dialysis of drug molecules. Applicability of theoretical equations of dialysis was tested and proved to be well applicable even if any stirring was not supplied.

But it was found that results of dialysis must be compared and discussed among the same condition of dialysis.

Dialysis from saturated solutions of benzocaine in Tween 80 solutions was studied and it was concluded that drug molecules in the micellar phase are transferred to the aqueous phase rapidly corresponding to decrease of the concentration of the drug in the aqueous phase even if any stirring is not supplied, then they participate in the dialysis to the outer fluid.

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55. Hisao Matsumoto[*1,*2]: Studies with Static Dialysis Method on the Release of Drugs from Nonionic Surfactant Solutions. III.[*3] Rapidity of Transfer of Drug Molecules from Micellar to Aqueous Phase.

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In the preceding paper,[*3] rate of dialysis of benzocaine was markedly decreased by the addition of Tween 80, and it was considered provisionally that it will be due to decrease of apparent dialysis coefficient, P'', which indicated inverse proportionality to the ratio, r.

But membrane permeability itself is not changed with nonionic surfactant. Therefore decrease of dialysis rate must be attributed to the decrease of drug concentration in the aqueous phase in the surfactant solution.

It was also evidenced that drug molecules solubilized in the micellar phase are rapidly transferred into the aqueous phase even if any stirring is not supplied, and that drugs only in this aqueous phase participate in dialysis.

In this report, theoretical equations were derived assuming that micellar to aqueous phase transfer of drug molecules occurs instantaneously corresponding to decrease of drug concentration in the aqueous phase, and the applicability was investigated.

When a drug is partitioned between micellar and aqueous phase with ratio, r (total to free), in a surfactant solution, and amount of dialyzed drug after time t is expressed as X_t, amount of drug in the aqueous phase in the inner solution, x_t' becomes

$$x_t' = \frac{x_0 - X_t}{r} \tag{1}$$

When unit A is used and volume of the outer fluid is limited to nV, concentration difference of the drug in the aqueous phase between inner and outer fluids, Δc, is given by

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[*3] Part II. H. Matsumoto, H. Matsumura, S. Iguchi: This Bulletin, 14, 391 (1966).

$$-\Delta c=\frac{x_0-X_t}{Vr}-\frac{X_t}{nV}=\frac{1}{rV}\left[x_0-\left(\frac{n+r}{n}\right)X_t\right] \qquad (2)$$

Substitution of Eq. (2) into Eq. (1) in the preceding paper[3] and integration give

$$\frac{nr}{n+r}\ln\frac{\left(\frac{n}{n+r}\right)x_0}{\left(\frac{n}{n+r}\right)x_0-X_t}=\left(\frac{AP}{\Delta lV}\right)t=P't \qquad (3)$$

where P' is apparent permeability coefficient and it is coincident with Eq. (4) in the preceding paper. Then, if this equation is applicable, P' must become constant independent to volume of the outer fluid and ratio, r.

For the sake of comparison, the other equation is derived assuming that transfer of drug molecules from the micellar phase does not occur entirely. At this time, amount of the drug in the aqueous phase in the inner solution at time t becomes

$$x_t'=\frac{x_0}{r}-X_t \qquad (4)$$

Concentration difference of the drug in the aqueous phase between inner and outer fluids given by

$$-\Delta c=\frac{1}{V}\left[\frac{x_0}{r}-X_t\right]-\frac{X_t}{nV}=\frac{1}{V}\left[\frac{x_0}{r}-\left(\frac{n+1}{n}\right)X_t\right] \qquad (5)$$

Hence, dialysis equation becomes

$$\frac{n}{n+1}\ln\frac{\left(\frac{n}{n+1}\right)\left(\frac{x_0}{r}\right)}{\left(\frac{n}{n+1}\right)\left(\frac{x_0}{r}\right)-X_t}=\left(\frac{AP}{\Delta lV}\right)t=P't \qquad (6)$$

When unit B, whose volume of the outer fluid is large enough to neglect the drug concentration in it during dialysis experiment, is used, concentration difference of the drug in the aqueous phase, Δc, given by

$$-\Delta c=\frac{1}{Vr}(x_0-X_t) \qquad (7)$$

Substitution of Eq. (7) into Eq. (1) in the preceding paper and integration give

$$r\ln\frac{x_0}{x_0-X_t}=\left(\frac{AP}{\Delta lV}\right)t=P't \qquad (8)$$

For the sake of comparison, assuming that transfer of the drug from the micellar phase does not occur entirely, equation becomes as follows:

$$\ln\frac{\left(\frac{x_0}{r}\right)}{\left(\frac{x_0}{r}\right)-X_t}=P't \qquad (9)$$

