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Physico-chemical Approach to Biopharmaceutical Phenomena. V.[1] Relationship between the Adsorption by Carbon Black from Aqueous Solution and the Biopharmaceutical Data of Barbituric Acid Derivatives[2]

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Discussions on biopharmaceutical phenomena were given on the bases of the adsorption by carbon black from aqueous solution and the permeation through Visking tube of barbituric acid derivatives.

The absorption of drug was considered to proceed through the two succesive steps: 1) adsorption or uptake onto the membrane surface and 2) transport through the membrane. The results showed that the absorption might be controlled by adsorption or uptake by membrane.

The permeation through Visking tube, as an experimental model for the transport process in the absorption, decreased with the increase of the molecular volume of barbituric acid derivatives, as was explained to be a simple diffusion through pore and was contrary to the actual absorption tendency.

Plotting the absorption data[6,11] against the adsorbability of barbituric acid derivatives by carbon black, a very good correlation was given, demonstrating that such an adsorption study was useful to estimate the absorbability *in vivo*.

Plotting the data of the binding to bovine serum albumin[13] against the adsorbability of barbituric acid derivatives by carbon black, a good correlation was given, suggesting that the adsorption by such a hydrophobic substance as carbon black might have some relation to the pharmacological action. Moreover, a fairly good correlation was observed between the existing pharmacological data and the adsorbability of barbituric acid derivatives by carbon black.

Biological membrane is generally considered to be composed of protein and lipid, though several models have been proposed for the structure.[4] Accordingly, the absorption of drug through membrane may have relation to the interaction between drug and such components. While the absorption of such physiologically essential substances as glucose and amino acids is controlled by active transport, the absorption of drug has been shown to be generally controlled by passive transport[5] and known to be seldom influenced by such a biological specificity as stereoselectivity. Accordingly, there remains a high possibility of making a physicochemical approach to an understanding of biopharmaceutical phenomena.

The absorption of drug may proceed through the two succesive steps: 1) adsorption or uptake onto the membrane surface and 2) transport through the membrane. The latter, in

1) Part IV: H. Nogami, T. Nagai, and H. Uchida, *Chem. Pharm. Bull.* (Tokyo), **17**, 168 (1969). This paper forms Part XXVI of "Studies on Powdered Preparations."
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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5) S. Mayer, R.P. Maickel, and B.B. Brodie, *J. Pharmacol. Exptl. Therap.*, **127**, 205 (1959).

general, is controlled by simple diffusion and thus its rate is expected to decrease with the increase of molecular volume. On the other hand, Kakemi, *et al.* reported that the absorption of barbituric acid derivatives increased with molecular volume.[6] Therefore, it seems difficult to explain the drug absorption on the basis of the diffusion through membrane.

If the drug is easily adsorbed on the membrane surface, its amount adsorbed is large, resulting in a high concentration gradient across the membrane, and thus the absorption rate is expected to be high. Considering that carbon black might have a similarity to the membrane in the surface chemical aspect because of its hydrophobic property[7] and that the adsorbability of barbituric acid derivatives by carbon black increased with the molecular volume (or the size of hydrophobic moiety),[1] the amount of the derivatives adsorbed (or uptaken) on the membrane surface under the given conditions may increase with the molecular volume and consequently the absorption rate may increase in the same way.

Based on the above consideration, the present study was attempted to discuss how the adsorption or uptake process might play an important role in the biopharmaceutical process concerning barbituric acid derivatives, investigating the permeations through Visking tube and through gelatin film.

Experimental

Materials——All the barbituric acid derivatives used were the same as those in the previous paper.[1] Gelatin of the purest reagent grade and Visking tubing (20/32″) were obtained commercially.

Preparation of Gelatin Film——A given amount of molten gelatin was poured on the stainless steel dish which was previously coated with liquid paraffin, and was solidified on drying at room temperature. This solidified film was soaked in 10% formalin solution overnight to make insoluble in water and then kept in a large amount of water until use.

Procedure and Apparatus for the Permeations through Visking Tube and through Gelatin Film—— The procedure was according to the usual equilibrium dialysis method using a glass cell, as shown in Fig. 1. The concentration of barbituric acid derivative was determined in the same way as described in the previous paper.

