of the 2-hydrogen, measured by NMR spectrum (τ 0.20), was within 3 min. (at 34°, pH of an aqueous solution of I (0.10 mole) was 3.70).

The Reaction of II with Potassium Cyanide in Deuterium Oxide——To a solution of II (0.50 g.) in D_2O (5.0 ml.) was added a solution of KCN (0.50 g.) in D_2O (2.0 ml.) with stirring. The precipitated oil was worked up as described above. The intensitive ratios of signal peaks of C-CH_3 groups to N-CH_3 groups of XVII and V obtained here in NMR spectra were 2.25/3.0 and 2.75/3.0, respectively.

The both 2-protons of the product XVII and V were not exchanged by deuterium in deuterium oxide under the same condition as the reaction was carried out.

The authors express their gratitude to Prof. Emeritus E. Ochiai of the University of Tokyo and Dr. K. Takeda, Director of this Laboratory, for their helpful guidance and encouragement. Thanks are also due to the members of the Physical Chemistry Department for the spectral measurements, to the members of the Analysis Room for the elemental analysis, and to Mr. S. Hashimoto for his technical assistance.

Summary

Reactions of 1-methyl- or 1,2-dimethyl-3-methoxybenzimidazolium iodide (I or II) with various nucleophilic reagents (CN^-, OH^-, RO^-, amines etc.) have been investigated.

Most of these reagents react with I to give the corresponding 2-substituted 1-methylbenzimidazole.

The reaction of II with these reagents generally affords 1,2-dimethylbenzimidazole (V) losing its methoxy group. Only with potassium cyanide, II undergoes substitution concurrently to give a mixture of 1,2-dimethyl-6-benzimidazolecarbonitrile and V.

The reaction mechanisms have been discussed as compared with those of N-alkoxypyridinium salt, and a possible zwitterion intermediate for the reaction of I is noted.

(Received September 22, 1965)

[Chem. Pharm. Bull. 14(4) 385~391 (1966)]

UDC 615.41-033

53. Hisao Matsumoto,*1,*2 Hisakichi Matsumura,*1 and Sadao Iguchi*3 : Studies with Static Dialysis Method on the Release of Drugs from Nonionic Surfactant Solutions. I. Permeation of Tween 80 through Cellulose Membrane.

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There are many reports describing that surfactants influence the availability of solubilized drugs.[1] It may be considered that the most important effect among them is on the permeability of drugs through biologic membrane, but its mechanism is not

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1) N. A. Allawala, S. Riegelman : J. Am. Pharm. Assoc., Sci. Ed., **42**, 267 (1953); C. A. Lawrence, A. L. Erlandson : *Ibid.*, **42**, 352 (1953).

well known. The effect may be divided into two classes. One is the change in membrane permeability characteristics brought by surfactants and another is interaction between drug and surfactant.[2] In this series of reports, the authors intended to investigate mainly the latter effect utilizing artificial semi-permeable membrane.

Kostenbauder[3] reported that cellulose membrane could not be used for equilibrium dialysis, because it was not impermeable to Tween, and then nylon membrane was used in their experiments. But it was pointed out that the nylon membrane has a tendency to bind phenolic compounds and swells gradually. Patel[4] found that thin rubber membrane was satisfactory for the dialysis experiment, but we found some defects in it. For example, it is difficult to maintain the thickness constant, and the membrane is easy to break. Therefore these membranes were considered to be unsuitable for kinetic investigation.

In this report, we re-examined at first on the permeation of nonionic surfactants through a cellulose membrane utilizing the new determination method which is based on the increase of the solubility of an oil soluble pigment in the presence of surfactant micelles.

With this method, it became evident that permeation of Tween and Brij type surfactants is negligible, although impurities, especially low molecular weight polyethylene glycol,[5] are dialyzed rapidly. Therefore the cellulose membrane could be used in equilibrium dialysis experiments, and kinetic dialysis experiment for the more extended period was also expected to be possible.

Experimental

Materials——yellow AB, sudan Ⅲ, oil orange SS, polyethylene glycol 400, phosphomolybdic acid and Tsuda reagent*4 were extra pure reagent grade. Ethyl *p*-aminobenzoate was recrystallized (m.p. 92～93). Tween 80*5 (polysorbate 80) and Nikkol BL-25*6 (Brij 35 type) were commercial products and used without any purification.

