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Adsorption of Local Anesthetics from Aqueous Solution. Analysis of Factors Affecting the Nerve Blocking[1,2]

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Evaluating the extent of the hydrophobicity of 8 local anesthetics by the adsorbability on carbon black, discussions were made what kind of role the hydrophobicity plays in the nerve blocking. Additionally, the relationship between the partition coefficient in *n*-octanol/buffer solution and the blocking potency was investigated.

The adsorbed amount (represented by a) increased with pH, while the reported data of minimum blocking concentration (MBC) decreased. There was no comparison between the increases of a and 1/(MBC) with pH, because the latter increase was so remarkable.

The blocking potency (represented by $-\log$ (MBC)) increased with the adsorbability (represented by $\log b$), where all the data were included between $\log b=2.8$, and $\log b=4.1$. The most available local anesthetics had the adsorbability around $\log b=4$.

The blocking potency increased with the decrease in the occupied area by one molecule.

The increasing tendency of partition coefficient with pH was closer to that of 1/(MBC) than that of a or b.

All the results suggested that a minimum adsorbability of drug is necessary in the nerve blocking and the behavior of drug under the surface of nerve membrane which may be related with the penetration forms a necessary factor affecting the activity.

The mechanism of drug action of local anesthetics has been investigated by various workers.[4–8] In any event, the deposition of drugs on the nerve membrane, which may be formed of the adsorption on and the penetration into the membrane, seems necessary to an onset of the drug effect.

In this series of works,[9–11] it has been demonstrated that the adsorption of organic medicinals by carbon black has relation to the intestinal absorption rate or the pharmacological activity, suggesting that the carbon black surface may be available as a model of biopharmaceutical system. Moreover, it has been elucidated that the adsorption by carbon black mentioned above proceeds on the basis of the hydrophobic interaction and thus the adsorbability on carbon black corresponds to the hydrophobicity of organic medicinals.

In the present study, evaluating the extent of the hydrophobicity of local anesthetics by the adsorbability on carbon black, discussions were made what kind of role the hydrophobicity plays in the mechanism of drug action. The drugs used in previous studies, *i. e.*, barbiturates,[9] sulfonamides[10] and phenothiazines,[11] were homologous in structure, respec-

1) This paper forms Part XVII of "Physico-chemical Approach to Biopharmaceutical Phenomena." Preceding paper, Part XVI: H. Umeyama, T. Nagai, and H. Nogami, *Yakuzaigaku*, **30**, 255 (1970).
2) This work is outlined in Abstracts of Paper, 90th Annual Meeting of the Pharmaceutical Society of Japan, Sapporo, July, 1970, Part IV-20.
3) Location: *Hongo, Bunkyo-ku, Tokyo.*
4) J.C. Skou, *Acta Pharmacol. Toxicol.* (Kbh), **10**, 281 (1954).
5) J.C. Skou, *Acta Pharmacol. Toxicol.* (Kbh), **10**, 325 (1954).
6) L. Hersh, *Molecular Pharmacol.*, 3, 581 (1967).
7) M.B. Feinstein, *J. Gen. Physiol.*, **48**, 357 (1964).
8) J.M. Ritchie and P. Greengard, *Ann. Rev. Pharmacol.*, **6**, 405 (1966).
9) H. Nogami, T. Nagai, and H. Uchida, *Chem. Pharm. Bull.* (Tokyo), **17**, 176 (1969).
10) H. Nogami, T. Nagai, and S. Wada, *Chem. Pharm. Bull.* (Tokyo), **18**, 348 (1970).
11) H. Nogami, T. Nagai, and N. Nambu, *Chem. Pharm. Bull.* (Tokyo), **18**, 1643 (1970).

tively, and their hydrophobicities were related to the side chain length or the non-polarity of substituent, but the present local anesthetics are not included in a homologue so that discussions should be centered on the relationship between the hydrophobicity and the anesthetic activity without regarding the structure.

Additionally, the relationship betweem the partition coefficient in *n*-octanol/buffer solution and the anesthetic activity was investigated on the consideration that the partition might have closer relation to the behavior of drug under the surface of nerve membrane than the adsorption.