Experimental

Materials——Emulgen 120[4] was commercial product. Sulfamin and chlorobutanol were recrystallized

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[4] Polyoxyethylene lauryl ether, Kao Soap Co., Tokyo.

from J. P. grade. The other reagents were the same as used in the preceding paper.

Dialysis Apparatus and Procedure——Unit A and unit B were used, and performed in the same manner as described in the preceding paper.

Determination of Methylparaben and Benzocaine——It was carried out as described in the preceding paper.

Determination of Sulfamin——It was carried out in the same procedure as benzocaine (Fig. 1).

Determination of Propylparaben——A sample solution containing Tween 80 was diluted appropriately with water and finally EtOH was added to give 80% EtOH solution. Then the absorbancy was measured at 256 mμ. Additive property of absorbancy in the presence of Tween 80 was confirmed (Fig. 2). In the dialysis experiments on propylparaben from surfactant solutions using unit A, small amounts of dialyzed propylparaben were exactly determined in the absence of Tween 80.

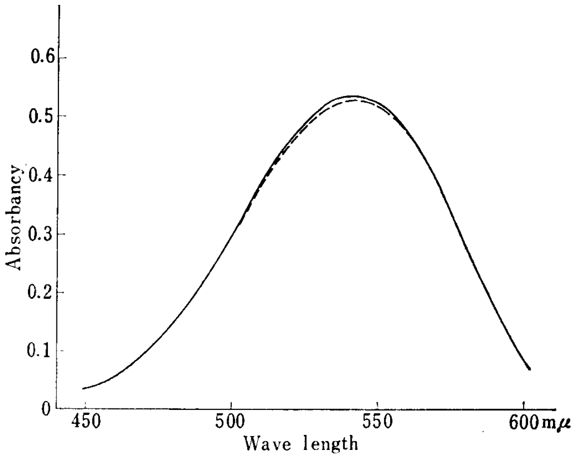

Fig. 1. Absorption Spectra of Color Developed Solutions of Sulfamin with Tsuda Reagent in the Presence of Tween 80

Sulfamin : 1x10^{-5}M

Tween 80 : ———— 0%, 0.04%

– – – – 0.08%

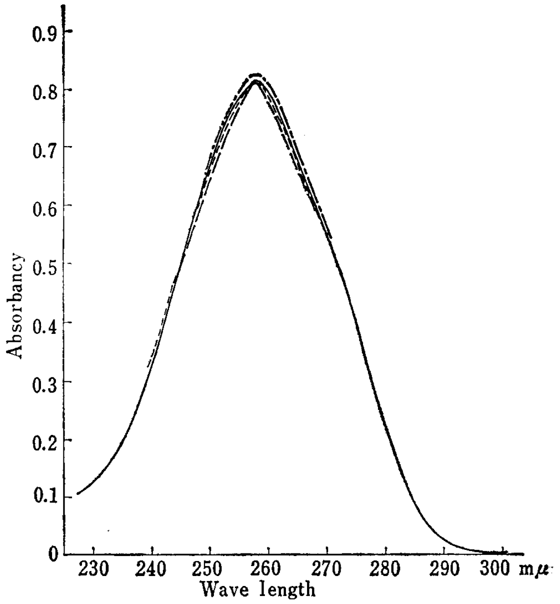

Fig. 2. Absorption Spectra of Propylparaben in the Presence of Tween 80

Solvent : 80% EtOH solution

Reference : Tween 80 wes added to the same concentration with sample solutions

Propylparaben : 0.5x10^{-4}M

Tween 80 : ———— 0% – – – – 0.6%

— — — 0.2% –·–·– 0.8%

Determination of Chlorobutanol——It was determined by alkaline hydrolysis followed by determination of chloride ion by titration with 0.01N AgNO$_3$.

Determination of Ratio, *r*——Ratios, *r*, of methylparaben and propylparaben were determined with equilibrium dialysis method at 30° as described in the preceding paper.[1] On sulfamin and chlorobutanol it was determined with solubility method as described in the preceding paper.

