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Physico-chemical Approach to Biopharmaceutical Phenomena. IV.[1) Adsorption of Barbituric Acid Derivatives by Carbon Black from Aqueous Solution[2)

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Following the previous discussions on the adsorption of tryptophan based on the hydrophobic bonding mechanism, an examination in detail was carried out in the adsorption of barbituric and thiobarbituric acid derivatives by carbon black from aqueous solution in relation to the size of hydrocarbon chains, making the first step to discuss the correlation between the adsorption and the biopharmaceutical data.

Equations were derived to express the relationship between the adsorption and the size of the respective molecule, considering the contributions of hydrophobic and hydrophilic moieties. The results were in good agreement with such equations, indicating that the larger the size of hydrophobic moiety, the more the molecule was adsorbed and that each of two 5-substituted hydrocarbon chains might adopt the parallel orientation to carbon black surface.

Calculating the thermodynamic functions of adsorption, it was shown that the larger the size of 5-substituted hydrocarbon chains, the less absolute value of enthalpy change and the greater entropy change were caused by the hydration of molecule accompanying the destruction of iceberg through the adsorption process.

A selectivity was not found for the adsorption of such geometrical isomers as pentobarbital and amobarbital by carbon black.

Correlating the adsorption with the solubility, the partition coefficient, and the surface tension depressing activity, it was established that carbon black was of hydrophobic surface and that the more hydrophobic the molecule, the easier it was adsorbed.

In the previous papers,[1,4,5) the adsorption of tryptophan by carbon black was explained on the basis of the hydrophobic bonding mechanism in which the hydrophobic moiety of molecule played an important role. Moreover, the adsorption of amino acids by carbon black increased with the size of hydrophobic moiety, *i.e.*, with the hydrophobicity of molecule.[1) Eventually, it was suggested that the adsorption by carbon black could be a model for biopharmaceutical phenomena in which hydrophobic bonding was considered to participate.

Intending to examine the above notion as an approach to an understanding of biopharmaceutical phenomena, barbituric acid derivatives may be useful to the samples because they include the molecules of various kinds of hydrocarbon chains offering the ones of various hydrophobicities and have been investigated by many workers in the fields of biopharmacy, pharmacology, biochemistry and others.

[1) Part III: H. Nogami, T. Nagai, and H. Uchida, *Chem. Pharm. Bull.* (Tokyo), **16**, 2263 (1968). This paper forms Part XXV of "Studies on Powdered Preparations." Preceding paper, Part XXIV: H. Nogami, T. Nagai, E. Fukuoka, and T. Yotsuyanagi, *Chem. Pharm. Bnll.* (Tokyo), **17**, 23 (1969).

[2) This work was presented at the 88th Annual Meeting of Pharmaceutical Society of Japan, Tokyo, April 1968, being taken in part from the thesis of Hiroshi Uchida for the degree of Doctor of Pharmaceutical Sciences, University of Tokyo, 1968.

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[4) H. Nogami, T. Nagai, E. Fukuoka, and H. Uchida, *Chem. Pharm. Bull.* (Tokyo), **16**, 2248 (1968).

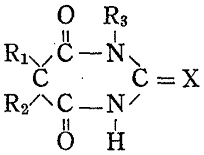
[5) H. Nogami, T. Nagai, and H. Uchida, *Chem. Pharm. Bull.* (Tokyo), **16**, 2257 (1968).

Accordingly, the present study was attempted to investigate the adsorption of barbituric acid derivatives by carbon black from aqueous solution in detail, characterizing the thermodynamic behaviors of the adsorption and correlating them with physical factors, such as solubility, partition coefficeint between organic and aqueous phases, and surface tension depressing activity. Therefore, this paper forms the previous portion before the correlation between the adsorption and the biopharmaceutical data will be discussed in the following.6)

Experimental

Materials——Carbon black used was the same as that in the previous paper.4) Barbituric and thiobarbituric acid derivatives used are listed in Table I. Cyclobarbital calcium, thiopental sodium and thiamylal sodium, which were supported by the companies described in Table I, were converted to the respective free bases by adding HCl solution, being recrystallized several times from aqueous solution. The other purified free bases supported were used without further treatment.

