[Chem. Pharm. Bull. 31(11)4040—4047(1983)]

Distribution of Fluocinolone Acetonide in Oil-in-Water Creams and Its Release from the Creams[1)]

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(Received January 17, 1983)

The distribution of fluocinolone acetonide (FA) in various creams and the release rate of FA from these creams were examined and the results were compared with the observed human vasoconstrictor activity of these creams. The creams were prepared by using various concentrations of surfactants and FA. The distribution of FA in the creams was examined by ultracentrifugation and ultrafiltration methods. The partition coefficients to the oil phase and surfactant phase were also measured. Then, the distribution of FA in the cream was calculated theoretically and these values were compared with the experimental data.

From the results for creams containing various concentrations of FA, the distribution of FA in cream is considered to depend simply on a distribution law. As the amount of polysorbate 80 in the cream was increased, the FA release rate and vasoconstrictor activity decreased. The reason was considered to be that FA was trapped in the surfactant phase, and so the free FA concentration in the aqueous phase decreased. In order to enhance the efficacy of the drug, therefore, it is necessary to select correct amounts and kinds of surfactants and to increase the free FA concentration in the aqueous phase.

Keywords——fluocinolone acetonide; oil-in-water cream; drug distribution; vasoconstrictor activity; ultracentrifugation; ultrafiltration

It is generally recognized that differences in the vehicle or base may affect the clinical efficacy of topical drugs. For that reason, many attempts have been made to investigate *in vitro* release and *in vivo* percutaneous absorption of drugs from ointments.[2)] In a previous paper,[3)] *in vitro* release of betamethasone 17-valerate from typical ointments using various membranes was compared with the percutaneous absorption (estimated from the vasoconstrictor activity).

In oil-in-water creams, the effects of the drug solubility[4)] and drug crystalline conversion in the base[5)] on the drug release have been studied. Another study dealt with the simulation of drug release from cream.[6)] A comparative study of the distribution of preservative in oil-in-water emulsions and the antimicrobial activity was also made.[7)] Although several studies have been carried out on drug release from creams, it appears that the relationship between the distribution of drug in each phase of the cream and drug release has hardly been studied. Therefore, in this study, in order to examine this relationship, the distribution of a drug, fluocinolone acetonide (FA), in creams containing various concentrations of surfactants and the drug was measured, and drug release from these creams was determined. Then, these results were compared with the observed human vasoconstrictor activity of the creams.

Experimental

Materials——FA and components of creams used were of pharmacopoeial grade (J.P. IX). Other materials used were of reagent grade.

Preparation of Creams——The formulae of tested creams are shown in Table I. They were prepared by adding purified water, heated to 70 °C, to the oil components and surfactant, in which FA had been dissolved, at the same

TABLE I. Formulation of Model Creams

Component	Formula No.	Composition (% (v/v)) 1	2	3	4	5	6	7	8	9	10
FA[a]		0.025							0.0125	0.05	0.1
Light mineral oil		15									
Isopropyl myristate		20									
Stearyl alcohol		10									
Polysorbate 80[a]		3	—	—	—	1	2	5	3		
Sodium lauryl sulfate[a]		—	3	—	—	—	—	—	—	—	—
Polyoxyethylene lauryl ether[a]		—	—	3	—	—	—	—	—	—	—
Benzalkonium chloride[a]		—	—	—	3	—	—	—	—	—	—
Purified water		52				54	53	50	52		

a) % (w/v).

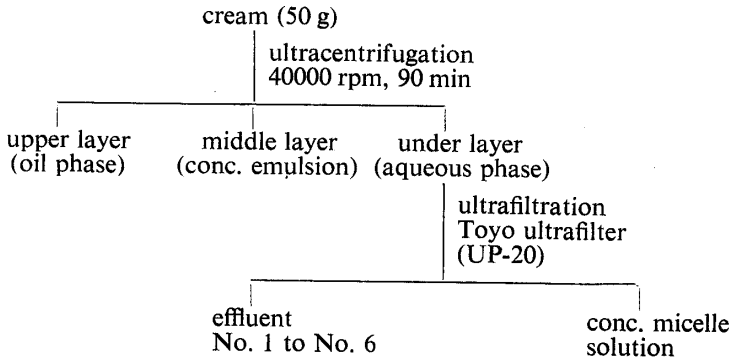

Fig. 1. Separation of the Phases of Creams

TABLE II. Conditions of High Performance Liquid Chromatography

Instrument	:	Hitachi type 633 HPLC
Column	:	LiChrosorb RP-18, 10 μm, (Merck Co., Ltd.) in 150 mm × 4 mm i.d. stainless steel column
Eluent	:	H_2O–MeOH (4:6)
Flow rate	:	1.0 ml/min
Detector	:	UV at 254 nm
Sensitivity	:	0.02 to 0.16 AUFS
Injection volume	:	10 μl
Column temperature	:	Ambient

