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Adsorption from Solution, Permeation through Cellulose Membrane, Partition Coefficient and Surface Tension of Several Antidepressants and Phenothiazines[1,2]

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The physico-chemical properties of several antidepressants were studied in comparison with those of tranquilizing phenothiazines, intending to obtain some information useful to understanding of biopharmaceutical and pharmacological mechanisms of antidepressants.

The adsorption isotherms of drug by carbon black (CB) were all observed in Langmuir type. The results suggested that the aromatic rings of drug molecules participate in the adsorption and that there is no special difference in behavior at the surface of CB between antidepressants and phenothiazines. Radii of drug molecule were evaluated by various methods, *i. e.*, r_1 from the molecular volume, r_2 from the assembled Stuart type molecular model, r_3 from the area occupied by one molecule at the surface of CB in the adsorption, and r_4 from the apparent diffusion constant in the cellulose membrane permeation, giving a good correlation between r_1 and r_4. Then the permeation through cellulose membrane was considered to belong to the simple diffusion through small pores. Contrary to such a previous investigation as in the case of barbiturates, there was no clear relationship among the parameters to indicate the hydrophobicity, *i. e.*, the adsorbability onto hydrophobic adsorbent CB, partition coefficient between *n*-octanol or chloroform and buffer solution, and the surface tension. It was found that both lytic effect of histamine-contraction and inhibitory effect of adrenaline-contraction have relation to the adsorbability onto CB from aqueous solution.

There have been reported a variety of data concerning phenothiazines as tranquilizers, including physico-chemical investigations.[4] Regarding antidepressants, which belong to neuroleptics and have the actions only in man, few investigations have been done concerning their pharmacological activities in animal, biopharmaceutical or physico-chemical properties. Although the relation between the chemical structure and pharmacological activities of antidepressants has been discussed by Pöldinger[5] and Camerman,[6] the clear elucidation has never been given. For example, while both antidepressants imipramine and amitriptyline have tricyclic moiety in molecule and are similar in structure to phenothiazines, the pharmacological activities are different. Furthermore, the combinations of antidepressants with phenothiazines have often been used clinically to depressive patients accompaning anxiety and enmity.

In the present study, therefore, the physico-chemical properties of several antidepressants were studied in comparison with those of tranquilizing phenothiazines, intending to obtain

1) This paper forms Part XXIX of "Physico-chemical Approach to Biopharmaceutical Phenomena." Preceding paper, Part XXVIII: J. Noda, H. Sakamoto, and T. Nagai, *Chem. Pharm. Bull.* (Tokyo), **23**, 445 (1975).

2) A part of this work was presented at the 94th Annual Meeting of Pharmaceutical Society of Japan, Sendai, April 1974.

3) Location: *Ebara-2-4-41, Shinagawa-ku, Tokyo, 142, Japan.*

4) *a*) H. Nogami, T. Nagai, and N. Nambu, *Chem. Pharm. Bull.* (Tokyo), **18**, 1643 (1970); *b*) N. Nambu, T. Nagai, and H. Nogami, *ibid.*, **19**, 808 (1971); *c*) *Idem*, *ibid.*, **19**, 1058 (1971); *d*) N. Nambu and T. Nagai, *ibid.*, **20**, 2463 (1972); *e*) *Idem*, *ibid.*, **22**, 1405 (1974).

5) W. Pöldinger, *Arzneim. -Forsch. Drug Res.*, **9**, 129 (1966).

6) A. Camerman and N. Camerman, *Science*, **168**, 1457 (1970).

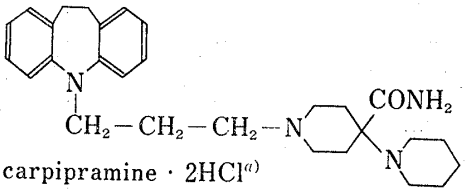
some informations useful to understanding of biopharmaceutical and pharmacological mechanisms of antidepressants.

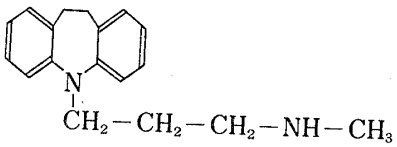
Experimental

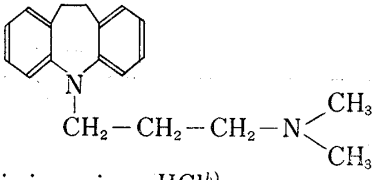
Materials——Carbon black (CB) marketed as "Seisei Shirasagi" by Takeda Chemical Ind., Ltd. was used after the activation by heating. The specific surface area of CB was 1084 m^2/g according to BET method. The cellulose membrane (Visking tubing) used was the same as that in the previous paper.[4b] Antidepressants and phenothiazines used are listed in Table I.