Results and Discussion

Applicability to Dialysis Equations for Unit A

At first, applicability of theoretical equations were tested using unit A. Lower concentration solution (10^{-3}M) of methylparabene, whose interaction[2] with nonionic surfactant is well known, was used as the inner solution containing 0 or 2% Tween 80, and it was dialyzed statically to 25 ml. of deionized water at 30°. The ratio, *r*, of methylparaben in 2% Tween 80 solution was relatively small (2.46) as shown in Fig. 10.

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[1] H. Matsumoto, *et al.* : This Bulletin, **14**, 385 (1966).

[2] N. K. Patel, H. B. Kostenbauder : J. Am. Pharm. Assoc., Sci. Ed., **47**, 289 (1958); S. M. Blaug, S. S. Ahsan : J. Pharm. Sci., **50**, 441 (1961); F. W. Goodhart, A. N. Martin : *Ibid.*, **51**, 50 (1962).

Fig. 3 shows remaining percentage of methylparaben with lapse of time. Dialysis of methylparaben in the Tween solution is restrained, but it decrease even after 120 minutes more than equilibrium value which is calculated assuming that only free paraben dialyzed into the outer fluid and distributed between inner and outer fluids.

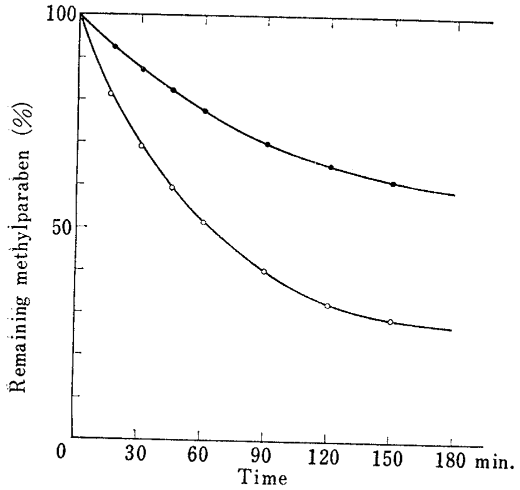

Fig. 3. Effect of Tween 80 on the Remaining Percentage of Methylparaben in the Inner Solution as a Function of Dialysis Period

Inner solution: $10^{-3}M$ methylparaben solution, 4 ml.
—○— in aqueous solution
—●— in 2% Tween 80 solution
Apparatus: unit A

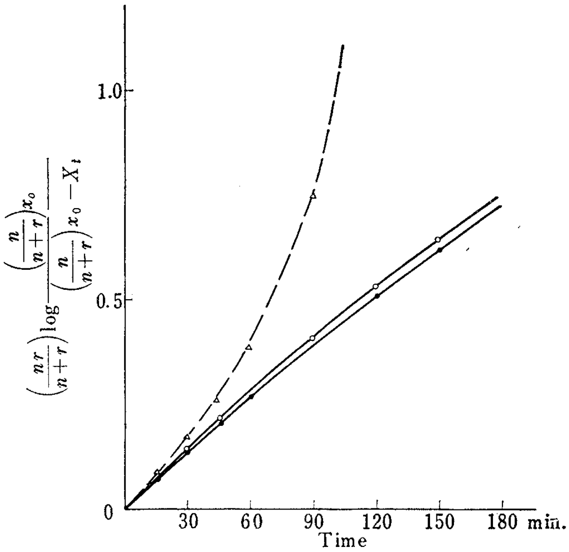

Fig. 4. Linear Relationship between Calculated Values with Eq. (3) and Dialysis Period

—○— aqueous solution (calculated with Eq. (3))
—●— 2% Tween 80 solution (calculated with Eq. (3))
—△— 2% Tween 80 solution (calculated with Eq. (6))

Then, above values were calculated with Eq. (3) and plotted in Fig. 4. It shows linear relationship both in aqueous and in 2% Tween 80 solutions, and these slopes,