temperature. The mixture was cooled to room temperature with stirring. FA was dissolved in these creams, since no FA crystals were observed microscopically during the experimental period of about 2 weeks.

Measurement of the Distribution of FA in Creams——The aqueous and the oil phases of the cream were separated by the ultracentrifugation and the ultrafiltration methods[7] as shown in Fig. 1. The oil phase was separated at 40000 rpm for 90 min at room temperature with a Hitachi 55 PA ultracentrifuge. FA in the separated oil phase was extracted with methanol. Since the separated aqueous phase contained the micelle phase of the surfactant, it was filtered with a Toyo ultrafilter (UP-20) and apparatus (Toyo Filter Co., Ltd., Tokyo). The concentrations of FA in the methanol and in the effluent of ultrafiltration were measured by high performance liquid chromatography (HPLC). The HPLC conditions are summarized in Table II.

Partition Coefficient of FA between Oil and Aqueous Phases——Fifty-five ml of a 10 μg/ml FA aqueous solution was added to the oil phase of 15 ml of light mineral oil, 20 ml of isopropyl myristate, and 10 ml of stearyl alcohol. This mixture was shaken for 24 h at 30 °C. The change in FA concentration in the aqueous phase was measured by HPLC under the conditions described above. The partition coefficient of FA between the oil and aqueous phases, K_o, is defined as

$$K_o=\frac{(FA)_o}{(FA)_{aq}}=\frac{(FA)_i-(FA)_{aq}}{(FA)_{aq}}\cdot\frac{V_{aq}}{V_o} \quad (1)$$

where $(FA)_o$, $(FA)_{aq}$ and $(FA)_i$ are the concentrations of FA in the oil phase, the aqueous phase, and the initial aqueous phase and V_o and V_{aq} are the volumes of the oil and aqueous phases, respectively.

Partition Coefficient of FA between Surfactant and Aqueous Phases——Ten μg/ml FA aqueous solutions containing various concentrations of surfactants were filtered with a Toyo ultrafilter (UP-20). The FA concentration in the effluents of these solutions was measured by HPLC. The partition coefficient of FA between the surfactant and aqueous phases, K_s, is defined as,

$$K_s=\frac{(FA)_s}{(FA)_{aq}}=\frac{(FA)_t-(FA)_{aq}V_{aq}}{(FA)_{aq}(V_s-V_{cmc})} \quad (2)$$

where $(FA)_s$ and $(FA)_{aq}$ are the concentrations of FA in the micelles of the surfactant and the aqueous phase, respectively, and $(FA)_t$ is the total concentration of FA in the solution. V_s, V_{cmc}, and V_{aq} are the partial volumes of the surfactant added to the solution, of the surfactant of the unformed micelles under the cmc, and of the aqueous phase, respectively. The partial volumes are calculated on the assumption that the specific gravity is approximately unity. Further, the partition coefficient of FA between the surfactant and the aqueous phases was determined by the solubility method.[8] An excess of FA was added to aqueous solutions of surfactants at various concentrations and the mixtures were shaken for 3 d. The solubility of FA in these solutions, S_t, and in purified water, S_{aq}, were measured by HPLC. The partition coefficient, K_s, is defined as,

$$K_s=\frac{S_t-S_{aq}\cdot V_{aq}}{S_{aq}\cdot(V_s-V_{cmc})} \quad (3)$$

FA Release from Cream——The technique employed was essentially the same as that described in a previous paper.[3] The membrane used was cellulose membrane (36/32 type, Union Carbide Corp., Chicago). The FA concentration in the acceptor solution was measured by HPLC under the same conditions after extraction with chloroform, followed by concentration. The FA release percentage from cream was plotted against the square root of time, and the release rate ($\%/\sqrt{h}$) was calculated from the slope of the straight line obtained.