Membrane——Seamless cellulose tubing,*7 size 24/32, was used. It was cut to every 8 cm. in length and folded twice every 0.2 cm. from the one edge. Then each was folded vertically in half at that edge and again inversely folded each side in turn. Next, it was folded in "S" character type and bound with cotton thread five times tightly. Then it was immersed in deionized water and spread to form a cellulose membrane bag. After two days' immersion, it was used for dialysis experiments.

Dialysis Apparatus and Procedure——Inner apparatus : The cellulose bag was rinsed with deionized water and placed between filter papers, and water was squeezed out completely. Then it was attached with a rubber ring at the lower end of a glass cylinder (1.5 cm. in diameter, 10 cm. in length). The cellulose bag was spread by air blowing and a sample solution (4.0 ml.) was pipetted into it as the inner solution.

Outer apparatus : Round bottomed test tubes (2.7 cm. in the inner diameter, 10 cm. in length) containing 25 ml. of deionized water as the outer fluid were used. They were held in a constant temperature water bath at 30°.

Procedure : The inner apparatus was immersed in the outer fluid to the same surface level of these solutions and fixed with a rubber stopper. At this time, it was determined as starting time of dialysis. During the dialysis experiments, any stirring was not supplied to both inner and outer fluids. After definite time intervals, the inner apparatus was picked up and analyzed.

Absorbancy Measurement of Surfactant Solutions Saturated with a Pigment——Excess quantities of a pigment were placed in measuring flasks (25 ml.) together with Tween 80, BL-25 or polyethylene glycol

*4 N-(2-diethylaminoethyl)-1-naphthylamine oxalate.
*5 Polyoxyethylene sorbitan monooleate, Atlas Powder Co., Wilmington, Del.
*6 Polyoxyethylene lauryl ether, Nikko Chemicals Co., Tokyo.
*7 Visking Co., Chicago, Ill.

2) G. Levy : "Prescription Pharmacy," Chap. 2 (1963). J. B. Lippin Cott Co., Philadelphia.
3) N. K. Patel, H. B. Kostenbauder : J. Am. Pharm. Assoc., Sci. Ed., **47**, 289 (1958).
4) N. K. Patel, N. E. Foss : J. Pharm. Sci., **53**, 94 (1964).
5) K. Nagase, K. Sakaguchi : Kôgyô Kagaku Zasshi, **64**, 1199 (1961).

(PEG) 400 solutions of varying concentration. These flasks were placed in a mechanical shaker and equilibrated at 30° for 72 hr. Then the saturated solutions were filtered through glass filter (G-4) at 30°, and the absorbancy was measured with spectrophotometer (Hitachi EPU-2 type) after appropriate dilution with water or 99% EtOH.

Determination of the Permeation Rate of Surfactants——Yellow AB solubilization method : Each sample (10 ml.) of the outer fluids obtained from the dialysis experiments of surfactant solutions was transferred into a measuring flask (25 m.) and excess quantity of yellow AB powder was added. Then it was equilibrated with the method described above and absorbancy of the filtrates was measured. From the relationship between absorbancy and concentration of surfactants, shown in Fig. 3, each concentration of the surfactants was determined and the rate of permeation was calculated.

Phosphomolybdic acid method : It was carried out colorimetrically with the method described by Takayama[6] with a little modification. After the color development, the absorbancy was measured in a 1.0 cm. glass cell at 470 mμ. Calibration curves were made using the same surfactant solutions (10 ml. of 10^{-3}~10^{-4}% standard solutions).

Determination of ratio, *r*, of Drugs in Surfactant Solutions——Solubility method : Excess quantities of benzocaine were placed in measuring flasks (25 ml.) together with Tween 80 solutions of varying concentration and equilibrated at 30°. Rate of total solubility in each Tween solution to the aqueous solution was plotted as ratio, *r*.

Equilibrium dialysis method employing cellulose membrane : The inner apparatus containing each sample (4.0 ml.) of $2.5\times10^{-3}M$ benzocaine in Tween 80 solutions of varying concentration were immersed in the outer apparatus containing 15 ml. of deionized water, and these were allowed to equilibrated for 18 hr. at 30° without any stirring. These conditions were proved to be enough to reach the equilibrium. Then the concentrations of benzocaine in inner and outer solutions were determined colorimetrically. The concentration of inner Tween solutions was corrected for the volume change after 18 hr. dialysis. The concentration ratio of benzocaine (inner/outer) was plotted as the value of ratio, *r*.