Experimental

Materials——Carbon black used was the same as described in a previous paper.[12] Dibucaine hydrochloride, quinine hydrochloride, phenyltoloxamine citrate, tetracaine hydrochloride,[13] lidocaine,[14] procaine hydrochloride, ephedrine hydrochloride and thymol of J.P. VII grade were used without further treatment, except for procaine hydrochloride which was decolored and recrystallized.

Procedure for Determination of the Adsorbed Amount by Batch Method——10 mg of carbon black (25 mg in the case of ephedrine) was added in 20 ml of the respective drugs in 5mM phosphate buffer solutions, and then the procedure was carried out in the same way as described in the previous paper.[12] It was examined preliminarily that the drugs were satisfactorily stable under the experimental conditions.

Quantitative Determination of Local Anesthetics——After diluting the sample with the same buffer solution as used for the adsorption experiment, the concentration of the drug was determined according to ultraviolet (UV) abosrption method using a Hitachi 124 spectrophotometer: dibucaine at 327 mμ; quinine, 235; phenyltoloxamine, 269; tetracaine, 227; lidocaine, 263; procaine, 290; ephedrine, 256; thymol, 273.

Determination of Partition Coefficent in *n*-Octanol/Buffer Solution——Ten ml of buffer solutions at various pH's containing 10^{-2}M tetracaine or 10^{-3}M procaine[15] was mixed with 10 ml of *n*-octanol, shaken for 3 days at 25°, and then the concentration in aqueous layer was determined to obtain the partition coefficient.

Results and Discussion

Langmuir Constants and Thermodynamic Functions of the Adsorption of Local Anesthetics by Carbon Black from Aqueous Solution

Since the adsorption isotherms by carbon black were described with Langmuir's equation, the Langmuir constants and the thermodynamic functions were obtained in the same way as described in the previous paper[12] and are listed in Table I, *i.e.*, a= the amount adsorbed when the entire surface is covered by a monolayer; b=the equilibrium constant of adsorption process; ΔG= the free energy change; ΔH= the enthalpy change; ΔS= the entropy change. Additionally, the occupied area by one molecule, A,[16] and the minimum blocking concentration (MBC)[4,17] are listed in the same table, which will be discussed later.

The temperature dependence of adsorption varied with the individual drug. Except for dibucaine and lidocaine, the adsorbed amount increased with temperature, as in the case of tryptophan,[12] though the adsorption is usually of exothermic process. The entropy change was positive in all case, suggesting that the structural change of iceberg around the adsorbate molecule takes place through the adsorption process, as was discussed in the previous paper.[12]

pH Dependence of the Adsorption of Local Anesthetics by Carbon Black from Aqueous Solution

pH dependences of basic properties of local anesthetics have been reported by Skou, *i.e.*, of the MBC against desheathed nerve fibers[4] and of the penetration into a monolayer

12) H. Nogami, T. Nagai, E. Fukuoka, and H. Uchida, *Chem. Pharm. Bull.* (Tokyo), **16**, 2248 (1968).
13) Supported by Tanabe Seiyaku Co., Ltd.
14) Supported by Sankyo Co., Ltd.
15) The difference in concentration between both drugs was due to the convenience for the experiment.
16) Calculated by the value of a and the specific surface area of carbon black (ref. 12).
17) D. Agin, L. Hersh, and D. Holtzman, *Proc. Natl. Acad. Sci.* U.S., **53**, 952 (1965).

Table I. Langmuir Constants, Thermodynamic Functions, Occupied Area by One Molecule, and Minimum Blocking Concentration (MBC) of Local Anethetics

