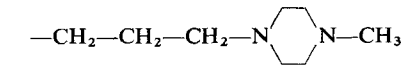
Part of this material was presented at the spring meeting of the Deutsche Pharmakologische Gesellschaft in Mainz 1970.[4]

## MATERIALS AND METHODS

*Materials.* The phenothiazine derivatives used are summarized in Table 1. Hydroxyzine was obtained from UCB Chemie, Sindorf (Atarax ®). Bovine serum albumin was purchased from Behringwerke, Marburg (quality: trocken, "reinst"). All other chemicals were of reagent grade. All binding measurements were made in the presence of 0·02 M phosphate buffer, pH 7·40, containing 0·15 M NaCl and 3 mM sodium thiosulfate (=standard buffer solution). Just before protein binding of a drug was determined the pH of the sample solution was measured and where necessary adjusted to pH 7·40 with 0·1 N HCl or 0·1 N NaOH.

TABLE 1 GENERIC NAME, MOLECULAR STRUCTURE, CHEMICAL NAME, TRADE NAME AND MOLECULAR WEIGHT OF THE PHENOTHIAZINE DERIVATIVES STUDIED



| Generic name | *x* | *y* | Chemical name | Trade name | Molecular weight |
|---|---|---|---|---|---|
| Promethazine |  | —H | 10-(2-dimethylaminopropyl)-phenothiazine hydrochloride | Atosil | 320·9 |
| Trimeprazine |  | —H | 10-(2-methyl-3-dimethylaminopropyl)-phenothiazine tartrate | Repeltin | 448·5 |
| Desmethylpromazine |  | —H | 10-(3-methylaminopropyl)-phenothiazine hydrochloride | — | 306·9 |
| Desmethylchlorpromazine |  | —Cl | 2-chloro-10-(3-methylaminopropyl)-phenothiazine hydrochloride | — | 420·9 |
| Perazine |  | —H | 10-[3-(4-methyl-1-piperazinyl)-propyl]-phenothiazine maleate | Taxilan | 571·7 |

*Methods.* The binding of the phenothiazine derivatives to albumin was studied in most experiments with the aid of gel filtration.[5] Experiments were usually performed on a 20 × 1·2 cm column of Sephadex® G-50 fine (Pharmacia) at 22°, equilibrated with the standard buffer solution, the flow rate being maintained at 18 ml/hr 20 ml of the albumin solution containing one or two drugs were loaded on the column. The pore size of the sephadex gel was such as to exclude albumin together with bound drug, which therefore pass rapidly down the column, while admitting free drug which slowly migrates as a separate zone (Fig. 1).

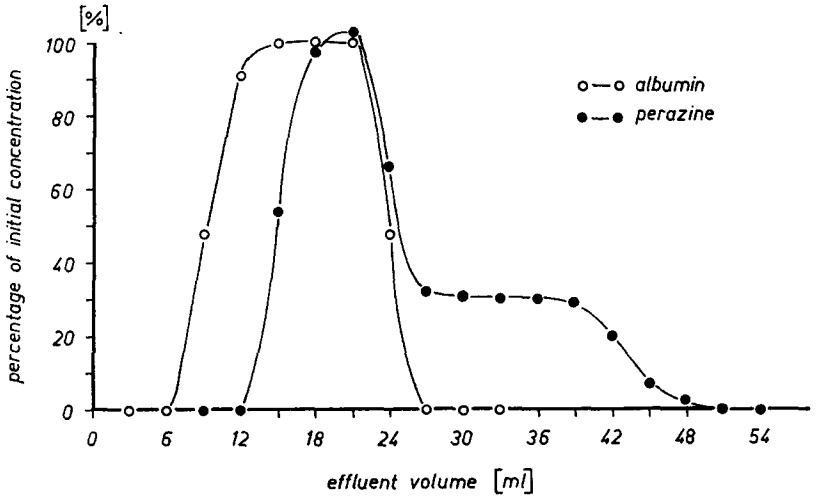



FIG. 1. Elution profile obtained in a gel filtration experiment on a 20 × 1·2 cm column of Sephadex G-50.

A 20-ml sample containing $10^{-4}$ M perazine and 1 g/100 ml bovine serum albumin was loaded on the column. Ordinate: percentage of the total initial concentration of albumin and perazine. Abscissa: effluent volume in ml.

The effluent from the column was divided into fractions of 3 ml. The albumin content was determined refractometrically. After extraction with *n*-heptane the phenothiazine derivatives were measured colorimetrically in 50% sulfuric acid containing 10 mg% $FeCl_3$.[5]

The results of a gel filtration experiment are shown in Fig. 1. Bound drug moves with the velocity of albumin. The plateau region following the albumin zone represents the unbound drug concentration which was in equilibrium with drug–albumin complex in the column. When experiments were performed in series for one substance, only the fractions after the protein zone were assayed for phenothiazine, which in Fig. 1 corresponds to an effluent volume between 25 and 50 ml.