Table I. Barbituric and Thiobarbituric Acid Derivatives in This Study



Compound	R_1	R_2	R_3	X
Barbital a)	ethyl	ethyl	H	O
Probarbital b)	ethyl	isopropyl	H	O
Allobarbital c)	allyl	allyl	H	O
Phenobarbital a)	ethyl	phenyl	H	O
Cyclobarbital d)	ethyl	cyclohexyl	H	O
Pentobarbital e)	ethyl	1-methyl butyl	H	O
Amobarbital f)	ethyl	isoamyl	H	O
Heptabarbital g)	ethyl	heptyl	H	O
Secobarbital a)	allyl	1-methyl butyl	H	O
Mephobarbital h)	ethyl	phenyl	CH_3	O
Thiopental e)	ethyl	1-methyl butyl	H	S
Thiamylal i)	allyl	1-methyl butyl	H	S

a) Supported by Grelan Pharmaceutical Co., Ltd.
b) Supported by E.R. Squibb & Son Inc.
c) Supported by Iwaki Seiyaku Co., Ltd.
d) Supported by Shionogi & Co. Ltd.
e) Supported by Tanabe Seiyaku Co., Ltd.
f) Supported by Dainippon Pharmaceutical Co., Ltd.
g) Supported by G.R. Geigy Soc., An.
h) Supported by Farbenfabriken Bayer AG.
i) Supported by Yoshitomi Pharmaceutical Industries Co., Ltd.

Procedure for Determination of the Adsorbed Amount by Batch Method——The adsorption procedure was carried out in the same way as described in the previous paper,4) except for the following points. 20 mg of carbon black was added in 10 ml of the respective aqueous solutions of barbital, probarbital, allobarbital and was added in 20 ml of the respective aqueous solutions of phenobarbital, cyclobarbital, pentobarbital, amobarbital, heptabarbital and secobarbital. 7 mg of carbon black, was added in 40 ml of aqueous solution of mephobarbital. Various amounts of carbon black were added in 50 ml of 10^{-4}M aqueous solution of thiopental and were added in 70 ml of 10^{-4}M thiamylal, because the solubilities were too low to prepare the solution of various concentrations appropriate to determine the adsorption isotherm by using a given amount of carbon black. It was examined preliminarily that the amount adsorbed per unit amount of carbon black was independent of the volume of solution or the amount of carbon black under the experimental conditions.

Determination of Solubility——The procedure was the same as described in the previous paper.5)

Analytical Method——The concentrations of barbituric and thiobarbituric acid derivatives were determined at 240 mμ and 305 mμ, respectively, according to UV absorption method after diluting with borate buffer pH 9.6, using a Hitachi Perkin-Elmer 139 spectrophotometer.

Measurement of Surface Tension——The surface tension of solution was measured at room temperature with a Du-Noüy surface tensiometer (Rigosha Manufacturing Co., Ltd.).

Results and Discussion

Adsorption of Barbituric and Thiobarbituric Acid Derivatives with Respect to the Hydrophobic and the Hydrophilic Moieties

Considering that the adsorption of tryptophan by carbon black had not depended distinctively on pH in the region of pH 3 to 9,[4] the adsorption experiments were carried out in aqueous solution without using a buffer.

Adsorption isotherms obtained at 40° are shown in Fig. 1. For the convenience of discussion, the term "adsorbability" is defined in this study as the amount adsorbed at the equilibrium concentration 0.25×10^{-3}M at 40°. The relationship between the adsorbability and the molecular volume at boiling point[7] is shown in Fig. 2, indicating that the larger the molecule, the more it was adsorbed. According to Traube's rule, the adsorption of an organic homologue from aqueous solution is expected to increase regularly with the hydrocarbon chain length, as has been reported on the adsorption of fatty acid by charcoal.[8] Barbituric acid derivatives except mephobarbital have the same hydrophilic moiety and thus the molecular volume may be correlated to the size of 5-substituted hydrocarbon chains. Therefore, the difference in adsorption shown in Fig. 2 was considered to be due to the difference in the effect of such nonpolar moiety, and subsequently the size of the moiety might have influence on the free energy change of adsorption, as will be discussed below.