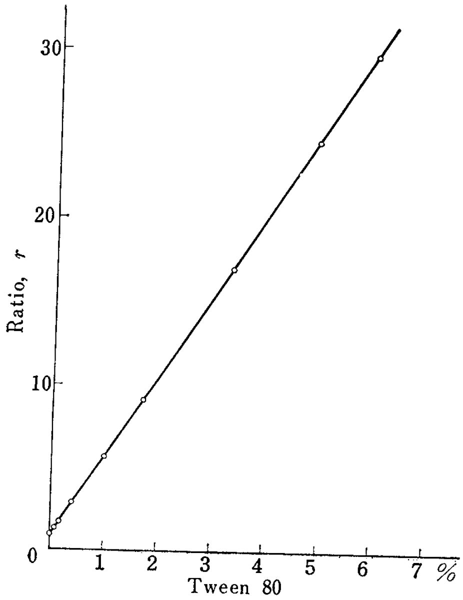

Fig. 5. Ratio, r, of Total to Free Propylparaben in Tween 80 Solutions

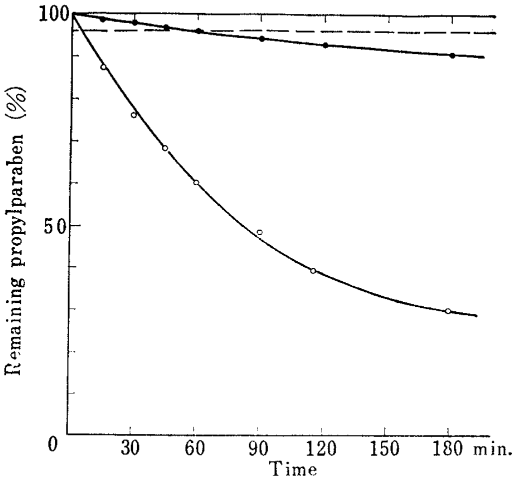

Fig. 6. Effect of Tween 80 on the Remaining Percentage of Propylpareben in the Inner Solution as a Function of Dialysis Period

Inner solution: $0.6\times10^{-3}M$ propylparaben solution, 4 ml.
—○— in aqueous solution
—●— in 2% Tween 80 solution
Apparatus: unit A

corresponding to apparent permeability coefficient, P', are showing the same value. Then it could be considered Eq. (3) is applicable.

For the sake of comparison, calcurated values with Eq. (6) were also plotted in Fig. 4, and it indicates marked change in the slope with lapse of time. Therefore it was confirmed Eq. (6) is not applicable at all.

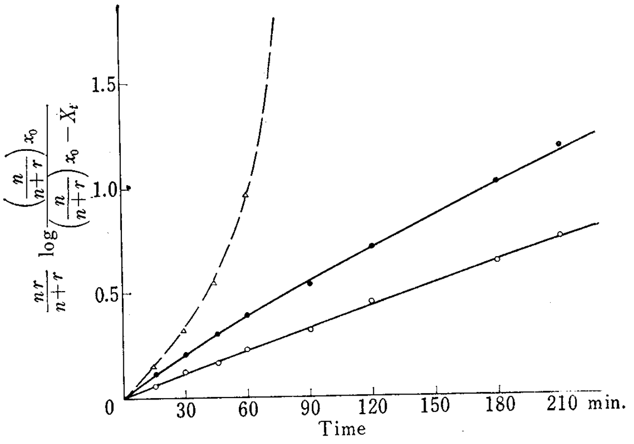

Fig. 7. Linear Relationship between Calculated Values with Eq. (3) and Dialysis Period

—○— aqueous solution (calculated with Eq. (3))
—●— 2% Tween 80 solution (calculated with Eq. (3))
—△— 2% Tween 80 solution (calculated with Eq. (6))

Next, as an example whose ratio, r, is very large, effect of Tween 80 on the dialysis of propylparaben was investigated. The ratio, r, was determined and illustrated in Fig. 5.

Fig. 6 shows the remaining percentage of propylparaben in the inner solution and the rate of dialysis of propylparaben becomes extremely small in the presence of Tween 80. But it decreases already after 60 minutes more than equilibrium value calculated assuming that only free paraben distributed between inner and outer fluids. Then these values were calculated with Eq. (3) and plotted in Fig. 7.

In both cases, aqueous and 4% Tween solutions, straight lines can be drawn through origin, but the slope in the case of the Tween solution is a little larger than that of aqueous solution. It means amounts of dialysis of propylparaben were larger than the theoretical values in the case of the Tween solution. Then it must be considered that some factors promoting the dialysis of propylparaben participated in addition to the instantaneous transfer of the drug from the micellar phase.