In order to control the gel filtration system several dialysis experiments* were performed for each drug. Sample solution (5 ml) containing albumin and one of the drugs was placed inside the dialysis tubing (Visking; 7 mm inflated diameter) which, after closure, were immersed in standard buffer solution (5 ml) in a glass tube (8 mm inside diameter). The dialysis tubings were agitated up and down mechanically (12 hr, 22°).

* H. Kurz, personal communication.

The displacement of some phenothiazines by acetylsalicylic acid and hydroxyzine was studied by means of gel filtration (Figs. 6 and 7). In these experiments the binding of the phenothiazines to albumin was determined as described above after the displacing agents were added.

For the partitioning, only *n*-octanol saturated with standard buffer and standard buffer saturated with *n*-octanol were used. One to three ml portions of octanol were used with 25-ml portions of standard buffer containing the drug ($10^{-4}$ M). After vigorous shaking on a mechanical shaker for 1 hr and centrifugation, the concentration of the sample in the water layer was determined; sample concentration in the octanol was obtained by difference. The analysis of the concentrations of the partitioned substances were made using a Beckman spectrophotometer DB. The absorbance of the drugs was measured at the following wavelengths: 246 nm for promethazine and trimeprazine, 248 nm for desmethylpromazine and perazine, 250 nm for desmethylchlorpromazine. The partition coefficient was calculated as $P = c_0/c_w$, where $c_0$ is the concentration of the drug in the organic phase and $c_w$ is the concentration of the drug in the aqueous phase. The partition coefficients are reported as mean values from six to twelve single determinations (Table 4).

TABLE 2. SYMBOLS, DIMENSIONS AND METHODS OF ANALYSIS OF THE PARAMETERS USED IN THIS STUDY

| Parameter | Symbol | Dimension | Method of analysis |
|---|---|---|---|
| Total concentration of phenothiazine derivative | $c$ | M | by weight, photometry |
| Concentration of free phenothiazine derivative | $c_f$ | M | gel filtration, dialysis |
| Concentration of bound phenothiazine derivative | $c_b$ | M | $c_b = c - c_f$ |
| Concentration of albumin | $c_a$ | g/100 ml M | by weight, refractometry |
| Percentage of free phenothiazine derivative | $\alpha$ | % | gel filtration, dialysis |
| Percentage of bound phenothiazine derivative | $\beta$ | % | $\beta = 100 - \alpha$ |
| Specific binding capacity | $\bar{r}$ | M/M | $\bar{r} = c_b/c_a$ |
| Regression coefficient | $m$ | — | Fig. 3 |
| Apparent binding constant | $k^*$ | $(10^{-5}\ \mathrm{M})^{1-m}$ | $k = c_b/c_f$, Fig. 3[25] |
| Association constant | $K_1$ | $10^4\ \mathrm{M}^{-1}$ | Scatchard plot, Fig. 4[26] |
| Free binding energy | $\Delta F^\circ$ | cal/M | $\Delta F^\circ = -RT \ln K_1$ |
| Partition coefficient | $P$ | M/M | Partition between *n*-octanol and buffer solution pH 7·40 |
| Logarithm of the calculated partition coefficient | $\Sigma\pi$ | M/M | calculation as described in the text |

Symbols, dimensions and methods of analysis of the values used to characterize the protein binding and the hydrophobic character of the drugs are summarized in Table 2.

## RESULTS

(1) Data of albumin binding: Data which characterize the binding of the phenothiazine derivatives to albumin are summarized in Table 3.

TABLE 3. BINDING OF SEVERAL PHENOTHIAZINE DERIVATIVES TO BOVINE SERUM ALBUMIN

| Phenothiazine derivative | $\beta$* | $m$† | $k$*† | $K_1 \times 10^{-4}$‡ | $-\Delta F°$§ |
|---|---|---|---|---|---|
| Promazine‖ | 49 | 0·915 | 1·12 | 0·9 | 5340 |
| Chlorpromazine‖ | 70 | 0·948 | 2·62 | 2·1 | 5830 |
| Promethazine | 62 | 0·980 | 1·70 | 1·3 | 5550 |
| Trimeprazine | 58 | 0·927 | 1·57 | 1·2 | 5500 |
| Desmethylpromazine | 50 | 0·988 | 1·01 | 0·8 | 5270 |
| Desmethylchlorpromazine | 64 | 0·962 | 1·97 | 1·5 | 5640 |
| Perazine | 75 | 0·975 | 3·13 | 2·1 | 5830 |

* $\beta$ is the percentage of bound drug in a 1% albumin solution with a total concentration $c = 10^{-4}$ M of phenothiazine derivative.