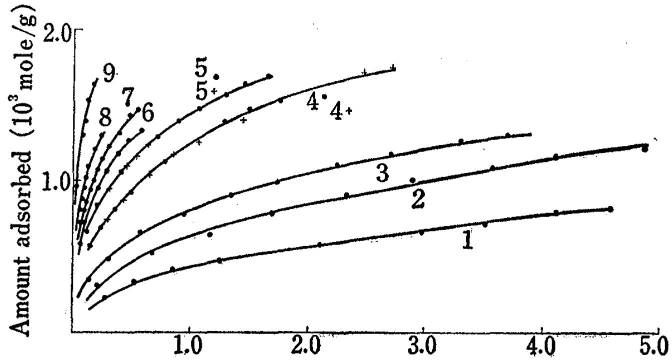


Fig. 1. Adsorption Isotherms of Barbituric and Thiobarbituric Acid Derivatives from Aqueous Solution by Carbon Black at 40°

1 barbital
2 probarbital
3 allobarbital
4• cyclobarbital
4+ phenobarbital
5• pentobarbital
5+ amobarbital
6 heptabarbital
7 secobarbital
8 thiopental
9 thiamylal

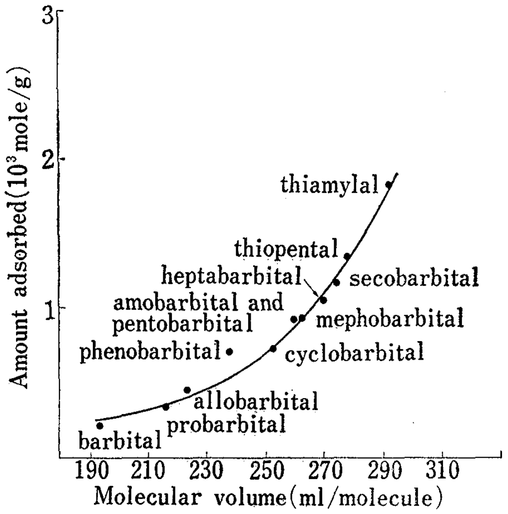


Fig. 2. Relationship between Adsorbability by Carbon Black and Molecular Volume of Barbituric and Thiobarbituric Acid Derivatives at 40°

Hydrocarbon chains in barbituric acid derivatives may have the same thickness l, and thus, the area in contact with carbon black surface is assumed to be $\kappa V/l$, where κ is the shape factor and V the sum of atomic volumes of hydrocarbon chain. Denoting f for the free energy change per unit area of molecule in contact, the free energy change per molecule, ΔF_n, may be expressed as

$$\Delta F_n = \kappa V f / l \qquad (1)$$

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[6] H. Nogami, T. Nagai, and H. Uchida, *Chem. Pharm. Bull.* (Tokyo,) **17**, 176 (1969).

[7] The value was calculated by summing the respective atomic volumes. Reference, L. Bas, *Proc. Chem. Soc.*, **22**, 322 (1906).

[8] E. Landt and W. Knop, *Z. Phys. Chem.*, **A162**, 331 (1932).

This free energy change is considered to be attributed to the nonpolar moiety. Assuming that the total free energy change, $\varDelta F$, consists of the respective contributions of hydrophobic and hydrophilic moieties, $\varDelta F$ is expressed as

$$\varDelta F=\varDelta F_n+\varDelta F_p \tag{2}$$

where $\varDelta F_p$ is the contribution of hydrophilic moiety.

Adsorption isotherms obtained as shown in Fig. 1 were in accordance with Langmuir equation expressed as

$$M=\frac{abC}{1+bC} \tag{3}$$

where M is the amount adsorbed at concentration C in solution, a the amount adsorbed when the entire surface is covered by a monolayer, and b the equilibrium constant of adsorption process.