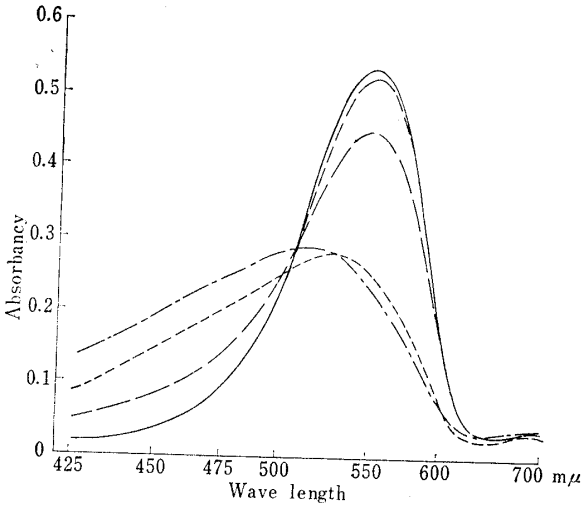

Fig. 1. Absorption Spectra of Color Developed Solution of Benzocaine with Tsuda Reagent in the Presence of Tween 80

Benzocaine : $1\times10^{-5}M$
Tween 80 : ——— 0%, 5×10^{-5}%
— — — 5×10^{-3}% ——— — 0.5%
—— —— 5×10^{-2}% ------ 5.0%

Sodium salicylate was dissolved at the same concentration ($10^{-3}M$) both in the inner Tween solutions and in the outer aqueous solutions, and equilibrated in the same manner as benzocaine, and then ratio, *r*, was determined.

Determination of Benzocaine in Tween 80 Solutions——Aliquot portions of a sample solution were pipetted into a graduated test tube and distilled water was added to make up to 5 ml. Then 2*N* HCl (2 ml.) and 0.2% $NaNO_2$ (0.4 ml.) were added, and shaken for 3 min. To this, 0.5% $NH_4SO_3NH_2$ (0.4 ml.) was added and it was shaken for 3 min. Then 0.5% Tsuda reagent (1 ml.) was added and mixed. After 30 min., absorbancy was measured at 550 mμ.

The calibration curve showed a straight line. Color development with this method was reduced a little in the presence of Tween 80. But, at the definite concentration of Tween 80, the calibration curve also indicated a straight line. Therefore accurate benzocaine concentration could be obtained by compensating for the inhibition rate of the color development in the presence of Tween 80 (below 0.08%) (Fig. 1).

Results and Discussion

Determination of Small Quantity of Surfactants with Yellow AB Solubilization Method

An oil soluble pigment, yellow AB, oil orange SS or sudan Ⅲ was solubilized up to its solubility at 30° into Tween 80 solutions of varying concentration, and each optical absorbancy of these filtrates was measured. Relation between absorbancy and concentration of the Tween solutions becomes linear as shown in Fig. 2. Especially, the filtrates of

6) Y. Takayama : Kôgyô Kagaku Zasshi, **60**, 872 (1957).

yellow AB did not show any absorbancy at 450 mμ in the absence of Tween and the most intense absorbancy among them with the increasing concentration of Tween 80. Then the Tween solution was further diluted with water to the extremely diluted concentration and each absorbancy of the filtrates solubilizing yellow AB was measured. The log-log plots of these results illustrate gently sloping curves at the extremely diluted parts and do not show a distinct critical micellar concentration as represented in Fig. 3.

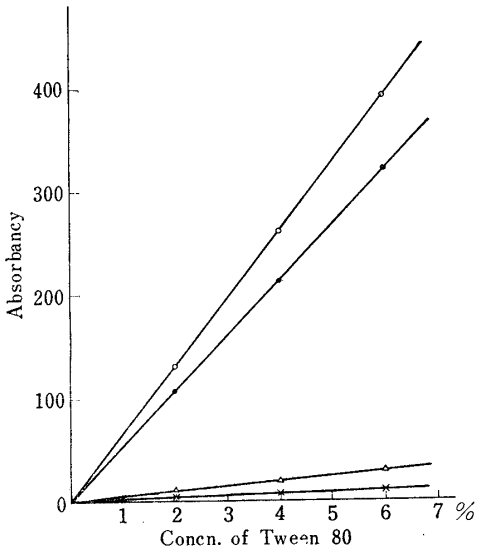

Fig. 2. Relationship between Concentration of Tween 80 and Absorbancy of the Solutions Saturated with Pigments