† $m$, the regression coefficient and $k$*, the apparent binding constant were obtained from Fig. 3. See also Table 2.

‡ $K_1$ is the association constant obtained from the Scatchard plot (Fig. 4).

§ $\Delta F°$ is the free binding energy.

‖ The values of these two drugs were obtained in a preliminary study.[18]

A two-factorial analysis of variance was used to test whether the differences in $\alpha$-values of the substances studied in this paper (factor 1) and the differences in $\alpha$-values at raising total concentrations of phenothiazines (factor 2) were statistically significant The corresponding $F$-tests both were significant at the 1 per cent level. The standard deviation of $\alpha$ was $s = 1{\cdot}75$.

Whereas the analysis of variance only gives an over-all evidence of these effects, specified differences between mean $\alpha$-values can be tested by the method of Tukey.[28] Confidence intervals evaluated by the Tukey method are of the form

$$|\bar{x}_i - \bar{x}_j| - T \leq |\mu_i - \mu_j| \leq |\bar{x}_i - \bar{x}_j| + T_P,$$

which means, that the "true difference" $\mu_i - \mu_j$ lies within the specified interval with probability 1 — P. If an absolute difference of two $\alpha$-values exceeds the $T$-value at a specified level of significance it can be considered as statistically significant. The following $T$-values were evaluated: $T = 4{\cdot}16$ (P < 5%), $T = 4{\cdot}99$ (P < 1%).

The protein binding was only slightly influenced by desmethylation of the $N$-atom in the basic side chain of the phenothiazine (promazine → desmethylpromazine; chlorpromazine → desmethylchlorpromazine). On the other hand the albumin binding of the phenothiazines increased significantly when a methyl group was present in the aliphatic part of the side chain (promazine → trimeprazine; → promethazine). Compared with promazine the increase of binding was in both cases nearly the same and apparently did not depend on the number of C-atoms between the two $N$-atoms. The enlargement of the side-chain in the case of perazine (methylated piperazine ring instead of a dimethylamino group) also led to an increase of the protein binding.

(2) Relation between albumin binding and the hydrophobic character of the phenothiazine derivatives: The hydrophobic character of the phenothiazine derivatives should be characterized according to Hansch *et al.*[6] by the partition coefficients between $n$-octanol and buffer solution. We renounced a correction of the partition coefficients with the degree of dissociation. Because most of the phenothiazines used are more than 99 per cent dissociated at pH 7·4 very large correction factors would be obtained and the scatter of the calculated $P$ values would be considerably increased.[7]

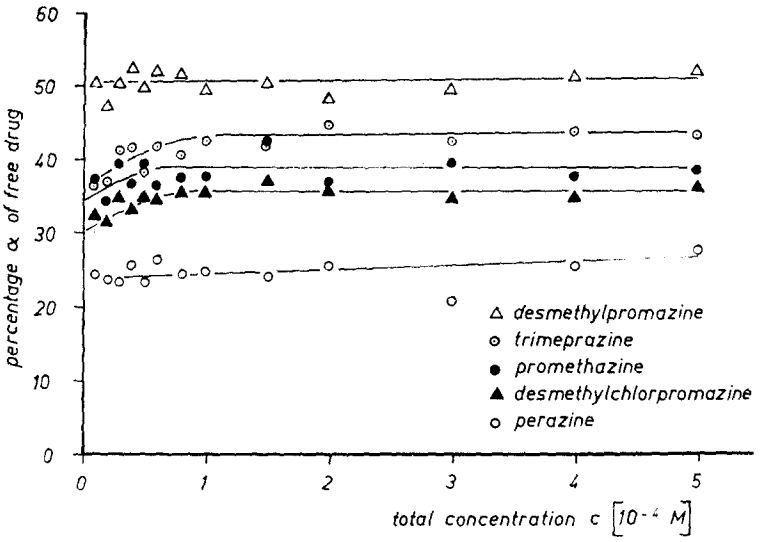



FIG. 2. Binding of varying concentrations of the phenothiazine derivatives to bovine serum albumin. Ordinate: percentage $\alpha$ of free phenothiazine derivative. Abscissa: total concentration $c$ of the phenothiazine derivative ($10^{-4}$ M). Binding measurements were carried out in a 1% albumin solution (pH 7·40; 22°). Each point represents the mean value of two experiments.