Since the discussion in this study is based on the data obtained in low equilibrium concentration where the plots are almost on the straight lines in Fig. 1, equation (3) approximates to

$$M=abC \tag{4}$$

The free energy change of adsorption is expressed as [4)]

$$\varDelta F=-\boldsymbol{R}T\ln b \tag{5}$$

Combining equations (1), (2), (4), and (5), the following equation is obtained.

$$\ln\frac{M}{C}=-\frac{\kappa f}{\boldsymbol{R}Tl}V_n-\frac{\varDelta F_p}{\boldsymbol{R}T}+\ln a \tag{6}$$

which is rewritten as

$$\ln M=\alpha+\beta V_n \tag{7}$$

$$\ln(1/C)=\alpha'+\beta V_n \tag{8}$$

where

$$\alpha=\ln aC-\frac{\varDelta F_p}{\boldsymbol{R}T},\quad \alpha'=\ln\,(a/M)-\frac{\varDelta F_p}{\boldsymbol{R}T},$$

and

$$\beta=-\frac{\kappa f}{\boldsymbol{R}Tl}.$$

Under such conditions that the equilibrium concentration (in the low region) and the temperature are identical, respectively, α and β are considered to be identical for various barbituric acid derivatives, and thus $\ln M$ is expected to be linearly related to V_n according to equation (7). This was demonstrated by the results obtained at the equilibrium concentration 0.25×10^{-3}M at 40°, as shown in Fig. 3. On the other hand, it is expected that $\ln C^{-1}$ is linearly related to V_n according to equation (8) under the same temperature and the same adsorbed amount, M, as demonstrated in Fig. 4 by the result obtained at $M=0.8\times10^{-3}$ mole/g at 40°. Consequently, the adsorption by carbon black could be related to the size of the hydrocarbon chains of barbituric acid derivatives. In the case of phenobarbital, the value obtained deviated over the straight line, as shown in Figs. 3 and 4. This seems due to the reason that benzene ring has different characteristics from straight hydrocarbon chain, *i.e.*, the differences in the thickness of chain and in the interaction with carbon black surface because of π-electron.

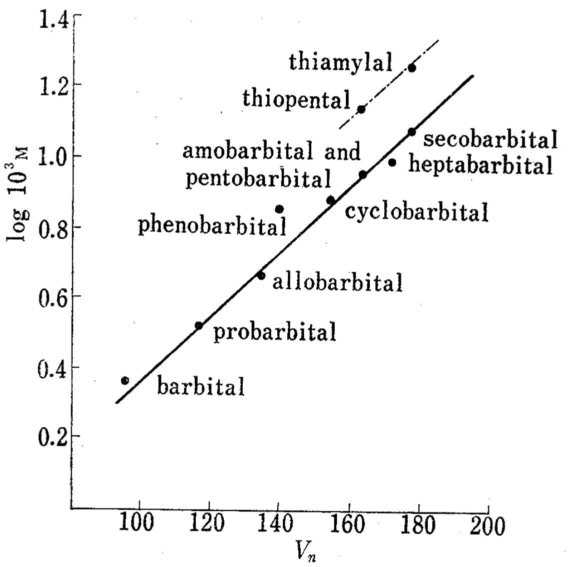

Fig. 3. Plots According to Equation (7) Under Equilibrium Concentration 0.25 mM at 40°

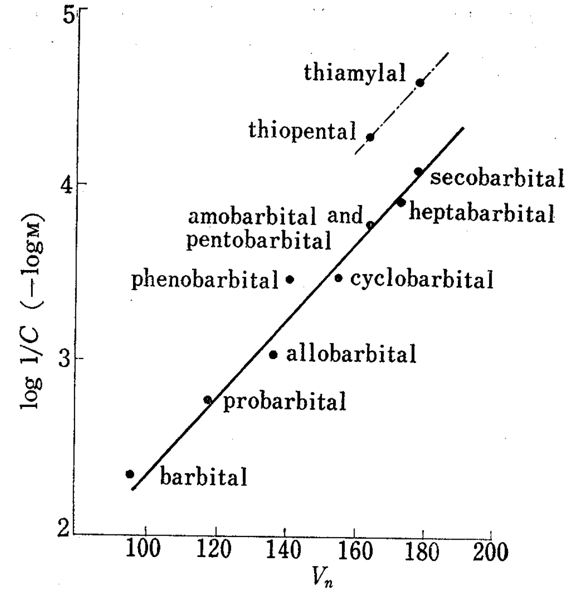

Fig. 4. Polts According to Equation (8) under $M = 0.8 \times 10^{-3}$ mole/g at 40°

Additionally, in the case of thiobarbituric acid derivatives, the values were considered to be on another straight line of the same slope, as shown in Figs. 3 and 4, meaning that β was the same as in case of barbituric acid derivatives, but ΔF_p was different.