—○— yellow AB at 275 mμ
—●— yellow AB at 450 mμ
—△— oil orange SS at 480 mμ
—×— sudan III at 506 mμ

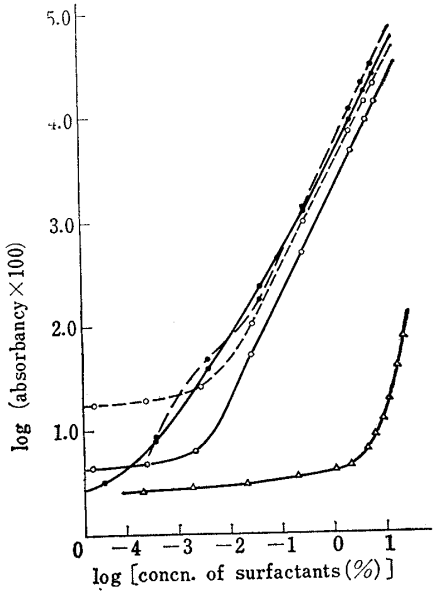

Fig. 3. Absorbancy of Yellow AB Solubilized up to the Solubility in Surfactant Solution to the Extremely Dilute Concentration

● Tween ○ BL-25 △ PEG 400
——— measured with aqueous dilution at 450 mμ
– – – – measured with EtOH dilution at 450 mμ

Absorbancy was also measured on BL-25 solutions saturated with yellow AB, and in this case, somewhat distinct critical micellar concentration was observed between 10^{-2} and 10^{-3} per cent of BL-25.

Furthermore, yellow AB was solubilized to PEG 400 solutions which may exist in

Table I. Determination of Concentration of Tween 80 with Yellow AB Solubilization Method in the Presence of Excess Amount of Polyethylene Glycol

Concn. of Tween 80 (%)	Coexisting PEG 400 (%)	PEG / Tween	Absorbancy at 450 mμ (Saturated with yellow AB at 30°)	Obtained concn. of Tween 80 (%) from Fig. 3.
0	0	—	0.027	0
4×10^{-4}	0	—	0.075	4.0×10^{-4}
4×10^{-4}	0.5×10^{-3}	1.25	0.078	4.4×10^{-4}
4×10^{-4}	1.0×10^{-3}	2.50	0.081	4.8×10^{-4}
4×10^{-4}	5.0×10^{-3}	12.5	0.083	5.0×10^{-4}
4×10^{-4}	1.0×10^{-2}	25.0	0.083	5.0×10^{-4}
4×10^{-4}	2.0×10^{-2}	50.0	0.089	5.6×10^{-4}
4×10^{-4}	4.0×10^{-2}	100.0	0.082	4.9×10^{-4}

the surfactants as one of the impurities, and the absorbancy of the filtrates was measured. As shown in Fig. 3, the absorbancy was negligible below 1% PEG concentration.

Then it became possible to determine small quantity of these surfactants about to 10^{-3}% order, even in the presence of polyethylene glycols (Table I).

Permeation of Nonionic Surfactants through Cellulose Membrane

A sample (4 ml.) of 4% Tween 80 solution in the cellulose bag was dialyzed toward 25 ml. of deionized water as the outer fluid during various period of time. Tween 80 concentration appeared in the outer fluid was determined with phosphomolybdic acid (PMA) method and also with yellow AB solubilization method. The former is sensible for any derivatives of polyethylene glycol and the latter is insensible for the polyethylene glycol chain itself.

The upper curve with closed circles in Fig. 4 represents the PMA method. On the other hand, bottom curve with open triangles shows very little permeation as the result of yellow AB solubilization method. Permeation of BL-25 was also detrmined and similar results with Tween 80 were obtained as shown in Fig. 4.