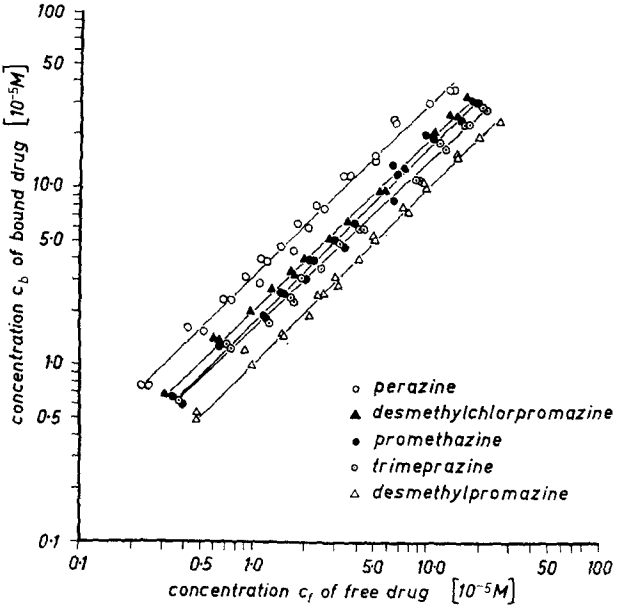



FIG. 3. Binding capacity of a 1% albumin solution for phenothiazine derivatives.
Ordinate: concentration $c_b$ of bound phenothiazine derivative ($10^{-5}$ M). Abscissa: concentration $c_f$ of free phenothiazine derivative ($10^{-5}$ M). Binding measurements were carried out in a 1% albumin solution (pH 7·40; 22°). Each point represents a single experiment. This plot is performed in order to obtain the binding constants $m$ and $k^{*}$.[25] See also Table 3. The equations of the regression lines in the double logarithmic system are for perazine: $y = 0{\cdot}495 + 0{\cdot}975x$; $r = 0{\cdot}996$; $n = 26$; desmethylchlorpromazine; $y = 0{\cdot}295 + 0{\cdot}962x$; r = 0·999; $n = 26$; promethazine: $y = 0{\cdot}230 + 0{\cdot}980x$; $r = 0{\cdot}994$; $n = 32$; trimeprazine: $y = 0{\cdot}196 + 0{\cdot}927x$; $r = 0{\cdot}998$; $n = 26$; desmethylpromazine: $y = 0{\cdot}004 + 0{\cdot}988x$; $r = 0{\cdot}998$; $n = 26$. $r$ = correlation coefficient, $n$ = number of experiments.

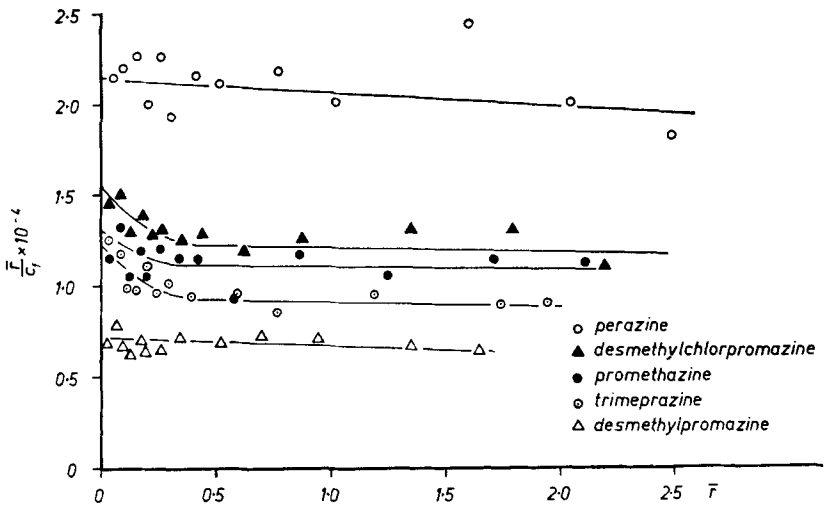



FIG. 4. Scatchard plot of the binding of the phenothiazine derivatives to bovine serum albumin. Ordinate: $r/c_f$ in $10^4$ $M^{-1}$. $c_f$ = molar concentration of free phenothiazine derivative in the albumin solution. Abscissa: $\bar{r}$ = number of moles of phenothiazine per mole of albumin. All measurements were made in 1% albumin solution (pH 7·40; 22°). Each point represents the mean value of two single experiments. For total binding constant $K_1$ see Table 3.