These results indicate that the adsorption of barbituric and thiobarbituric acid derivatives was influenced by the size of hydrocarbon chain and also by the hydrophilic moiety, *i.e.*, barbituric (or thiobarbituric) acid ring, as was in agreement with the proposed equations. Considering that the results in the cases except for phenobarbital and thiobarbituric acid derivatives were on the same straight line according to equations (7) and (8), each of the two 5-substituted hydrocarbon chains might adopt the parallel orientation to carbon black surface.

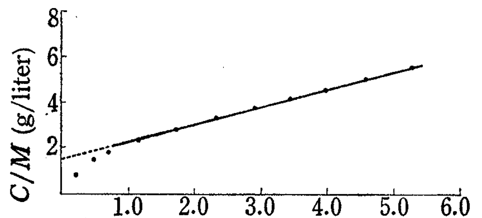

Fig. 5. Langmuir Plot of Adsorption of Barbital of Aqueous Solution by Carbon Black at 30°

Langmuir plots deviated to the lower part in the low equilibrium concentration, *e.g.*, as shown in Fig. 5. Such a phenomenon has been observed in the adsorptions of tryptophan by carbon black from aqueous solution and of carboxylic acids by polystyrene from aqueous solution, as has been discussed on the bases of the surface heterogeneity and the change with concentration in orientation of the adsorbed molecule, respectively.[4,9]

Thermodynamic Functions of the Adsorption of Barbituric and Thiobarbituric Acid Derivatives by Carbon Black from Aqueous Solution

Since the adsorption of the derivatives decreased with the increase of temperature at the same equilibrium concentration, the temperature dependence was usual, contrary to the case of tryptophan.[4] The thermodynamic functions were calculated in the same way as described in the previous paper,[4] and the value obtained at 40° are shown in Table II. The enthalpy decreased through the adsorption process, that is, the process was exothermic. The absolute value of free energy change increased with molecular volume, as was expected also from Fig. 2. The entropy change was positive in all cases, as was reported on the adsorption of tryptophan by carbon black from aqueous solution in the previous paper.[4] This increase

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[9] H. Schneider, G. C. Kresheck, and H. A. Scheraga, *J. Phys. Chem.*, **69**, 1311 (1966).

Table II. Thermodynamic Functions of Adsorption of Barbituric Acid Derivatives from Aqueous Solution by Carbon Black at 40°

Barbituric acid derivative	ΔF kcal/mole	ΔH kcal/mole	ΔS e.u.
Barbital	-3.70	-3.4	1.0
Probarbital	-3.86	-2.0	6.1
Allobarbital	-4.20	-1.2	9.9
Phenobarbital	-4.40	-2.3	7.0
Cyclobarbital	-4.58	-1.9	8.0
Pentobarbital	-4.95	-1.6	11
Amobarbital	-5.05	-0.6	15
Secobarbital	-5.25	-0.9	14

of entropy was considered to be caused by breakdown of the iceberg enclosing the molecule in solution, as was discussed in the case of tryptophan.[4,5] It is expected that the larger the size of 5-substituted hydrocarbon chains, the larger the size of the surrounding iceberg is. That is, the larger the size of 5-substituted hydrocarbon chains, the less absolute value of enthalpy change and the greater entropy change are caused by the dehydration of molecule accompanying the destruction of the iceberg. Therefore, the result shown in Table II was considered to be quite reasonable.

Adsorption of Pentobarbital and Amobarbital by Carbon Black from Aqueous Solution

It is shown in every figure that there was no difference in adsorption behaviour between pentobarbital and its geometrical isomer, amobarbital. The difference shown in Table II seemed due to the experimental errors. Therefore, the result corresponded to the case of tryptophan, in which both D- and L-tryptophan showed the same behavior in the adsorption by carbon black, while they showed a difference in the adsorption by Keratin.[4] Accordingly, it is apparent that carbon black has no property to select both stereoisomers and geometrical isomers for adsorption, while stereoselective adsorptions by various naturally occuring substances have been reported.[10-12]

Adsorption of Barbituric and Thiobarbituric Acid Derivatives in Relation to Solubility, Partition Coefficient, and Surface Tension Depressing Activity

Among many factors having influence on the adsorption from solution, solubility, partition coefficient, and surface tension depressing activity might be important ones.