Drug	Temperature	a	b	$-\Delta G$	ΔH	ΔS	A	$-\log$ (MBC)
Dibucaine	25°	1.74×10^{-3}	1.02×10^{4}	5.47	−1.02	14.9	1.19×10^{-18}	4.2
	37°	1.82×10^{-3}	9.55×10^{3}	5.65	−1.02	14.9	1.14×10^{-18}	
Quinine	25°	1.36×10^{-3}	1.21×10^{4}	5.57	19.57	84.3	1.53×10^{-18}	3.6
	37°	1.15×10^{-3}	4.35×10^{4}	6.58	19.57	84.3	1.81×10^{-18}	
Phenyltoloxamine	25°	1.45×10^{-3}	1.60×10^{4}	5.73	5.21	36.7	1.43×10^{-18}	3.2
	37°	1.48×10^{-3}	2.25×10^{4}	6.17	5.21	36.7	1.40×10^{-18}	
Tetracaine	25°	1.62×10^{-3}	1.23×10^{4}	5.58	7.89	45.1	1.28×10^{-18}	2.9
	37°	1.62×10^{-3}	2.06×10^{4}	6.12	7.89	45.1	1.28×10^{-18}	
Lidocaine	25°	1.75×10^{-3}	3.58×10^{3}	4.85	−2.68	7.2	1.19×10^{-18}	1.96
	37°	1.89×10^{-3}	3.00×10^{3}	4.93	−2.68	7.2	1.10×10^{-18}	
Procaine	25°	1.14×10^{-3}	7.02×10^{3}	5.24	1.35	22.1	1.82×10^{-18}	1.67
	37°	1.04×10^{-3}	7.66×10^{3}	5.51	1.35	22.1	2.00×10^{-18}	
Ephedrine	25°	7.22×10^{-4}	1.44×10^{3}	4.31	0.42	15.8	2.87×10^{-18}	0.8
	37°	5.53×10^{-4}	1.48×10^{3}	4.50	0.42	15.8	3.77×10^{-18}	
Thymol	25°	5.26×10^{-3}	7.17×10^{2}	3.89	9.19	43.9	3.95×10^{-19}	0.52
	37°	4.82×10^{-3}	1.31×10^{3}	4.42	9.19	43.9	4.31×10^{-19}	

a: Langmuir constant in mole/g
b: Langmuir constant in liter/mole
ΔG: free energy change in kcal/mole
ΔH: enthalpy change in kcal/mole
ΔS: entropy change in e.u.
A: occupied area by one molecule in m²/molecule
MBC: reported by Skou and Agin, *et al.*[17] in mM

Table II. Langmuir Constants, Partition Coefficient and Minimum Blocking Concentration (MBC) of Tetracaine and Procaine at Various pH's

	pH	a	b	P_x	MBC
Tetracaine	6.0	1.411×10^{-3}	5.787×10^{3}	0.182	0.0415
	6.5	1.578	7.921	0.228	0.0205
	7.0	1.806	12.30	0.458	0.0105
	7.35	1.818	12.22	0.698	0.004
	8.0	2.241	13.73	1.146	0.002
Procaine	6.0	0.814	4.095	0.044	23
	6.5	0.998	4.660	0.141	10.5
	7.0	1.103	6.146	0.473	4.6
	7.35	1.149	5.440	0.876	2.8
	8.0	1.189	8.010	1.369	1.3

a: Langmuir constant in mole/g at 25° for tetracaine and at 22° for procaine
b: Langmuir constant in liter/mole at 25° for tetracaine and at 22° for procaine
P_x: partition coefficient in *n*-octanol/buffer at 25°
MBC: reported by Skou in mM.

formed of the extracted lipid of nerve fibers.[5] In the present study, pH dependence of the adsorption of local anesthetics by carbon black was investigated with a view to finding a relationship between the adsorbability and the reported data described above.

The values of a and b obtained from the adsorption isotherms of tetracaine at 25° and procaine at 22° at different pH's are listed in Table II, where the values of MBC reported by Skou[4] are also listed. a and b increased with pH, while MBC decreased. The increase in a and b with pH might be due to the increase in the amount of undissociated molecule, giving the demonstration that the adsorption of these drugs proceeds on the basis of the hydrophobic interaction. Moreover, the hydrophobic and hydrophilic balance of molecule was considered sensitive to the dissociation in the present cases, as was discussd in the cases of phenothiazines,[11] though pH had no distinct effect on the adsorption of tryptophan[12] and sulfonamides[10] in neutral or weakly alkaline media.

Since 1/(MBC) may correspond to the extent of the anesthetic activity or the blocking potency, the decrease in MBC with the increase in a or b means that the adsorbability seems to have relation to the activity. Plotting the ratios of the values of a, b and 1/(MBC) at various pH's to those at pH 6.0 against pH in Fig. 1 and 2 for tetracaine and procaine, respectively, the blocking potency, 1/(MBC), increased strikingly with pH, while a and b did not increase so remarkably. This result showed that factors other than the adsorption may give more effect on the durg action. In other words, the behavior of drug under the surface of nerve membrane should be inportant in the appearance of drug action, which might be related with the penetration of drug, as will be discussed later.