Vasoconstrictor Activity——The vasoconstrictor activities of No. 1 and No. 5 to No. 10 creams which contained polysorbate 80 (shown in Table I) were measured using the technique described in a previous paper.[3] About 50 mg of each cream was applied at random to two sites on the flexor surface of the right forearm of ten healthy volunteers, using adhesive plaster for patch tests (Torii Pharm. Co., Ltd., Tokyo). The total number of test sites was 20 per cream.

At 4 h after the application of the creams, the plaster was removed and the site was wiped with gauze containing 70% ethanol. Then, at 6, 8, and 24 h after application, the blanching of the test site was graded as showing no blanching, slight blanching, distinct blanching, or very distinct blanching (scored as 0, 1, 2, and 3, respectively). The average score of each cream was obtained by dividing the total score by the number of test sites.

Results and Discussion

Concentration of Free FA in Aqueous Phase of Creams

FA is considered to be distributed in the oil phase, the surfactant phase on the interface of the emulsion, and the aqueous phase, which includes the micelles of the surfactant. FA in the aqueous phase may be separated into free FA and the contents in the micelles. Since only a small amount of the aqueous phase could be obtained from cream by ultracentrifugation, it was difficult to determine the free FA concentration in the aqueous phase by dynamic dialysis[9] or equilibrium dialysis.[10] For that reason, the free FA concentration in the aqueous phase of cream was measured by the ultrafiltration method.[7] One of the disadvantages of this method was that FA was adsorbed on the ultrafilter or the apparatus. However, if the first portion of effluent was discarded, the effect of adsorption could be neglected, as shown in Fig. 2. Another possible disadvantage is that a part of the micelles may be filtered through the ultrafilter, and therefore this possibility was checked by comparing the partition coefficient obtained by this method with that obtained by the solubility method for surfactant solutions. When FA solutions containing various concentrations of surfactants were ultrafiltered, the FA concentrations in the effluents were found to be as shown in Fig. 3. The solubility of FA in various surfactant solutions is shown in Fig. 4. The partition coefficients of FA between the surfactant and aqueous phases obtained by the ultrafiltration and solubility methods are

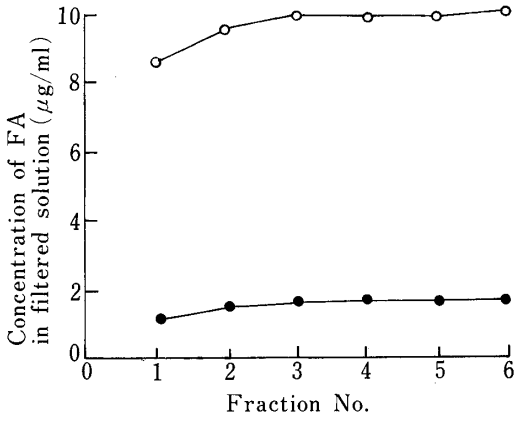

Fig. 2. Ultrafiltration of FA Solution (○) and Solution Containing 1% Polysorbate 80 (●)

The fraction volume was 1 ml.

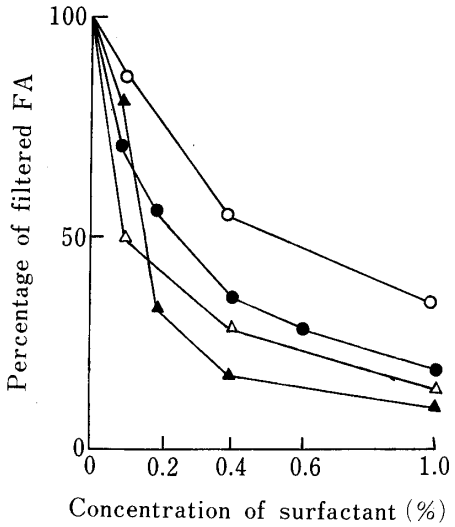

Fig. 3. Separation of Free FA (%) from FA Solution Containing Surfactant by Ultrafiltration

●, polysorbate 80; ▲, sodium lauryl sulfate; ○, polyoxyethylene lauryl ether; △, benzalkonium chloride.