In Relation to Solubility——Since the adsorption from solution is considered to be a portion of reverse process of dissolution, a reverse relationshiop is generally expected between the adsorbability and the solubility. For example, Hansen and Craig described that the adsorption isotherms of homologous series of fatty acid on Spheron 6 were superimposable on each other, if the amount adsorbed was plotted against the relative concentration, *i.e.*, the ratio of equilibrium concentration to solubility.[13] It is shown in Table III that the less soluble the derivative, the more it was adsorbed, but a complete correlation was not given. Solubility is affected not only by the state of solution, but by that of solid or crystal. Accordingly, the solubility could not be a primary factor on the adsorption here.

In Relation to Partition Coefficent——Hansch, *et al.* found that there was a good correlation between biological activity and log P_X or $\pi = \log(p_X/p_H)$ representing the relative hydrophobicity where P_H and P_X are the partition coefficients of parent substance and derivative,

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[10] W. Bradley and D. Easty, *J. Chem. Soc.*, **1951**, 499.

[11] M. Ohara, K. Ohta, and T. Kwan, *Bull. Chem. Soc. Japan*, **37**, 76 (1964).

[12] S. Koga, M. Morita, K. Konishi, and Y. Kuroki, Presentation No. 4R3 26 at the 20th Annual Meeting of Chemical Society of Japan, Tokyo, March 1967.

[13] R. S. Hansen and R. P. Craig, *J. Phys. Chem.*, **58**, 211 (1954).

Table III. Characteristics of Barbituric and Thiobarbituric Acid Derivatives

Compound	1	2	3	4	5	6
Barbital	184	194	61.0	5.6	0.65	0.23
Probarbital	198	216	12.1	11	0.95	0.31
Allobarbital	208	234	13.7	—	1.05	0.46
Phenobarbital	232	238	9.00	20	1.42	0.70
Cyclobarbital	236	253	9.14	30	—	0.75
Pentobarbital	226	261	7.64	48	1.95	0.90
Amobarbital	226	261	3.82	48	1.95	0.90
Mephobarbital	246	263	—	—	—	0.96
Heptabarbital	238	271	1.17	—	—	0.99
Secobarbital	238	275	7.90	125	2.15	1.17
Thiopental	242	279	—	—	—	1.3[a)]
Thiamylal	254	294	0.375	—	—	1.8[a)]

1. molecular weight
2. molecular volume (ml/molecule)
3. solubility in water at 40° (10^2M)
4. m_1m_2 estimated by surface tension measurement
5. log P_X P_X is the partition coefficient (C. Hansch and S.M. Anderson, *J. Medicinal Chem.*, **10**, 745 (1967))
6. amount adsorbed by carbon black at 0.25 mM equilibrium concentration at 40° (10^3 mole/g)

a) value estimated by extrapolation

respectively, using the values obtained in 1-octanol/water system.[14)] Using the values of P_X[14)] a good correlation with the adsorbability was given as shown in Fig. 6.