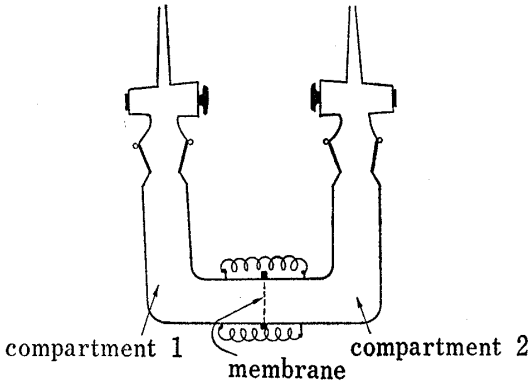

Fig. 1. Permeation Apparatus

Results and Discussion

Permeation of Barbituric Acid Derivatives through Visking Tube

If the drug absorption is controlled by transport process, its rate may decrease with the increase of molecular volume, because the diffusion constant decreases with the increase of molecular volume. As a model experiment concerning such a transport process, the permeation of barbituric acid derivatives through Visking tube was investigated, which might be assumed to be based on a diffusion through pore.

Theoretical equations of permeation are derived as follows:[8]

$$\log(C_0-2C_2)=Kt+\log C_0 \tag{1}$$

$$K=-\frac{2A}{2.303Vl}\times D \tag{2}$$

6) K. Kakemi, T. Arita, R. Hori, and R. Konishi, *Chem. Pharm. Bull.* (Tokyo), **15**, 1534 (1967).
7) H. Nogami, T. Nagai, E. Fukuoka, and H. Uchida, *Chem. Pharm. Bull*, (Tokyo), **16**, 2248 (1968).
8) L.M. Lueck, D.E. Wurster, T. Higuchi, A.P. Lemberger, and L.W. Busse, *J. Am. Pharm. Assoc., Sci. Ed.*, **46**, 694 (1957).

where C_0 is the initial concentration in the compartment 1 from which the material permeates to compartment 2, C_2 the concentration in the compartment 2 at the time t, A the effective surface area of membrane, V the volume of solution in the respective compartments, l the length of pore, K the permeation rate constant, and D the diffusion constant.

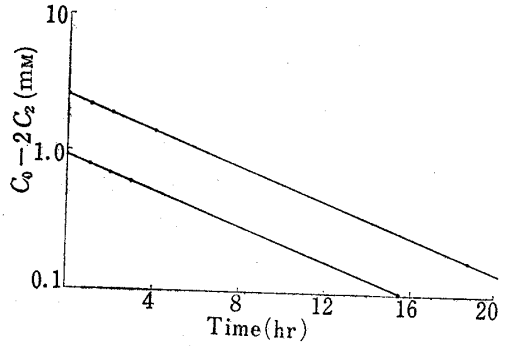

Fig. 2. Permeation of Phenobarbital through Visking Tube at 40°

Fig. 2 shows, for example, the permeation data of phenobarbital plotted according to equation (1). The other barbituric acid derivatives gave the same relationship. Consequently it was concluded that the permeation through Visking tube was controlled by simple diffusion through pore. Since the permeation rate constant, K, under given experimental conditions depends only on the diffusion constant, D, the ratio of K to the diffusion constant in water, D^*, should be constant regardless of kind of the derivatives. The values of D^* at 40° were estimated by Wilke's method,[9] using D^* of barbital 1.17×10^{-5} cm^2/sec at 40° which was estimated from the data by Nogami, *et al.*[10] The values of D expressed in equation (2) were considered to be different from the respective values of D^*, but the ratio D/D^* should be constant regardless of kind of barbituric acid derivatives. The ratio K/D^* indicated in the last column of Table I seemed constant, considering the experimental error

Table I. Permeation of Barbituric Acid Derivatives through Visking Tube at 40°

	C_1^0 10^3M	C_1^2 10^3M	C_2^2 10^3M	$C_1^2+C_2^2$ 10^3M	$\log C_1^0/(C_1^0-2C_2^2)$ K -20 hr	D^* 10^5cm^2 sec	K/D^*
Barbital	2.30	2.03	0.262	2.292	1.12	1.17	0.96
Probarbital	2.36	2.10	0.260	2.360	1.08	1.14	0.95
Allobarbital	2.37	2.10	0.260	2.360	1.08	1.12	0.98
Phenobarbital	2.38	2.12	0.262	2.382	1.08	1.08	1.00
Cyclobarbital	2.35	2.10	0.250	2.350	1.04	1.04	1.00
Pentobarbital	2.30	2.07	0.230	2.300	0.97	1.03	0.89
Amobarbital	2.28	2.07	0.211	2.281	0.89	1.03	0.87
Secobarbital	2.36	2.14	0.220	2.360	0.90	0.99	0.92

C_1^0: concentration in the compartment 1 at time $=0$
C_1^2: concentration in the compartment 1 at time$=$ 2 hr
C_2^2: concentration in the compartment 2 at time $=2$ hr
D^*: diffusion constant in water estimated by Wilke's method, using D^* of barbital to be 1.17×10^{-5} cm^2/sec which was estimated from data by Nogami, *et al.*[10]

to obtain K and the estimation error of D^*. The above result also showed that the permeation through Visking tube was controlled by simple diffusion through pore and that the permeation rate constant decreased with the increase of molecular volume.