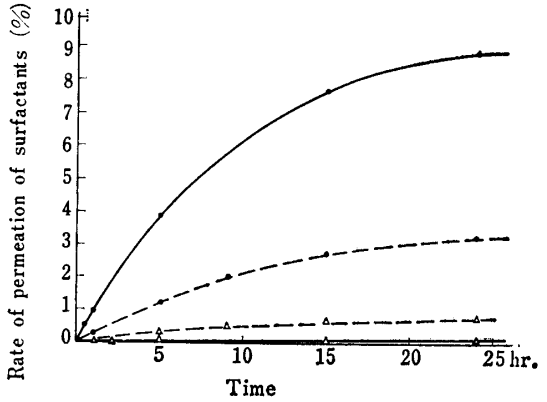

Fig. 4. Permeation of Nonionic Surfactants through Cellulose Membrane with Lapse of Time

Inner solution : ——— 4% Tween 80, 4 ml.
— — — — 4% BL-25, 4 ml.
Outer fluid : deionized water, 25 ml.

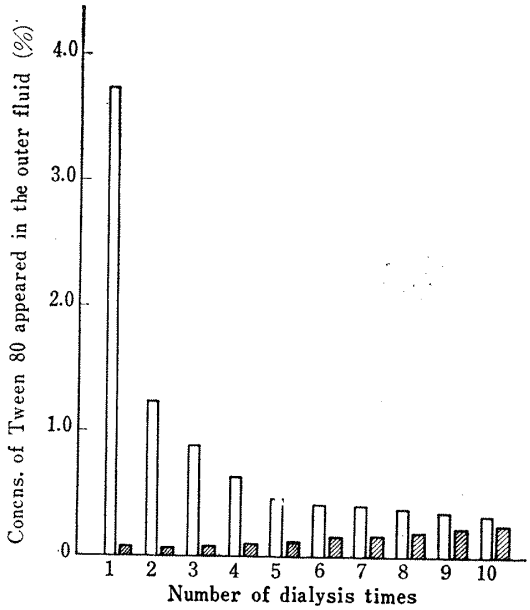

Fig. 5. Permeation of Tween 80 through Cellulose Membrane as the Results of Repeated Dialysis

Inner solution : 4 ml. of 4% Tween 80
Outer fluid : 25 ml. of deionized water
Period of once dialysis : 14 hours
□ with PMA method ($\times 10^{-2}$%)
▨ with yellow AB solubilization method ($\times 10^{-3}$%)

In order to ascertain the relatively rapid permeation of polyethylene glycol, a sample (4 ml.) of 4% Tween 80 solution was dialyzed toward 25 ml. of deionized water and the outer fluid was replaced with fresh outer fluid every 14 hours and analyzed.

As shown in Fig. 5, the quantity of permeation at the first time dialysis is very large with the result of PMA method, but it decreases rapidly with the repeating of the dialysis. Whereas it shows nearly equal permeation over the repeating with the result of yellow AB method. A little increase of permeation is considered to be due to the increase of contact area of the membrane owing to the volume change of the inner Tween solution.

From these results, it may be reasonably considered that impurities, especially low molecular weight polyethylene glycol, permeate rapidly through the cellulose membrane, but the quantity of permeation of nonionic surfactant molecules is extremely small. These results are contrary not only to the report of Kostenbauder,[3] but also to the report of Nishida[7] who stated that free polyethylene glycol and nonionic surfactant molecules permeate through cellulose membrane accompanied each other.

Since the permeation of nonionic surfactant molecules was evidenced to be negligible in the above experiments, kinetic dialysis experiments for relatively long period must become possible. It will also be expected that cellulose membrane may be utilized for the purification of nonionic surfactants which has been very laborious.

Equilibrium Dialysis Method with Cellulose Membrane

Change in volume of inner solutions as the results of the transfer of water from the outer fluid by osmotic pressure and evaporation out of the inner solution were recognized, and determined by weighing the inner apparatus before and after the equilibrium dialysis for 18 hours. Changes in solubilizing ability owing to the loss of impurities during the dialysis experiment were also tested as following manner. Samples of Tween 80 solutions were dialyzed for 18 hours and solubility of yellow AB in these solutions was measured before and after the dialysis. It was ascertained that solubilizing abilities of these inner solutions remained almost unaffected. Hence, it became sure that equilibrium dialysis method employing the cellulose membrane is also possible.