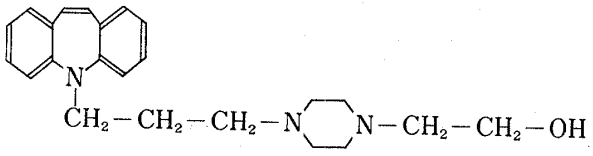
Table I. Drugs Used in This Study

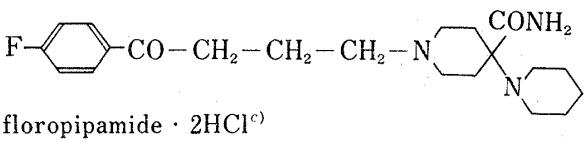
Antidepressants

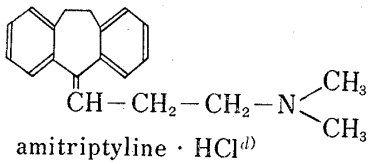

carpipramine · 2HCl[a]


desipramine · HCl[b]


imipramine · HCl[b]


opipramol · 2HCl[b]


floropipamide · 2HCl[c]


amitriptyline · HCl[d]

Phenothiazines

promazine·HCl,[e] chlorpromazine·HCl,[a] chlorpromazine-sulfoxide·HCl,[a] isothipendyl·HCl[f]

a) supplied by Yoshitomi Pharmaceutical Co., Ltd.
b) supplied by Fujisawa Pharmaceutical Co., Ltd.
c) supplied by Eizai Co., Ltd.
d) supplied by Yamanouchi Pharmaceutical Co., Ltd.
e) supplied by Banyu Pharmaceutical Co., Ltd.
f) supplied by Sumitomo Chemical Co., Ltd.

Quantitative Determination of Drugs——The phenothiazines used were determined according to ultraviolet (UV) absorption method using a Hitachi 323 spectrophotometer at the same wavelength as already reported.[4a] The antidepressants used were also determined according to UV absorption method using the same spectrophotometer at following wavelengths: carpipramine, 252 mμ; desipramine, 252.5; imipramine, 253; opipramol, 255; floropipamide, 248; amitriptyline, 240.

Determination of the Amount Adsorbed by Batch Method——This was done at pH 6.00 at 30° in the same way as described in the previous paper.[4a]

Determination of the Permeability through Cellulose Membrane——This was done at pH 6.00 and 7.00 at 30° in the same way as described in the previous paper.[4b]

Determination of Partition Coefficient——Ten ml of 1/30 M phosphate buffer solution (pH 6.00) containing 10^{-3} M of the drug was mixed with 5 ml *n*-octanol or 3—4 ml chloroform and shaken for 2.5 hr at 25°, and then the concentration in the aqueous layer was determined in the same way as described above.

Measurement of Surface Tension——The surface tension was measured by Wilhelmy method using a Kyowa Electro Surface Balance (Kyowa Scientific Co., Ltd.) for 1/30 M phosphate buffer solution (pH 6.00) containing 10^{-3} M of each drug at 30°.

Results and Discussion

Adsorption from Aqueous Solution

The adsorption isotherms of drugs by CB were all observed in Langmuir type as shown in Fig. 1, then Langmuir constants a and b were calculated by Langmuir's equation.[4a] The values obtained are shown in Table II. There was no clear difference in the saturated amount a between antidepressants and phenothiazines. Generally, b values of antidepressants were smaller than those of phenothiazines except for desipramine. The chemical structure of floropipamide only is not tricyclic among them, as was considered to be the reason why its b value was about one-tenth of the others. This result might suggest that aromatic rings of drug molecules participate in the adsorption by CB. The b value of isothipendyl was smaller than those of other phenothiazines, suggesting that the rings of both compounds participate in the adsorption by CB and the hydrophobicity of benzene ring is larger than that of pyridine ring. The a values of imipramine and amitriptyline were larger than those of desipramine and imipramine, respectively. This result might suggest that a drug molecule becomes more hydrophobic by the substitution of methyl group for the hydrogen atom in the former or that of diphenylmethane group for the diphenylamine group in the latter.