Adsorption of barbituric acid derivatives by Visking tube was negligible as shown in Table I. This might be expected because of small surface area. Considering that Visking tube is composed of cellulose and that the adsorbent of large surface area is suitable to clarify the adsorption feature, the adsorptions of barbital (of the highest permeability) and secobarbital (of the lowest permeability) by cellulose were investigated, resulting in a rather negative adsorption, as was expected because of the hydrophilic property of cellulose. If the interaction between the drug and the pore wall took place, it should give effect on

9) C.R. Wilke, *Chem. Eng. Progress*, **45**, 218 (1949).
10) H. Nogami, T. Nagai, E. Fukuoka, and T. Yotsuyanagi, *Chem. Pharm. Bull.* (Tokyo), **17**, 23 (1969).

the permeation rate. Considering the above adsorption data, the permeation of barbituric acid derivatives through Visking tube might be free from such an interaction. However, this problem was not disclosed completely.

Permeation through Gelatin Membrane

With the intention of discussing the permeation through the substantial part of membrane aside from pore, the experiments were carried out using gelatin membrane, as the result is shown in Fig. 3. There was not a significant difference among the permeation rate constants of barbituric acid derivatives. Although the reason for this result was unknown in detail, it might be due to the denaturation of gelatin.

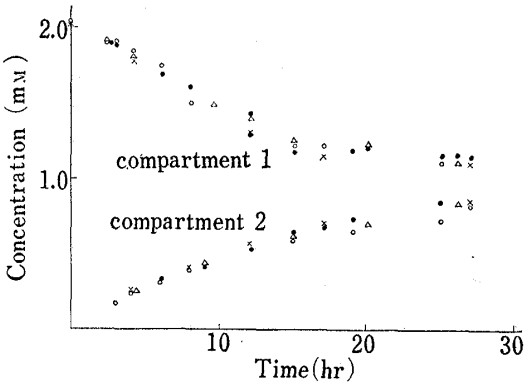

Fig. 3. Permeation of Barbituric Acid Derivatives through Gelatin Membrane

× barbital △ phenobarbital
● pentobarbital ○ secobarbital

Correlation between the Adsorption by Carbon Black from Aqueous Solution and the Biopharmaceutical Data of Barbituric Acid Derivatives

The absorption rate constant[6] and the fraction absorbed for a given period[11] of barbituric acid derivatives have been reported, both values increasing with molecular volume contrary to the permeation through Visking tube described above. Therefore, the transport process might be a secondary factor in the drug absorption, and the adsorption or uptake by membrane surface might have a possibility to be related to the drug absorption.

In the previous paper,[1] a good correlation was given between the adsorbability by carbon black[12] and the partition coefficient in 1-octanol/water system of barbituric acid derivatives, suggesting that carbon black could be a model of membrane surface for the

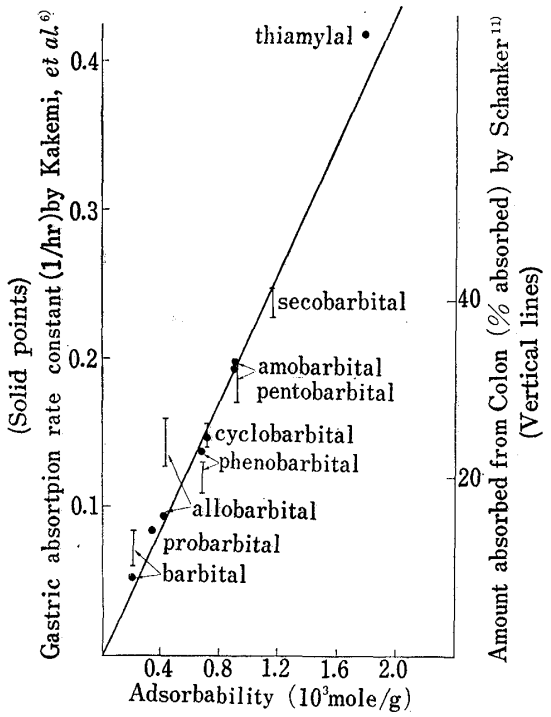

Fig. 4. Relationship between The Absorption Data and The Adsorbability of Barbituric Acid Derivatives