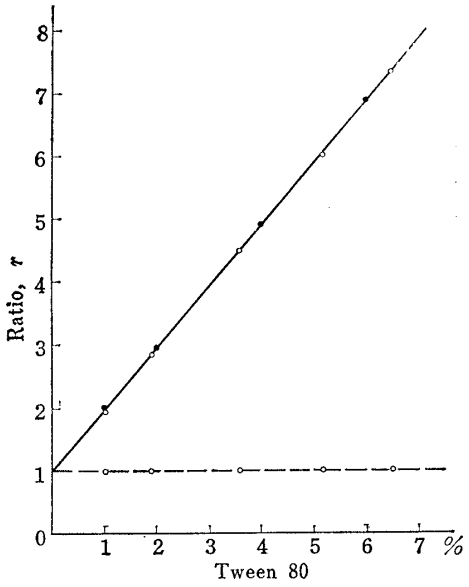

Fig. 6. Ratio, r, of Total to Free Drugs in Tween 80 Solutions

——— Benzocaine
— — — Sodium salicylate
● Obtained with solubility method at 30°
○ Obtained with equilibrium dialysis method employing cellulose membrane at 30°

Then benzocaine was selected as an example having the interaction with nonionic surfactants, which was previously investigated with ultraviolet absorption spectrum by Riegelman,[8] and the ratio, r, of total to free benzocaine in Tween 80 solutions was determined with this method. The results are illustrated in Fig. 6 and well agreed with those obtained with solubility method.

Degree of interaction of sodium salicylate with Tween 80 was also measured with the equilibrium dialysis method and illustrated in Fig. 6. It shows sodium salicylate has not any interaction with Tween 80.

Summary

A method of determining the small quantities of nonionic surfactants (Tween and Brij types) in the presence of polyethyene glycol was devised utilizing the increase of solubility of yellow AB.

With this method, permeation of nonionic surfactants through cellulose membrane was investigated and compared with the results obtained with phosphomolybdic acid method.

7) M. Nishida, *et al.*: Yakuzaigaku, **24**, 53 (1964).
8) S. Riegelman: J. Am. Pharm. Assoc., Sci. Ed., **49**, 339 (1960).

As the results, it became evident that impurities, especially low molecular weight polyethylene glycol, permeate rapidly, but permeation of nonionic surfactant molecules is negligible.

To obtain the magnitude of interaction between benzocaine or sodium salicylate and Tween 80, equilibrium dialysis experiments were successfully carried out employing the cellulose membrane.

(Received August 26, 1965)

[Chem. Pharm. Bull. 14(4) 391~398 (1966)] UDC 615.41-033 : 615.781

54. Hisao Matsumoto,[*1,*2] Hisakichi Matsumura,[*1] and Sadao Iguchi[*3] : Studies with Static Dialysis Method on the Release of Drugs from Nonionic Surfactant Solutions. II.[*4] Dialysis of Benzocaine Saturated in Tween 80 Solutions.

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According to the theory of micelle formation, it is considered that drugs in surfactant solutions are partitioned between "micellar phase" and surrounding "aqueous phase", and pointed out that efficiency of phenolic preservatives used in nonionic surfactant solutions is displayed not by the partitioned to the micellar phase but only by the remained in the aqueous phase.[1] Therefore it is recognized that the preservatives must be added to maintain their concentrations above the effective level in the aqueous phase.

If drugs in the aqueous phase in the surfactant solution are removed rapidly by some causes, it will be naturally expected that the drug molecules in the micellar phase distribute to the aqueous phase when sufficient time or stirring is supplied. Without these conditions, it is not yet thoroughly investigated whether such transfer of the drug molecules rapidly occurs or not.

In the past literature, Allawala[2] evidenced utilizing characteristics of bactericidal action of iodine that such transfer occurs instantaneously. But there remain some questions whether it was carried out without any stirring or not. Nishida[3] reported as the results of dialysis experiments of short time period that transfer of chlorobutanol from micellar phase did not occur even if moderate stirring was added and the drug in the aqueous phase rapidly removed by the dialysis.

At this time, the authors investigated these phenomena in more detail by prolonging the dialysis period and utilizing the static dialysis method which is not supplied any stirring. For it is considered these mechanism must have close relation to the absorption of various drugs through biologic membrane from solutions containing nonionic surfactants.

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*4 Part I. H. Matsumoto, H. Matsumura, S. Iguchi : This Bulletin, **14**, 385 (1966).
1) M. Matsumoto, M. Aoki : *Ibid.*, **10**, 251 (1962); W.P. Evans : J. Pharm. and Pharmacol., **16**, 323 (1963); A.G. Mitchell : *Ibid.*, **16**, 533 (1964).