From the above results, it was evidenced that drug molecules in the micellar phase are transferred to the aqueous phase instantaneously without any stirring and the theoretical equation derived from this assumption is well applicable when the ratio, r, is relatively small.

Applicability to Dialysis Equations for Unit B

Theoretical equations for unit A were a little complicated and inconvenient to consider the meaning, but equations for unit B are indicating rather simplicity. Then dialysis experiments using unit B were also carried out.

A sample of $10^{-3}M$ benzocaine in aqueous or 2% Tween solution was dialyzed to examine the applicability to the equations. Fig. 8 shows the remaining percentage of benzocaine in the inner solution with lapse of time. These values were calculated with Eq. (8) and plotted in Fig. 9. Straight lines could be drawn through origin and these slope values, corresponding to apparent permeability coefficient, P', agreed each other. Therefore the validity of Eq. (8) was also confirmed.

When concentration of a drug at the beginning and at time t in the inner solution is expressed as c_0 and c_t respectively, and r in Eq. (8) is transferred to the right part, the equation becomes

$$\ln\frac{c_0}{c_t}=\left(\frac{P'}{r}\right)t=P_d t \qquad (10)$$

$$\frac{P'}{r}=P_d \qquad (11)$$

where P_d is defined as apparent decreasing coefficient. Eq. (10) is identical with that

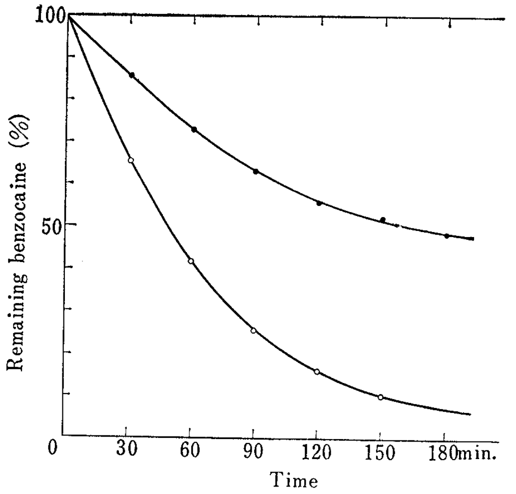

Fig. 8. Effect of Tween 80 on the Remaining Percentage of Benzocaine in the Inner Solution as a Function of Dialysis Period

Inner solution : $10^{-3}M$ benzocaine, 4 ml.
—○— in aqueous solution
—●— in 2% Tween 80 solution
Apparatus : unit B

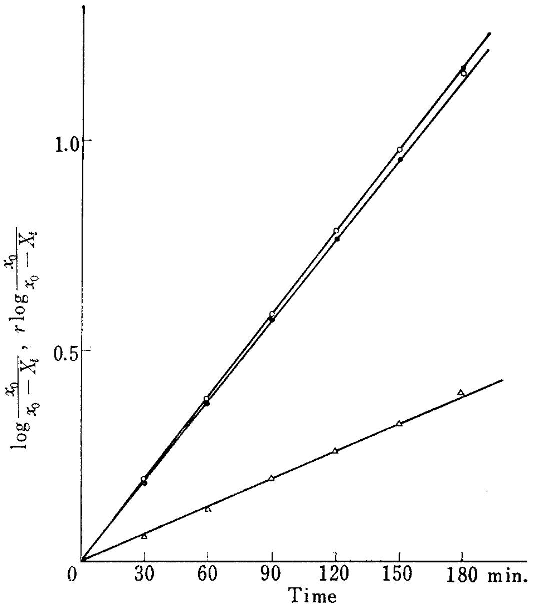

Fig. 9. Linear Relationship between Calculated Values with Eq. (8) and (10) and Dialysis Period

—○— aqueous solution (calculated with Eq. (8))
—●— 2% Tween 80 solution (calculated with Eq. (8))
—△— 2% Tween 80 solution (calculated with Eq. (10))

for the first order reaction, and Eq. (11) indicates the value of P_d becomes $1/r$ of apparent permeability coefficient, P'.

Lower plots with open triangles in Fig. 9 are calculated values with Eq. (10) and the slope value corresponds to P_d. Then the value of P_d becomes $1/r$ of P' of the aqueous solution. Therefore it is considered Eq. (10) is also applicable and relationship between P_d and ratio, r, becomes a hyperbola curve. Applicability to this relationship was examined using various drugs in the following.