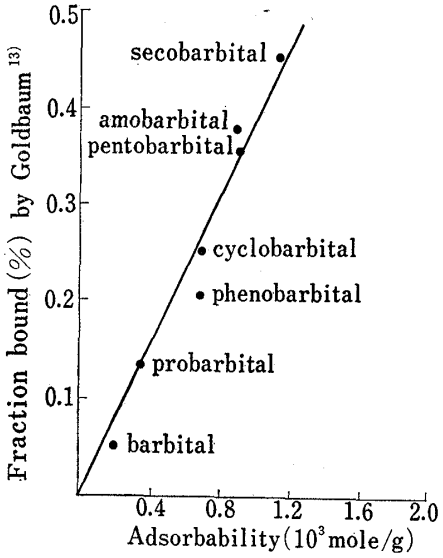

Fig. 5. Relationship between The Binding to Bovine Serum Albumin and The Adsorbability of Barbituric Acid Derivatives

11) L.S. Schanker, *J. Pharmacol. Exptl. Therap.*, **126**, 283 (1959).
12) The term adsorbability is defined in this study as the amount adsorbed at the equilibrium concentration 0.25×10^{-3} M at 40°.

absorption process. Plotting the absorption data by Kakemi, *et al.*,[6] and by Schanker[11] against the adsorbability of barbituric acid derivatives by carbon black, very good correlations were given as shown in Fig. 4. Therefore, it might be concluded that the drug absorption was controlled by adsorption or uptake by membrane and that the investigation on the adsorbability of drug by carbon black *in vitro* was useful to estimate the absorbability *in vivo*. Moreover, it was demonstrated that this kind of physicochemical study would be significant as an approach to biopharmaceutical phenomena.

Various kinds of biological phenomena, particularly pharmacological ones, have been considered to have relation to protein binding of drug. Plotting the data of the binding to bovine serum albumin by Goldbaum[13] against the adsorbability of barbituric acid derivatives by carbon black, a good correlation was given as shown in Fig. 5. Accordingly, it might be expected that the adsorption by such a hydrophobic substance as carbon black had some relation to the pharmacological action. Table II shows the adsorption by carbon black in

Table II. Relationship between Pharmacological Data and Adsorbability of Barbituric Acid Derivatives

Barbiturate	1	2	3	4	5	6
Barbital	0.23	110	480	150	33	22
Probarbital	0.31	70	130	100	—	9.2
Allobarbital	0.46	41	—	—	40	—
Phenobarbital	0.70	15	43.5	80	—	12.3
Cyclobarbital	0.75	11	—	—	—	—
Pentobarbital	0.90	6.1	8.5	—	55	0.1
Amobarbital	0.90	5.0	7.5	40	83	0.2
Secobarbital	1.71	1.2	6.5	—	90	0.1

1. adsorption (10^3mole/g) by carbon black at 0.25 mM equilibrium concentration at 40°
2. concentration (10^{-4}M) producing 50% inhibition of Arbacia egg cell division in sea water [14]
3. concentration (10^{-4}M) producing 50% inhibition of rat brain cortex oxygen consumption [15]
4. inability to rise of rabbit when shaken (dose mg./kg. of body weight) [16]
5. effect on DPNH oxidase (%) at final concentration (10^{-3}M) [17]
6. delay in onset of anesthetic action (in minute) following intravenous administration to mice [18]

relation to pharmacological data reported by the respective workers,[14–18] where a fairly good correlation may be found. Considering these facts, the appearance of pharmacological action would be related to the adsorption process. However, there are a number of barbituric acid derivatives having no pharmacological activity, which have similar chemical structures to the usually employed remedy, being anticipated to be adsorbed well by carbon black, and which are not also explained by the structure-activity correlation studied by Hansch, *et al.*[19] Therefore, it is very difficult to estimate the pharmacological effect completely by physico-chemical methods only.

Finally, the physico-chemical approach by adsorption study attemped in this paper was useful to estimate the extent and mechanism of biopharmaceutical phenomena, showing that some biological process could be explained physico-chemically.

13) L.R. Goldbaum and P.K. Smith, *J. Pharmacol. Exptl. Therap.*, **111**, 197 (1954).
14) G.H.A. Clowes, A.K. Kletch, and M.E. Krahl, *J. Pharmacol. Exptl. Therap.*, **68**, 312 (1940).
15) F.A. Fuhrman and J. Field, *J. Pharmacol. Exptl. Therap.*, **77**, 392 (1943).
16) H.A. Shonle and A. Moment, *J. Am. Chem. Soc.*, **45**, 243 (1923).
17) M.L. Cowger, R.F. Labbe, and B. Mackler, *Arch. Biochem. Biophys.*, **96**, 583 (1962).
18) T.C. Butler, *J. Pharmacol. Exptl. Therap.*, **74**, 118 (1942).
19) C. Hansch and S.N. Anderson, *J. Med. Chem.*, **10**, 745 (1967).