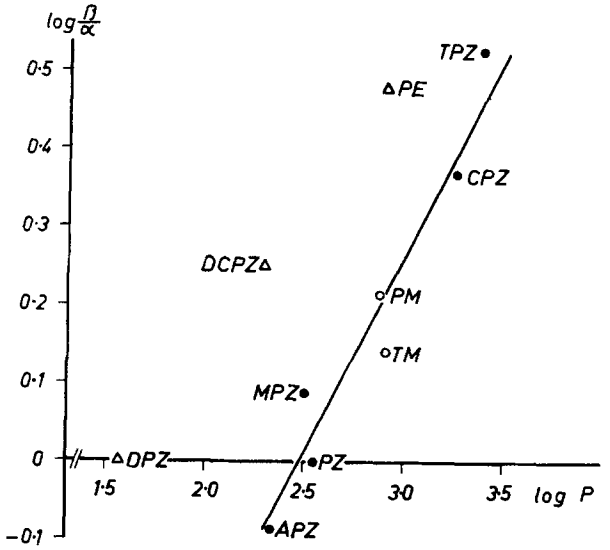



FIG. 5. Relationship between albumin binding and partition coefficients of phenothiazine derivatives, Ordinate: log $\beta/\alpha$· $\beta$ = per cent phenothiazine derivative bound, $\alpha$ = per cent free. Binding measurements were made in a 1% bovine serum albumin solution (pH 7·40; 22°). Abscissa: log $P$. $P$ = partition coefficient between *n*-octanol and buffer solution, pH 7·40. APZ = acepromazine, PZ = promazine, MPZ = methopromazine, TM = trimeprazine, PM = promethazine, CPZ = chlorpromazine, TPZ = triflupromazine, PE = perazine, DCPZ = desmethylchlorpromazine, DPZ = desmethylpromazine. The equation of the regression line: log $\beta/\alpha = -1{\cdot}3131 + 0{\cdot}5299$ log $P$ is calculated only for APZ, PZ, MPZ, CPZ and TPZ.

Moreover we had some doubt whether the p$K$-values published for phenothiazines by different authors could be compared.[17,24]

A measure for the change of the hydrophobic character of a substance after replacement of one hydrogen is the substituent constant $\pi$ defined by Hansch *et al.*[6] as $\pi = \log P_s - \log P$ where $P$ is the partition coefficient of a parent compound and $P_s$ is that for a derivative. $\Sigma\pi$ values were calculated from the measured partition coefficient of promazine and published substituent constant $\pi$ values (see example below). Fujita *et al.*[8] have determined several substituent constants for benzene derivatives. We used these constants for the calculation of $\Sigma\pi$ values of our substances (Table 4).

TABLE 4. COMPARISON OF OBSERVED ($P$, log $P$) AND CALCULATED ($\Sigma\pi$) PARTITION COEFFICIENTS OF SEVERAL PHENOTHIAZINE DERIVATIVES

| No. | Phenothiazine derivative* | $P$† | log $P$ | $\Sigma\pi$‡ |
|---|---|---|---|---|
| 1 | Promazine | 357 ± 16 (8) | 2·55 | — |
| 2 | Acepromazine | 218 ± 4 (9) | 2·34 | 2·27 |
| 3 | Methopromazine | 317 ± 21 (8) | 2·50 | 2·67 |
| 4 | Chlorpromazine | 1814 ± 107 (12) | 3·25 | 3·31 |
| 5 | Triflupromazine | 2461 ± 257 (10) | 3·39 | 3·62 |
| 6 | Promethazine | 757 ± 23 (6) | 2·88 | 2·37 |
| 7 | Trimeprazine | 813 ± 31 (6) | 2·91 | 2·87 |
| 8 | Desmethylpromazine | 38 ± 1 (6) | 1·58 | 1·84 |
| 9 | Desmethylchlorpromazine | 200 ± 6 (6) | 2·30 | 2·55 |
| 10 | Perazine | 803 ± 36 (6) | 2·90 | 3·15 |

* Drugs 1–5 were examined in a preliminary paper.[14]

† The partition coefficients $P$ were determined between *n*-octanol and buffer solution pH 7·40. Mean values ± S.E.M., number of experiments in parenthesis.

‡ The calculated $\Sigma\pi$ values were obtained by summation of group $\pi$ values, and log $P$ of promazine as explained in the text.

The following example illustrates the calculation of the $\Sigma\pi$ value of chlorpromazine:

$$\Sigma\pi_{\text{chlorpromazine}} = \log P_{\text{promazine}} + \pi_{\text{CL}} = 2{\cdot}55 + 0{\cdot}76 = 3{\cdot}31.$$

The function log $\beta/\alpha$, where $\beta$ is the percentage of phenothiazine bound and $\alpha$ is the percentage of free drug (total concentration was $10^{-4}$ M) is used for correlation of the albumin binding results with log $P$. We preferred $\beta/\alpha$ for this type of correlation because it is directly analogous to an organic solvent–water partition coefficient. The following equation was calculated from the data for five phenothiazine derivatives substituted in position 2 of the phenothiazine nucleus: $\log \beta/\alpha = -1{\cdot}3131 + 0{\cdot}5299 \log P$ (correlation coefficient $r = 0{\cdot}9760$). With the aid of this equation and of $\Sigma\pi$ values from Table 4 it is possible to calculate the albumin binding of the phenothiazine derivatives surprisingly exactly. However, a large discrepancy is found between observed and calculated $\alpha$ values for the desmethylderivatives, perazine and promethazine (Table 5).