TABLE III. Partition Coefficients of FA for Various Surfactants

Surfactant	Partition coefficient, K_s[a)]	
	Ultrafiltration method	Solubility method
Polysorbate 80	488±37[b)]	600±56[b)]
Sodium lauryl sulfate	2529±176	5350±339
Polyoxyethylene lauryl ether	228±36	847±170
Benzalkonium chloride	964±123	4611±368

a) Calculated from Eq. (2), or Eq. (3), using V_{cmc} values of 0.0 for polysorbate 80, 0.002 for sodium lauryl sulfate, 0.0 for polyoxyethylene lauryl ether, and 0.001 for benzalkonium chloride, which were estimated from Fig. 4. These K_s values are averages calculated from several data shown in Figs. 3 and 4.

b) Mean±S.D.

summarized in Table III. In the case of the solution containing polysorbate 80, the partition coefficients obtained by the two methods showed good agreement, but in the case of the solutions containing sodium lauryl sulfate, polyoxyethylene lauryl ether, and benzalkonium chloride, the value obtained by the ultrafiltration method was smaller than that obtained by the solubility method. For this reason, it is considered that the micelles of these surfactants were partially filtered through the ultrafilter. As a consequence, ultrafiltration of the aqueous phase in cream was performed only for the creams containing polysorbate 80.

Effect of Surfactants on Drug Release from Cream

The FA release percentages for No. 1 to No. 4 creams, which contained 3% surfactant, are shown in Fig. 5. The results show that the FA release percentage increased with decrease in the partition coefficient of FA between the surfactant and aqueous phases (obtained by the solubility method), as shown in Table III. For No. 1 and No. 5 to No. 7 creams which contained various concentrations of polysorbate 80, the FA release percentage decreased as the amount of surfactant increased, as shown in Fig. 6. These results show that if the cream

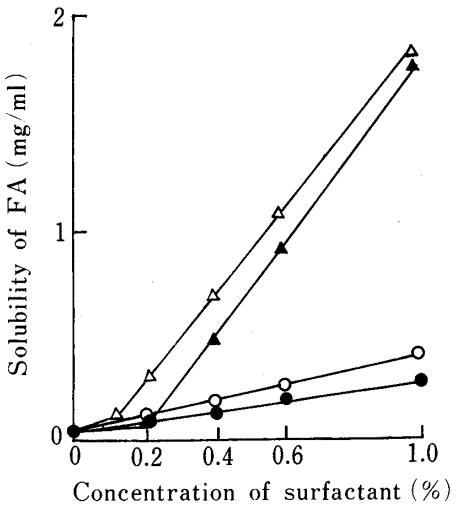

Fig. 4. Solubility of FA in Various Surfactant Solutions at 30 °C

●, polysorbate 80; ▲, sodium lauryl sulfate; ○, polyoxyethylene lauryl ether; △, benzalkonium chloride.

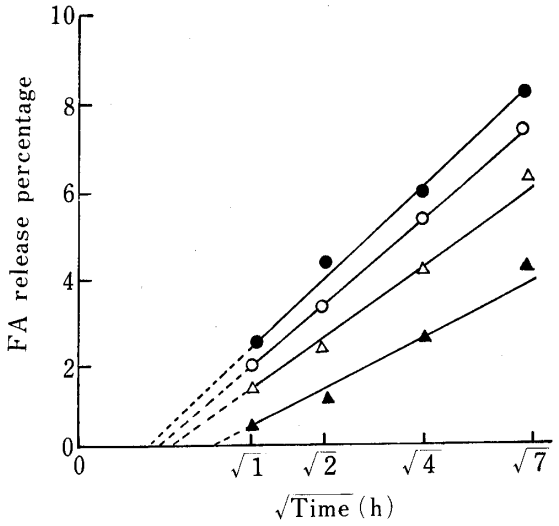

Fig. 5. Release of FA from Creams Containing Various Surfactants (3%) at 30 °C

●, polysorbate 80; ▲, sodium lauryl sulfate; ○, polyoxyethylene lauryl ether; △, benzalkonium chloride.