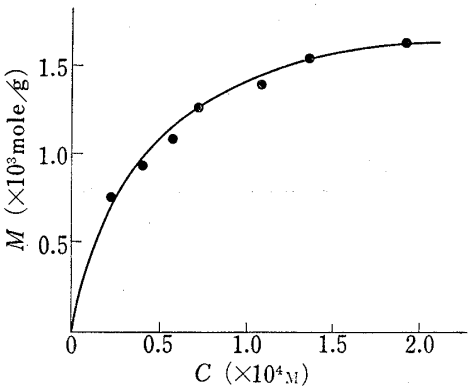


Fig. 1. Adsorption Isotherm of Amitriptyline by Carbon Black from Aqueous Solution at 30°

M: amount adsorbed
C: equilibrium concentration

Table II. Langmuir Constants a and b in the Adsorption by Carbon Black

Drugs	a ($\times 10^3$ mole/g)	b ($\times 10^{-4}$M^{-1})
Antidepressants		
Carpipramine	1.54	2.36
Desipramine	1.36	4.70
Imipramine	1.80	1.48
Opipramol	1.51	1.77
Floropipamide	1.15	0.17
Amitriptyline	2.05	2.07
Phenothiazines		
Promazine	1.70	3.36
Chlorpromazine	1.70	4.37
Chlorpromazine-sulfoxide	1.13	3.18
Isothipendyl	1.30	2.23

The area occupied by one molecule on the surface of CB was calculated from Langmuir constant a and the specific surface area of CB. The results obtained are shown in Table III.

As shown in Table III, the area of each drug was almost 100Å^2 in the plain buffer solution, and decreased with the addition of sodium chloride as might be due to the dehydration at the surface of CB. The projected area of each drug molecule obtained using the assembled Stuart type model was 63—85Å^2 as shown in Table III and was smaller than the value mentioned above. Vilallonge[7] mentioned that "from a study of monolayers adsorbed at the air/0.1M HCl interface, the following values for the area occupied by a molecule at great surface pressures have been found, promazine 66Å^2 and imipramine 50Å^2." In the adsorption from aqueous solution by CB, water molecule also may be adsorbed on the surface of CB, giving the large area compared with that obtained by model or at the air/0.1M HCl interface.

In the cases of local anesthetics, the area occupied by one molecule on cell membrane obtained *in vitro* differs from that in the cases of general anesthetics, and this fact is considered to be closely related to the difference in actions between both anesthetics.[8] In the present

7) F. Vilallonge, E. Fried, and J. A. Izquierdo, *Arch. Intern. Pharmacodyn.*, **130**, 260 (1961).
8) P. Seeman, *Pharmacological Reviews*, **24**, 583 (1972).

Table III. The Area Occupied by One Molecule in the Adsorption by Carbon Black and the Cross-sectional Area Obtained by Assembled Stuart Type Molecular Model (Å²)

	In the adsorption (plain buffer)	In the adsorption (with 1 M NaCl)	By model
Antidepressants			
Carpipramine	116.9	81.5	77.1
Desipramine	132.4	91.4	76.3
Imipramine	100.0	94.2	65.2
Opipramol	119.2	119.2	84.4
Floropipamide	156.5	162.2	74.7
Amitriptyline	88.0	101.1	76.9
Phenothiazines			
Promazine	105.9	79.0	68.9
Chlorpromazine	106.2	83.0	81.6
Chlorpromazine-sulfoxide	159.3	146.4	81.6
Isothipendyl	138.5	113.2	63.2

study, however, there was no clear difference in the area occupied by one molecule between antidepressants and phenothiazines, suggesting that there is no special difference in behavior at the surface of CB between these two groups.

Permeation through Cellulose Membrane

The values of cellulose membrane permeability of each drug at pH 6.00 and 7.00 are shown in Table IV. P is the permeability constant and D_{app} is the apparent diffusion constant.[9]

Table IV. Permeability Constant P and Apparent Diffusion Constant D_{app} through Cellulose Membrane at 30° (membrane constant: 0.042; volume of solution: $\pi \times 2.15^2$ cm²; thickness of the membrane: 8.6×10^{-3} cm; initial concentration: 1×10^{-3} M)[4b]