(3) Displacement of some phenothiazines from their albumin binding: Hydroxyzine was able to displace perazine from its albumin binding sites, but the fraction of free chlorpromazine was not enhanced in the albumin solution (Fig. 6, Table 6).

TABLE 5. COMPARISON OF OBSERVED AND CALCULATED α VALUES OF SEVERAL PHENOTHIAZINE DERIVATIVES

| No.* | Phenothiazine derivatives | α (%) observed† | α (%) calculated‡ |
|---|---|---|---|
| 1 | Promazine | 51 | — |
| 2 | Acepromazine | 55 | 53 |
| 3 | Methopromazine | 45 | 44 |
| 4 | Chlorpromazine | 30 | 27 |
| 5 | Triflupromazine | 23 | 20 |
| 6 | Promethazine | 38 | 53 |
| 7 | Trimeprazine | 42 | 38 |
| 8 | Desmethylpromazine | 50 | 69 |
| 9 | Desmethylchlorpromazine | 36 | 48 |
| 10 | Perazine | 25 | 31 |

* Drugs 1–5 were examined in preliminary studies.[14, 27]

† Binding studies were carried out in a 1% bovine serum albumin solution (pH 7·40; 22°).

‡ Calculated α values were obtained from $\Sigma\pi$ values (Table 4) and the equation $\log \beta/\alpha = -1{\cdot}3131 + 0{\cdot}5299\ \Sigma\pi$ of the regression line in Fig. 5.

Perazine was displaced by acetylsalicylic acid to a greater extent than chlorpromazine whereas the percentage of free desmethylchlorpromazine did not significantly rise after addition of acetylsalicylic acid to the albumin solution (Fig. 7, Table 6).

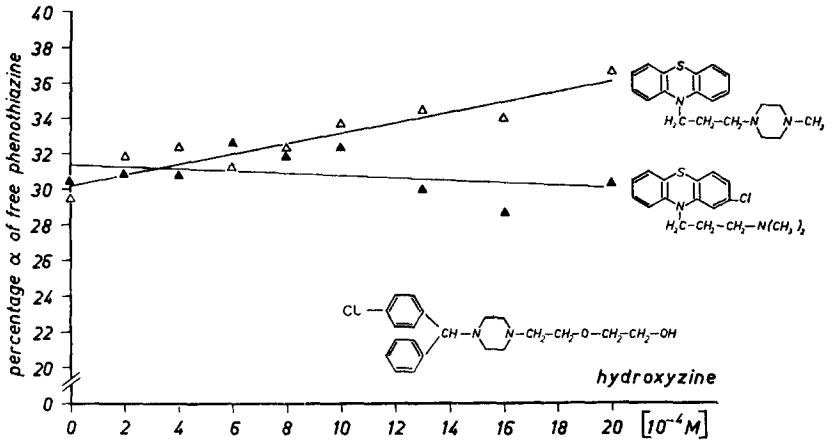



FIG. 6. Influence of hydroxyzine on the binding of perazine and chlorpromazine to bovine serum albumin.

Ordinate: percentage α of free perazine and chlorpromazine respectively in the albumin solution. Abscissa: total concentration of hydroxyzine ($10^{-4}$ M). All measurements were made in 1% albumin solution (pH 7·40; 22°) containing $10^{-4}$ M perazine or chlorpromazine and varying concentrations of hydroxyzine. Each point represents the mean value of two single experiments. For statistical evaluation see Table 6.

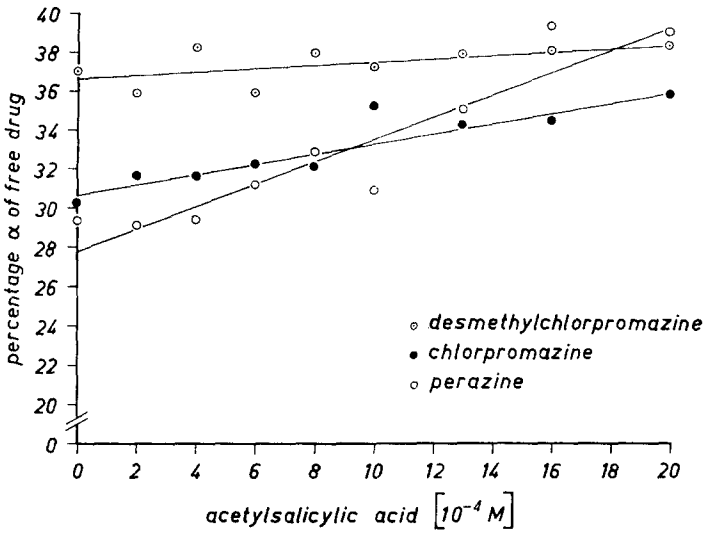



FIG. 7. Displacement of perazine, chlorpromazine and desmethylchlorpromazine by acetylsalicylic acid from binding to bovine serum albumin.