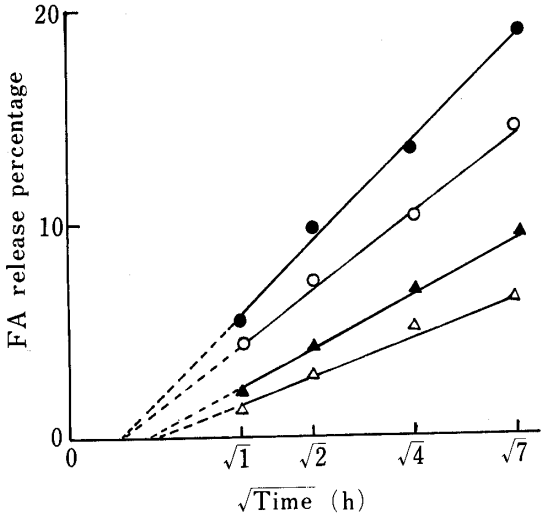

Fig. 6. Release of FA from Creams Containing Various Concentrations of Polysorbate 80 at 30 °C

Concentration of polysorbate 80; ●, 1%; ○, 2%; ▲, 3%; △, 5%.

contains a surfactant with a large partition coefficient for the drug and contains a high concentration of surfactant, the drug is trapped in the surfactant phase, resulting in a depression of drug release.

Distribution of FA in Creams

For No. 1 and No. 5 to No. 7 creams, which contained various concentrations of polysorbate 80, the FA distributions in the aqueous and oil phases were measured by the ultracentrifugation and ultrafiltration methods. The FA distribution in the surfactant phase was determined by subtraction, as the residue after deduction of FA in the aqueous and oil phases from total FA. On the other hand, the FA distribution in these creams was calculated from the partition coefficients to the oil phase, K_o, which was determined from Eq. (1) as 3.5, and to the surfactant phases, K_s, which was obtained by the solubility method. The surfactant phase in cream is considered to involve the interface of emulsion and micelles, but in the calculation we used K_s values for micelles, since the FA distributions in these phases could not be measured separately. The FA concentration in cream, $(FA)_t$, is expressed as

$$(FA)_t=(FA)_{aq}V_{aq}+(FA)_oV_o+(FA)_sV_s \quad (4)$$

where $(FA)_{aq}$, $(FA)_o$, and $(FA)_s$ are the concentrations in pure aqueous phase, oil phase, and surfactant phase including the interface of emulsion and micelles in the aqueous phase, respectively.

The FA concentration in the pure aqueous phase, *i.e.* free FA concentration in the aqueous phase, can be expressed by using K_o of Eq. (1) and K_s of Eq. (2) or Eq. (3), as follows,

$$(FA)_{aq}=\frac{(FA)_t}{K_oV_o+K_sV_s+V_{aq}} \quad (5)$$

The FA distribution in creams obtained by both experiment and calculation is shown in Fig. 7. These data are in good agreement. Therefore, it was considered that the estimation of FA

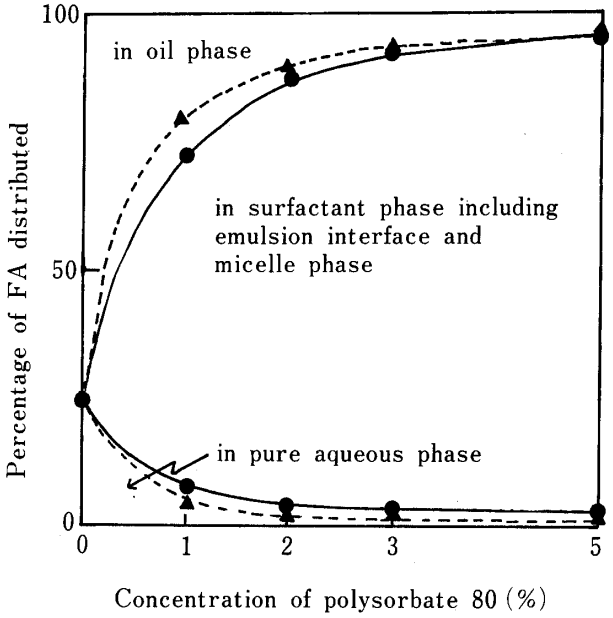

Fig. 7. Distribution of FA in Creams
●, experimental data; ▲, calculated values.