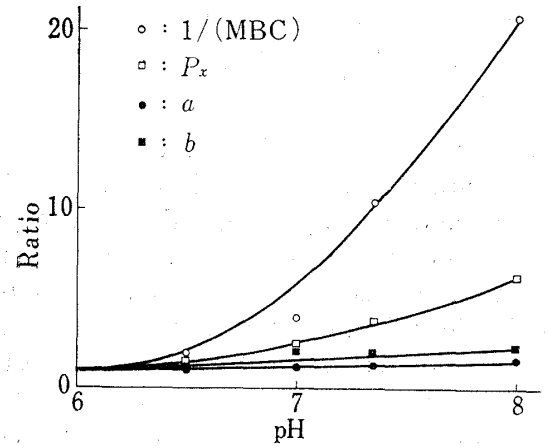

Fig. 1. Plots of Ratios of Langmuir Constants, a and b, Blocking Potency, 1/(MBC), and Partition Coefficient, P_x, to Those at pH 6.0 against pH for Tetracaine

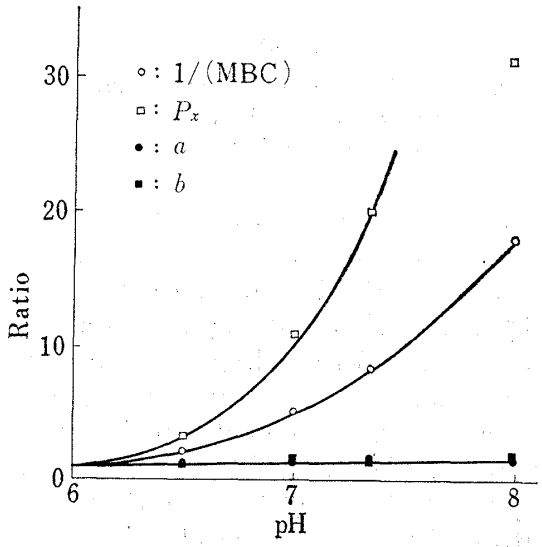

Fig. 2. Plots of Ratios of Langmuir Constants, a and b, Blocking Potency, 1/(MBC), and Partition Coefficient, P_x, to Those at pH 6.0 against pH for Procaine

Comparing the above two drugs in a and 1/(MBC), a for tetracaine was less than twice that for procaine, while 1/(MBC) for the former was 500—600 times that for the latter. Regarding the difference in structure, tetracaine has p-butylaminobenzoyl and 2-dimethylaminoethyl groups instead of p-aminobenzoyl and 2-diethylaminoethyl groups of procaine, respectively, and thus the former may be more hydrophobic. As a result, this increase in hydrophobicity had little effect on the increase in adsorbability compared with the effect on the increase in blocking potency. Therefore, it was suggested that the alkyl substituents at both N's in aminobenzoyl and 2-aminoethyl groups may play an important role in a static or dynamic behavior of the drug under the surface of membrane, having relation to the drug action.

General Relationship between Adsorbability and Minimum Blocking Concentration (MBC) of Local Anesthetics

The present 8 drugs have a variety of structure, and thus it should be better to discuss the adsorbability on the basis of b which is a function of the adsorption energy.

Plotting $-\log(\mathrm{MBC})$ against $\log b$, the former increased strikingly with the latter, where all the data were included between $\log b=2.8$ and $\log b=4.1$, as shown in Fig. 3.
Therefore, it seemed possible that a certain adsorbability of drug at least is necessary to the nerve blocking and also it is interesting that the most available local anesthetics such as dibucaine, phenyltoloxamine, tetracaine, lidocaine and procaine have the adsorbability around $\log b=4$.