Dialysis of Various Drugs

Prior to dialysis experiments, ratio, r, of each drug in surfactant solutions was determined at 30° and plotted in Fig. 10. On chlorobutanol, the ratio was determined in Emulgen 120 solutions to compare with Nishida's report.[3] The ratio of methylparaben was also determined at 12° and indicated a little larger value than at 30°. All these solutes except sodium salicylate were undissociated form when simply dissolved in these surfactant solu tions.

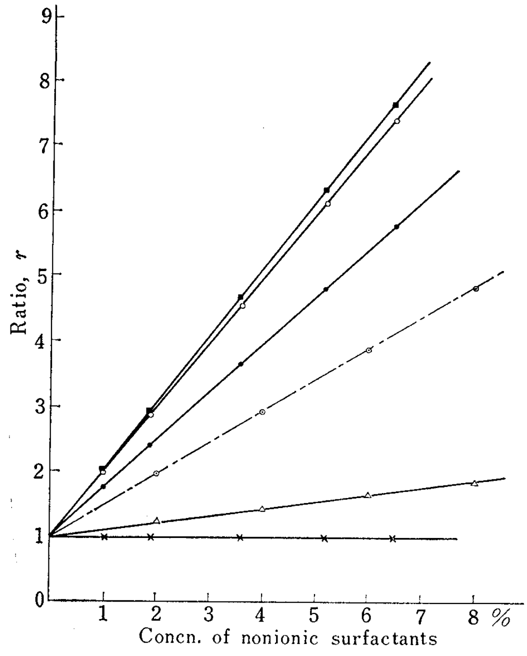

Fig. 10. Ratio, r, of Various Drugs in Nonionic Surfactant Solutions

● methylparaben (30°) ■ methylparaben (12°)
○ benzocaine (30°) △ sulfamin (30°)
× sodium salicylate (30°) ⊙ chlorobutanol (30°)
——— in Tween 80 solutions
—-— in Emulgen 120 solutions

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[3] M. Nishida, *et al.* : Yakuzaigaku, **22**, 257 (1962).

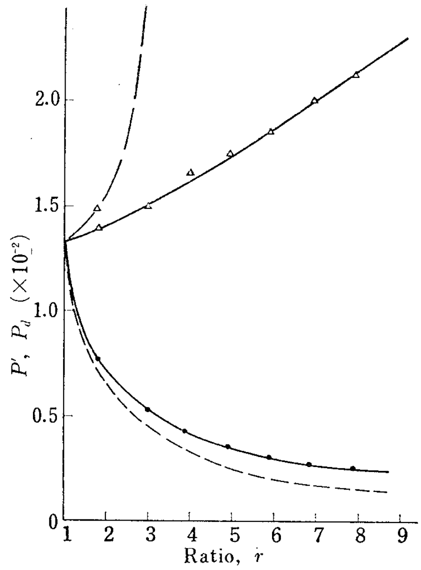

Fig. 11. Relationship between P' or P_d and Ratio, r, of Benzocaine in Tween 80 Solutions

Inner solution : $10^{-3}M$ benzocaine
Apparatus : unit B
——●—— P_d, with Eq. (10)
— — — theoretical curve of P_d, with Eq. (11)
——△—— P', with Eq. (8)
– –△– – P', with Eq. (9)

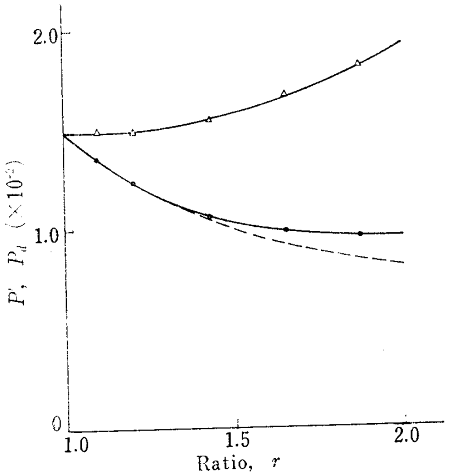

Fig. 12. Relationship between P' or P_d and Ratio, r, of Sulfamin in Tween 80 Solutions