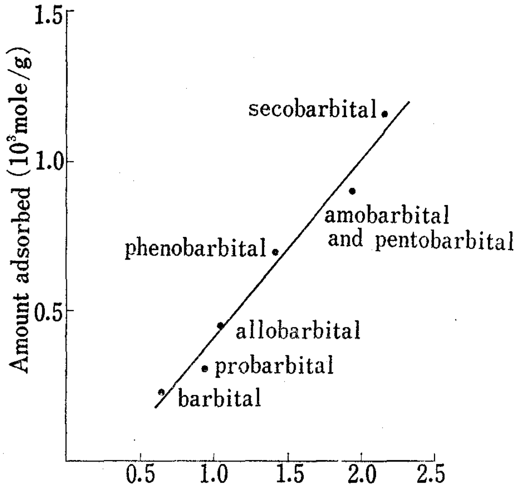

Fig. 6. Relationship between Adsorbability and Partition Coefficient

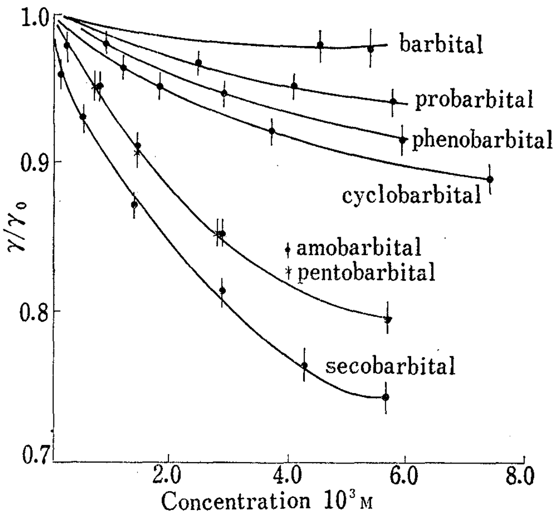

Fig. 7. Surface Tension Depression of Aqueous Solution of Barbituric Acid Derivatives

In Relation to Surface Tension Depressing Activity——If the adsorption of barbituric acid derivatives by carbon black from aqueous solution takes place through the orientation of hydrocarbon chain to the surface as described before, it may be correlated with the adsorption to air–solution interface because of a similarity in phenomena, which is represented by the surface tension depressing activity. Sorby, *et al.* showed by a statistical analysis that the correlation coefficient between the extent of adsorption of phenothiazine derivatives by activated carbon and the surface tension depression of solution was −0.843, being significant by *t*-test at 5% level, but such a correlation was not obtained by polar adsorbents such as talc and kaolin.[15)] It has been reported that barbituric acid derivatives depress the surface ten-

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14) C. Hansch and S.N. Anderson, *J. Medicinal Chem.*, **10**, 745 (1967).

15) D.L. Sorby, E.M. Plein, and J.D. Benmaman, *J. Pharm. Sci.*, **55**, 785 (1966).

sion.[16] The values obtained decreased with the increase of concentration of derivative, as shown in Fig. 7.

According to Szyskowski's experimental equation,[17] which was given to the solution of a homologous series, such as fatty acid and alcohol, the surface tension depression of solution is expressed as

$$\gamma_0-\gamma=m_1 \ln (1+m_2C) \tag{9}$$

where γ_0 and γ are the surface tensions of the solvent and the solution of concentration C, respectively, and m_1 and m_2 are the constants for the homologous series and for the derivative, respectively. If C is low, equation (q) is modified to

$$\gamma_0-\gamma=m_1m_2C \tag{10}$$

As shown in Fig. 7, the values of γ/γ_0 at $C=0.25\times10^{-3}$M and $C=0.50\times10^{-3}$M seem to be on the respective straight lines. The mean of the values of m_1m_2 obtained according to equation (10) at the above two concentrations are listed in Table III. Since log m_1m_2 is considered to correspond to the energy of adsorption of solute from solution to air–solution interface, *i.e.*, the escaping tendency from solution, it may indicate the surface tension depressing activity and may be correlated to the adsorbability of the respective molecule by carbon black. Fig. 8 shows such a correlation between the data obtained.

The results obtained in relation to the above three physical factors indicate that carbon black is of hydrophobic surface and that the more hydrophobic the molecule, the easier it is adsorbed.

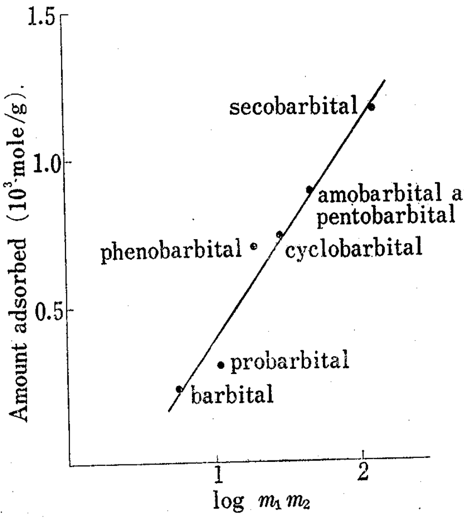

Fig. 8. Relationship between Adsorbability and m_1m_2 at 40°

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[16] R.J. Kuffner, M.T. Bush, and L.J. Bircher, *J. Am. Chem. Soc.*, **79**, 1587 (1957).

[17] B. von Szyszkowski, *Z. phys. Chem.*, **64**, 385 (1908).