Drugs	pH 6.00		pH 7.00	
	$P \times 10^7$ (cm²/sec)	$D_{app} \times 10^6$ (cm²/sec)	$P \times 10^7$ (cm²/sec)	$D_{app} \times 10^6$ (cm²/sec)
Antidepressants				
Carpipramine	1.10	2.62	1.79	4.27
Desipramine	1.89	4.50	1.60	3.81
Imipramine	1.83	4.45	2.23	5.58
Opipramol	1.29	3.07	1.72	4.10
Floropipamide	1.54	3.67	1.85	4.41
Amitriptyline	1.85	4.40	1.61	3.84
Phenothiazines				
Promazine	1.60	3.81	1.67	3.97
Chlorpromazine	1.73	4.12	1.53	3.64
Chlorpromazine-sulfoxide	1.51	3.60	1.94	4.62
Isothipendyl	2.72	6.48	1.89	4.50

each value is the mean of three experimental runs

The order of D_{app} was 10^{-6} and there was no clear difference in D_{app} between antidepressants and phenothiazines. The cellulose membrane permeability constant P was different between

9) According to M. Nakagaki (ed.), "Yakubutsu no Seitainai Iko (Drug Transport in Body)", Nankodo, Tokyo, 1969, there is given the following equation: $P=fbD$, where P is the permeability constant, f membrane constant, b partition coefficient. However, it was difficult to determine the exact value of b in the present system, and thus it seemed reasonable to take $D_{app}=bD$.

pH 6.00 and 7.00 without any clear relationship. This result might be explained on considering that the interaction of drugs with cellulose membrane has no relation with the pH in bulk solution. D_{app} of floropipamide did not differ especially from those of others, though only this compound is not tricyclic and takes a long conformation, and thus is expected to have a large D_{app} value on the assumption that the permeation through cellulose membrane is a simple diffusion process through pores of the membrane.

Radii of Drug Molecules Evaluated by Various Methods

Radii of drug molecules evaluated by various methods are shown in Table V.

TABLE V. Radii of One Molecule Obtained by Various Methods (Å)

Drugs	r_1	r_2	r_3	r_4
Antidepressants				
Carpipramine	5.98	4.95	6.10	9.97
Desipramine	5.05	4.93	6.49	5.80
Imipramine	5.18	4.55	5.64	5.87
Opipramol	5.75	5.18	6.16	8.51
Floropipamide	5.59	4.88	7.06	7.12
Amitriptyline	5.16	4.95	5.29	5.94
Phenothiazines				
Promazine	5.12	4.68	5.81	6.86
Chlorpromazine	5.20	5.10	5.81	6.34
Chlorpromazine-sulfoxide	5.23	5.10	7.12	7.26
Isothipendyl	4.77	4.49	6.64	4.03

correlation coefficient: r_1—r_2, 0.529; r_1—r_3, 0.049; r_1—r_4, 0.941; r_2—r_3, 0.110; r_2—r_4, 0.580; r_3—r_4, 0.030

r_1 is the radius calculated from the molecular volume at boiling point on the assumption that the molecule is spherical. r_2 is the radius evaluated from the projected area obtained using the assembled Stuart type molecular model on the assumption that the projection of the molecule is circular. r_3 is the radius calculated from the area occupied by one molecule at the surface of CB in the adsorption from aqueous solution on the assumption that the projection of the molecule is circular. r_4 is the radius calculated according to Stokes-Einstein's equation from the apparent diffusion constant D_{app} in the cellulose membrane permeation. Radii evaluated by various methods were all several Å and were reasonable on the consideration of the size of drug molecules.

Calculating the correlation coefficient among these radii, a clear relation existed only in r_1—r_4, that is, between the radius calculated from molecular volume and that in cellulose membrane permeation, having the correlation coefficient 0.941. The smaller the size of the molecule, the larger was the apparent diffusion constant in the cellulose membrane permeation, showing the permeation belongs to the simple diffusion process through small pores.

Relation between Hydrophobicities of Drug Molecules

Glasser[10] mentioned that the hydrophobic interaction plays an important role in the binding of antidepressants to protein. Kitler[11] also mentioned that antidepressants show a large interfacial tension lowering activity in pH 2.00 buffer/cyclohexane system, although this extent is not larger than that of phenothiazines. The larger the interfacial tension lowering activity, the larger is the surface potential and also the transferring tendency to artificial lipid liquid/aqueous interface, and thus this parameter has often been used to indicating the hydrophobicity. Additionally, the adsorbability onto hydrophobic adsorbent

10) H. Glasser and J. Krieglstein, *Naunyn-Schmiedebergs Arch. Pharmakol.*, **265**, 321 (1970).
11) M.E. Kitler and P. Lamy, *Pharm. Acta Helv.*, **46**, 483 (1971).

CB, partition coefficient between *n*-octanol and chloroform/buffer solution have often been used as an indicator of the hydrophobicity. In the present study, therefore, the relationship was studied among these parameters. The results obtained are shown in Table VI.