Ordinate: percentage α of free phenothiazine derivative. Abscissa: total concentration of acetylsalicylic acid ($10^{-4}$ M). All measurements were made in a 1% albumin solution (pH 7·40, 22°) containing one of the phenothiazine derivatives ($10^{-4}$ M) and varying concentrations of acetylsalicylic acid. Each point represents the mean value of two single experiments. For statistical evaluation see Table 6.

## DISCUSSION

Several authors demonstrated a correlation between the hydrophobic character and protein binding of low molecular weight substances,[6,7,9–14] if the hydrophobic character of the substances was characterized according to Hansch[6] between *n*-octanol and water. These results suggest that hydrophobic interactions[15,16] play an important role in protein binding of organic compounds. However, a good correlation between protein binding and partition coefficients can be shown only for substances of structurally related groups.[7] Hence not only hydrophobic interactions can be present in protein binding of organic molecules, but other binding mechanisms such as ionic binding, hydrogen binding or steric effects, etc. must be also involved.[7,14]

Several observations suggest that phenothiazines are bound to albumin by hydrophobic interactions of their benzene rings:

(1) The albumin binding of the phenothiazine derivatives with substituents in position 2 of the phenothiazine nucleus increases with their hydrophobic character.[14] The substituents seem not to influence the degree of dissociation of the dimethylamino group significantly.[17]

(2) Simple aromatic substances, like benzoic acid or aniline are able to displace phenothiazines from their binding sites on the albumin molecule.[18,19] It is apparent from these studies that competition occurs between the benzene rings which are part of the structure of all substances used.

(3) A comparative study of the albumin binding of promazine and chlorpromazine revealed that only one of the phenothiazine benzene rings could be attached to albumin.[18]

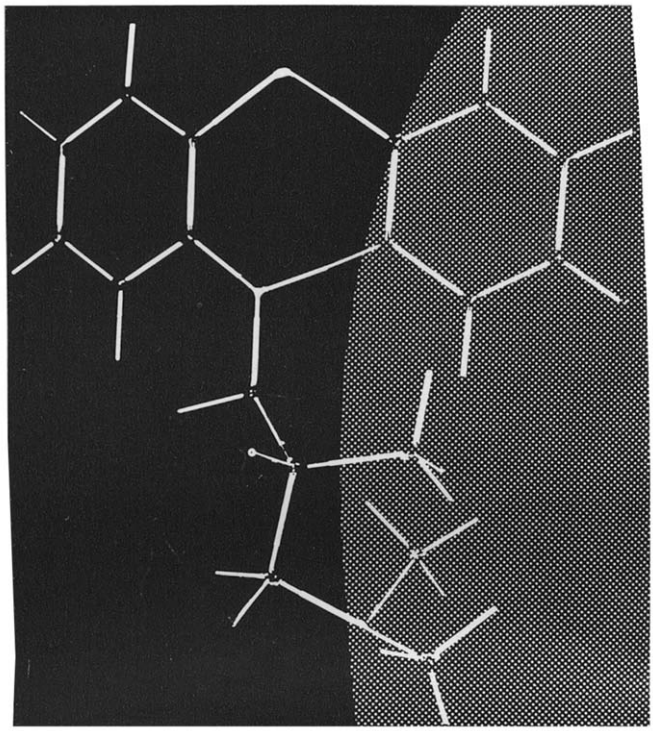


FIG. 8. Model of trimeprazine attached to the albumin surface.
One benzene ring, the methyl group in the aliphatic side chain, and the dimethylamino group are associated with the albumin molecule. The dotted zone represents the albumin molecule.

B.P.—*facing page* 994

TABLE 6. DISPLACEMENT OF CHLORPROMAZINE, DESMETHYLCHLORPROMAZINE, AND PERAZINE FROM THEIR ALBUMIN BINDING BY HYDROXYZINE AND ACETYLSALICYLIC ACID

| Combination No. | | Equation of the regression line* | $n^c$† | $r^d$‡ | Significance of $r$ | Comparison of the regression coefficients |
|---|---|---|---|---|---|---|
| 1 | Perazine (A)§<br>Hydroxyzine (B) | $y = 30{\cdot}20 + 0{\cdot}30x$ | 18 | 0·7279 | $P < 1\%$ | 1 with 2:<br>$P < 1\%$ |
| 2 | Chlorpromazine (A)<br>Hydroxyzine (B) | $y = 31{\cdot}40 - 0{\cdot}07x$ | 18 | −0·1990 | not significant | |
| 3 | Perazine (A)<br>Acetylsalicylic acid (B) | $y = 27{\cdot}89 + 0{\cdot}58x$ | 18 | 0·8696 | $P < 1\%$ | 3 with 4:<br>$P < 1\%$ |
| 4 | Chlorpromazine (A)<br>Acetylsalicylic acid (B) | $y = 30{\cdot}73 + 0{\cdot}26x$ | 18 | 0·8598 | $P < 1\%$ | 4 with 5:<br>$P < 5\%$ |
| 5 | Desmethylchlorpromazine (A)<br>Acetylsalicylic acid (B) | $y = 36{\cdot}56 + 0{\cdot}09x$ | 18 | 0·3309 | not significant | |