Inner solution : $10^{-3}M$ sulfamin
Apparatus : unit B
——●—— P_d, with Eq. (10)
— — — theoretical curve of P_d, with Eq. (11)
——△—— P', with Eq. (8)
– –△– – P', with Eq. (9)

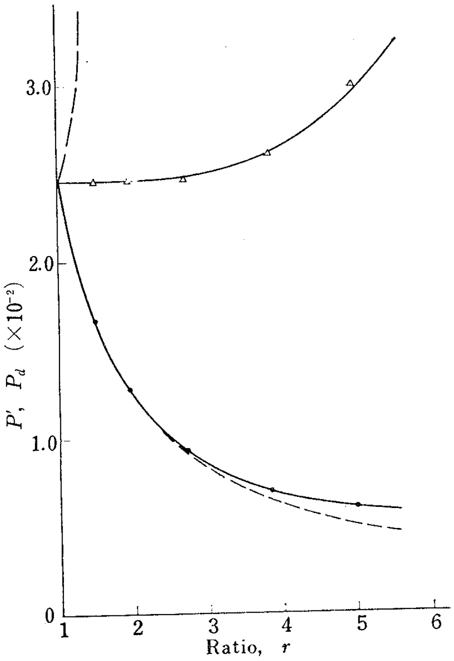

Fig. 13. Relationship between P' or P_d and Ratio, r, of Chlorobutanol in Emulgen 120 Solutions

Inner solution : $7.8\times10^{-2}M$ chlorobutanol
Apparatus : unit B
——●—— P_d, with Eq. (10)
— — — theoretical curve of P_d, with Eq. (11)
——△—— P', with Eq. (8)
– –△– – P', with Eq. (9)

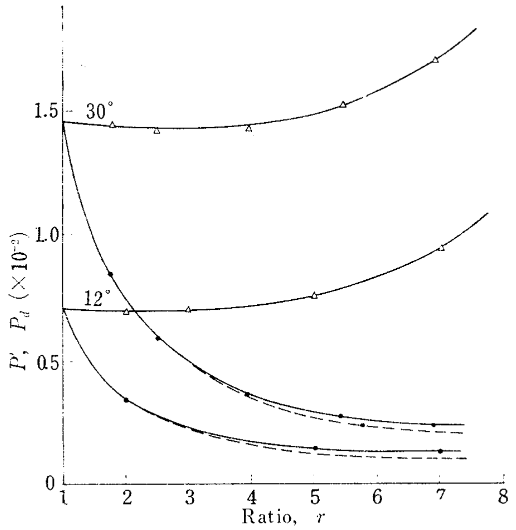

Fig. 14. Relationship between P' or P_d and Ratio, r, of Methylparaben in Tween 80 Solutions

Inner solution : $10^{-3}M$ methylparaben
Apparatus : unit B
——●—— P_d, with Eq. (10)
— — — theoretical curve of P_d, with Eq. (11)
——△—— P', with Eq. (8)

Using these drugs in surfactant solutions as inner solutions, static dialysis experiments were performed for 60 minutes. From these results, values of P_d were calculated with Eq. (10) and plotted against ratio, r, in Figs. 11, 12, 13 and 14. Theoretical hyperbola curves were obtained calculating with Eq. (11) using the value of P' in the case of the aqueous solution as the inner solution.

In all cases, each value of P_d agrees considerably well with the theoretical hyperbola curve, but these plots are showing tendency to become a little larger with increasing concentration of surfactants. Then each value of P_d was multiplied by each ratio, r, and they are also plotted in these figures. As mentioned above, P' must become constant independent to the ratio, r, but these plots indicate gradual increase.

To investigate the cause of these increase, P' was plotted against concentration of surfactants in Fig. 15. It shows degree of the increase is not parallel each other, and in the case of sodium salicylate the plots show a parallel line with axis of abscissa. Therefore it seems that these increases of P' do not relate directly to the surfactant concentration. On the other hand, P' values are plotted against ratio, r, in Fig. 16 and it shows obvious correlation between them.