Table VI. Langmuir Constant *a* in the Adsorption by Carbon Black, Partition Coefficient and Surface Activity

Drugs	$a^{a)}$ ($\times 10^3$ mole/g)	P.C.$^{b)}$ (Octanol)	P.C.$^{c)}$ (Chloroform)	$\Delta\pi^{d)}$
Antidepressants				
Carpipramine	1.54	4.43×10	1.40×10^3	3.35
Desipramine	1.36	9.4×10^{-1}	3.00×10	3.58
Imipramine	1.80	1.29×10	2.24×10^2	2.56
Opipramol	1.51	1.02×10^2	8.63×10	2.91
Floropipamide	1.15	9.0×10^{-1}	4.00	2.18
Amitriptyline	2.05	2.57×10	2.83×10^2	3.01
Phenothiazines				
Promazine	1.70	1.29×10	2.76×10^2	3.38
Chlorpromazine	1.70	7.45×10	4.64×10^2	3.02
Chlorpromazine-sulfoxide	1.13	1.5×10^{-1}	9.62	1.62
Isothipendyl	1.30	4.35	7.57×10	−0.17

a) Langmuir constant in the adsorption by carbon black
b) partition coefficient in *n*-octanol/phosphate buffer at pH 6.00 system
c) partition coefficient in chloroform/phosphate buffer at pH 6.00 system
d) log value of surface tension lowering of 10^{-3}M of each drug in phosphate buffer at pH 6.00
Correlation coefficient: r_3-P.C. (octanol), −0.381; r_3-P.C. (chloroform), −0.804; $r_{a-\Delta\pi}$, −0.504

The values of the correlation coefficient between these parameters are shown at the foot of Table VI, but there was no clear relation between them. In the cases of barbiturates,[12] for example, there was a clear relation between these parameters. In order to give some explanation for these circumstances, much more informations from biological sides may be required, which may be obtained in the future.

Relation between Physico-chemical Properties and Pharmacological Data

Ahtee[13] and Theobald[14] studied pharmacological properties of antidepressants and phenothiazines *in vitro*. The relation between the adsorbability by CB (represented by Langmuir constant *a*) and the lytic effect of histamine-contraction[14] and the inhibitory effect of adrenaline-contraction[14] of antidepressants are shown in Fig. 2. Regarding both the lytic and the inhibitory effects, as the relative concentration to give a certain activity increased, the adsorbability decreased. The size of data was only four, but there was considered to be some relation between the adsorbability and the pharmacological effect.

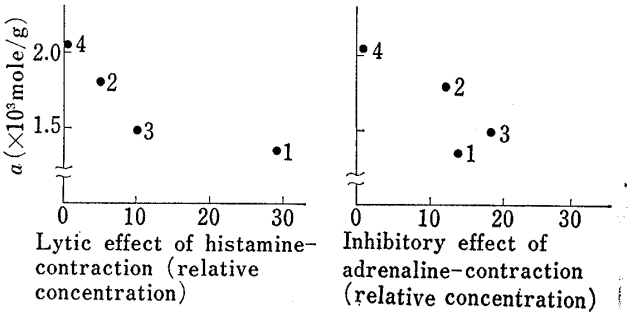


Fig. 2. Relationship between Adsorbability Represented by Langmuir Constant *a* and Biological Activities by Theobald[14]

1. desipramine; 2. imipramine; 3. opipramol; 4. amitriptyline

12) H. Nogami, T. Nagai, and H. Uchida, *Chem. Pharm. Bull.* (Tokyo), **17**, 168 (1969).
13) L. Ahtee, *Ann. Med. Exp. Biol. Fenn.*, **44**, 431 (1966).
14) W. Theobald, O. Büch, and H.A. Kunz, *Arzneim. -Forsch.*, **15**, 117 (1965).

From this result, antidepressants, as well as phenothiazines,[4] were considered to have some behavior at the active sites similar to that at the surface of CB, which might have relation to the onset of pharmacological actions.

Acknowledgement The authors gratefully acknowledge for the generous supplies of the materials to Yoshitomi Pharmaceutical Co., Ltd., Yamanouchi Pharmaceutical Co., Ltd., Fujisawa Pharmaceutical Co., Ltd., Eizai Co., Ltd., Banyu Pharmaceutical Co., Ltd. and Sumitomo Chemical Co., Ltd. Thanks are also given to Mr. Toyozo Kobayashi, Miss Michiko Nagasawa, Suzuko Okada, Chieko Okano, and Mariko Takita for their assistance in the experimental work.