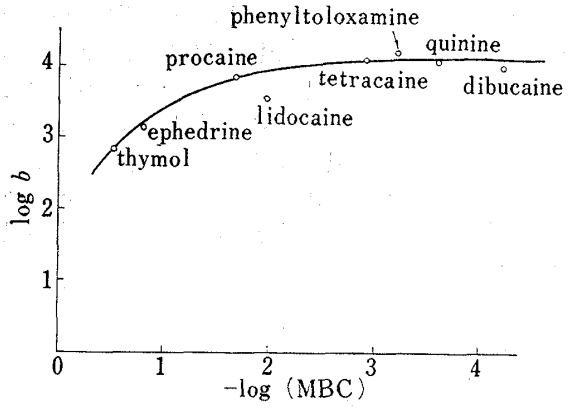

Fig. 3. Relationship between Langmuir Constant, b, and Blocking Potency, 1/(MBC) of Local Anesthetics

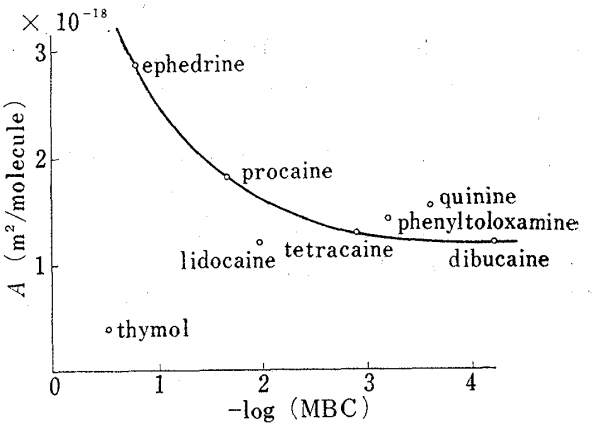

Fig. 4. Relationship between Occupied Area by One Molecule, A, and Blocking Potency, 1/(MBC), of Local Anesthetics

Anyhow, a linear relationship was not observed between the adsorbability and the blocking potency. Then, plotting the occupied area by one molecule[16] against $-\log(\mathrm{MBC})$ in Fig. 4, it was shown that the blocking potency increased with the decrease in occupied area, except for thymol. This result suggested that the smaller the molecular cross-sectional area when adsorbed, the easier the drug may penetrate into membrane to reach the active site. Thymol gave the smallest value in the occupied area by one molecule and almost 100% was partitioned into *n*-octanol in the present experimental system, while $-\log(\mathrm{MBC})$ for it was also smallest, as shown in Fig. 4. This is considered due to its low intrinsic activity. In other words, it may penetrate completely into the nerve membrane, but may not cause a very great change in nature of the membrane effective to the nerve blocking. All the other present local anesthetics have amino groups in their structure, and thus the above result supports that amino groups are important for the drug affecting the nervous system to an onset of the drug effect.

It seems to follow from the above results that a minimum adsorbability of drug is required and the change in nature of the nerve membrane caused by the penetrating drug is necessary to the nerve blocking.

Relationship between Partition Coefficient and MBC

As described already, the behavior of drug under the surface of nerve membrane which might be related with the penetration was considered to be important in the nerve blocking. Although both the statical and kinetical factors should be taken into consideration to discuss the penetration, the partition coefficient in *n*-octanol/buffer solution was investigated in the present study as an approach to understanding the phenomenon on the consideration that the partition might have closer relation to the behavior of drug under the surface of nerve membrane than the adsorption. *n*-Octanol has often been used in discussing the relationship between the partition coefficient and the biological activity of various drugs.[18]

Plotting the ratios of the obtained partition coefficients at various pH's to that at pH 6.0 in Fig. 1 and 2 for tetracaine and procaine, respectively, the increasing tendency of partition coefficient with pH was closer to that of 1/(MBC) than that of a or b, suggesting again that the penetration is more important in the nerve blocking than the adsorption. The curves of partition coefficient and 1/(MBC) in Fig. 1 and 2 did not only coincide with each other, but the former came in lower part from the latter in the case of tetracaine while in the upper part in the case of procaine. This difference between partition coefficient and 1/(MBC) might be due in part to the difference in the experimental conditions. In other words, MBC was obtained

18) For example, B.B. Brodie, H. Kurz, and L.S. Schanger, *J. Pharmacol. Ext. Ther.*, **130**, 20 (1960); C. Hansh, K. Kiehs, and G.L. Lawrence, *J. Am. Chem. Soc.*, **87**, 5770 (1965).

under biological conditions while the partition coefficient under physico-chemical and moreover statical ones. Thus, there remains a possibility of obtaining an improved correlation between partition coefficient and 1/(MBC) by more precise investigations.

Although it may be too early to conclude that the partition correlates to the penetration of drug, the above results shown in Fig. 1,2 and 4 suggest that the penetration into the nerve membrane forms a necessary factor affecting the anesthetic activity.

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