* The equations presented here correspond to the regression lines in Figs. 6 and 7.
† $n$ = number of single experiments.
‡ $r$ = correlation coefficient.
§ The phenothiazine derivative displaced is designated with (A), the displacing drug with (B).

It was demonstrated for several different substances that protein binding increases with each further $CH_3$ or $CH_2$ group in the molecule.[7,20–23] Accordingly, the stronger protein binding of trimeprazine in comparison to promazine agreed very well with findings described in the literature. However, the amount of bound promethazine is also higher than that of promazine and this cannot be understood immediately from the results described above. Promethazine differs from promazine only by the position of the dimethylamino group in the propyl side chain (see Table 1), but the length of the aliphatic side chain is the same in both drugs. In addition, promethazine and trimeprazine, which differ by a $CH_2$ group from each other, show about the same affinity to albumin (Table 3). This might be explained by supposing that the aliphatic chain between the two *N*-atoms is not in a position to contribute to the binding of the phenothiazine derivatives used (see Fig. 8). Against that, a methyl group in the aliphatic side chain may lie sufficiently close to the surface of the albumin molecule to intensify the binding of the drug molecule by hydrophobic interaction (Fig. 8). Thus the same degree of binding of promethazine and trimeprazine on the one hand and about the same increase of binding of these two drugs in comparison to promazine on the other would be explicable.

Several authors have shown that the protein binding of a substance decreased after desmethylation or desacetylation of the dimethylamino group.[11,22] Therefore, one would have expected a lower degree of binding of the desmethylderivatives than of their parent compounds. But after desmethylation of promazine and chlorpromazine the binding of these substances to albumin was only little changed (Table 3) whereas the partition coefficients diminished to one-tenth of those of the parent compounds (Table 4). Similar results have been reported for imipramine and desipramine.[14] This discrepancy of protein binding and hydrophobic character after desmethylation of the phenothiazine derivatives is clearly shown in Fig. 5 and in the $\alpha$ values calculated in Table 5. The calculated $\alpha$ values of desmethylpromazine, desmethylchlorpromazine, and perazine, differ considerably from those experimentally determined. Therefore the binding of the side chain in position 10 of the phenothiazine nucleus must be governed by other factors in addition to hydrophobic interactions. The structure of the side chain suggests some evidence of ionic binding. This idea is supported by the p*K*-values of the monodesmethyl metabolites, which were higher than those of the parent compounds.[17,24] It appears that after desmethylation ionic binding forces increase to the same extent as hydrophobic binding forces decrease, or, the methyl groups of the *N*-atom do not participate directly in binding at all. Furthermore, didesmethylpromazine and didesmethylchlorpromazine still show the same degree of binding as promazine and chlorpromazine respectively.*

The participation of the side chain in the total binding of the phenothiazine derivatives is also indicated in the displacing experiments (Figs. 7, 8; Table 6). Hydroxyzine which like perazine possesses a piperazine ring displaces perazine but not chlorpromazine from its albumin binding sites. Acetylsalicylic acid can compete with the benzene rings of the phenothiazine ring system. It provides additional support for the occurrence of stronger ionic binding of desmethylchlorpromazine to albumin, since chlorpromazine and not desmethylchlorpromazine is displaced significantly from albumin (Table 6; Fig. 7). However, the results obtained from such displacing

* J. Krieglstein and F. Lier, to be published.

experiments may be complicated by additional factors, often working contrary to each other,[19] so that clear results are scarcely to be expected from this technique (Fig. 8).

The results obtained from these and from previous studies[14,18,19,27] allow the following assumptions of the binding of phenothiazine derivatives to albumin (Fig. 8). Only one of the phenothiazine benzene rings is inserted into a hydrophobic crevice in the albumin,[18] while the basic dimethylamino group is associated with a negatively charged group on the protein surface. Between these two parts of the drug molecule attached to the albumin the aliphatic side chain is extended and obviously does not directly take part in binding. Only a further substituent, such as the methyl group of trimeprazine, is able to enhance the binding of the molecule by hydrophobic interaction in this case. The methyl groups on the nitrogen might also be bound by hydrophobic interactions.

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