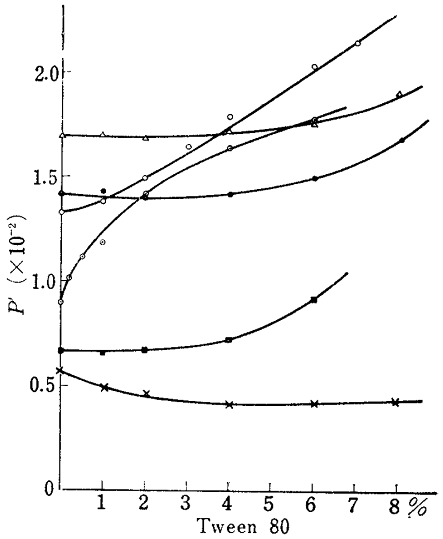

Fig 15. Plots of P' of Various Drugs against Concentration of Tween 80

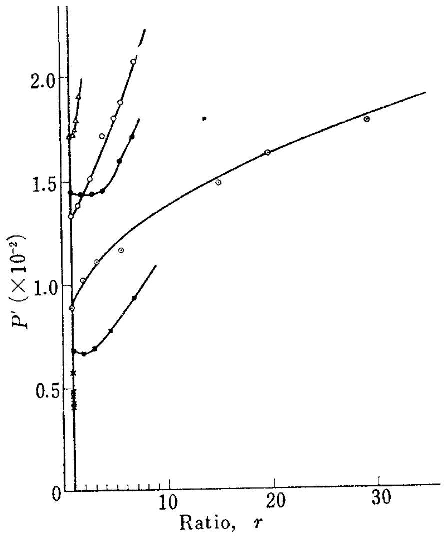

Fig. 16. Plots of P' of Various Drugs against Ratio, r

—●— methylparaben (30°)
—⊙— propylparaben (30°)
—△— sulfamin (30°)
—■— methylparaben (12°)
—○— benzocaine (30°)
—×— sodium salicylate (30°)

From these results, it will be reasonable to consider that these increasing phenomena of P' resulted from the effect of direct dialysis of drug molecules through the cellulose membrane from micellar phase in which great part of the drug is partitioned. Therefore the direct dialysis may be neglected when the value of ratio, r, is relatively small, and it may be considered Eqs. (3), (8), (10) and (11) are well applicable.

Instantaneous transfer of various drug molecules from micellar to aqueous phase in nonionic surfactant solutions becomes surely evident from above investigations at 30° even in the absence of stirring. But, if the transfer is due to thermal movement of drug molecules, delay from the instantaneous transfer will be suspected when temperature of the surfactant solution is lowered.

Then dialysis experiment was also carried out on methylparaben at 12°, and the results are plotted in Fig. 14. It shows the value of P' of methylparaben at 12° is smaller than the value at 30°, but well applies to Eqs. (8) and (10). Therefore it was evidenced that delay of the transfer does not appear even at 12°.

If the micelles of nonionic surfactant were considered to be shell-like structure and drug molecules were partitioned into these, it will be suspected that drug molecules in the shell are transferred rapidly to the aqueous phase. But Riegelman5) evidenced with ultraviolet spectra that benzocaine is solubilized in the palisade layers in the nonionic surfactant solution. Then it seems that these drugs are also solubilized in the relatively outer part of the micelles and easily transferred to the aqueous phase in the nonionic surfactant solutions.

The author expresses his deep gratitude to Prof. S. Iguchi of Kyushu University for guidance and encouragement through the course of this work.

Summary

In order to investigate the rapidity of transfer of drug molecules from micellar to aqueous phase in nonionic surfactant solutions, theoretical equations were derived assuming that the transfer occurs instantaneously corresponding to the decrease of drug concentration in the aqueous phase.

Applicability to the equations was examined with static dialysis of methylparaben, propylparaben and benzocaine in Tween 80 solutions using two kinds of dialysis apparatus at 30°.

It was proved that these results were well applicable to the equations and the transfer is instantaneous. But a little increase of dialysis beyond the theoretical value was observed when drug molecules were partitioned predominantly into the micellar phase and the ratio, r, became large. This phenomenon was attributed to direct dialysis from the micellar phase. Therefore the direct dialysis could be neglected when the ratio, r, was relatively small.

When volume of the outer fluid was enough large, decrease of drug concentration in the inner surfactant solution also became theoretically the first order process and its apparent decreasing coefficient, P_d, became P'/r. These relationships were well applied to the dialysis of various drugs.

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5) S. Riegelman: J. Am. Pharm. Assoc., Sci. Ed., **49**, 339 (1960).