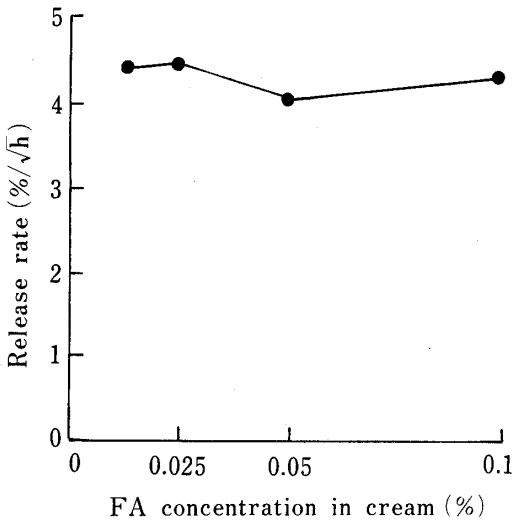

Fig. 8. Release Rate from Creams Containing Various Concentrations of FA

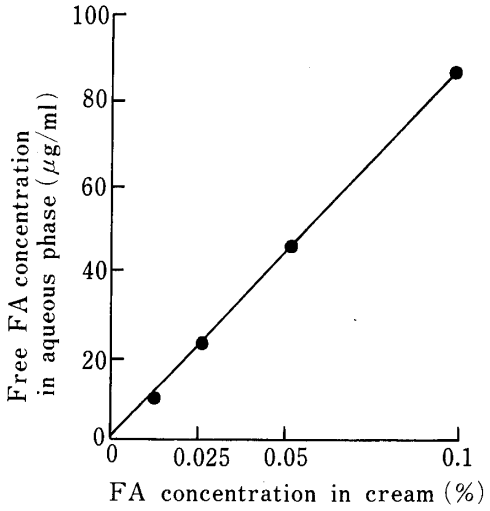

Fig. 9. Relationship between Free FA Concentration in Aqueous Phase and FA Concentration in Cream

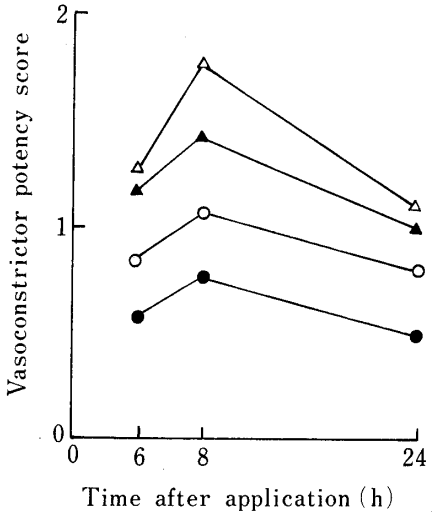

Fig. 10. Vasoconstrictor Potency of Creams Containing Various Concentrations of FA
Concentration of FA in creams: ●, 0.0125%; ○, 0.025%; ▲, 0.05%; △, 0.1%.

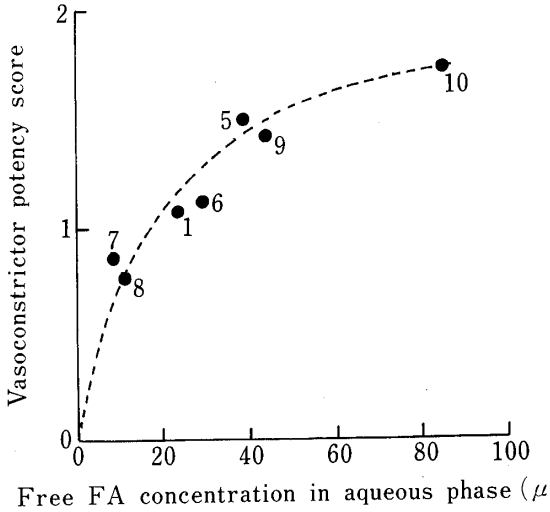

Fig. 11. Relationship between Free FA Concentration in Aqueous Phase and Vasoconstrictor Potency at 8 h after Application

The numbers show the formula No. of creams in Table I.

distribution in cream by using K_o and K_s was reasonable. The free FA concentration in the aqueous phase decreased as the amount of polysorbate 80 was increased and most of the FA was present in the surfactant phase in cream containing 5% polysorbate 80. It was considered that the FA distribution was dominated by the amount of surfactant in these model creams.

Effect of Drug Concentration

The FA release and free FA concentration in the aqueous phase were measured in No. 1 and No. 8 to No. 10 creams which contained various concentrations of FA. The release rates ($\%/\sqrt{h}$) for these creams were independent of the FA concentration in the cream from 0.0125 to 0.1%, as shown in Fig. 8. However, the free FA concentration increased as the FA concentration in the cream was increased, as shown in Fig. 9. These results showed that FA distribution in creams depends simply on a distribution law. However, FA in the aqueous phase of No. 9 and No. 10 creams which contained 0.05 and 0.1% FA may be dissolved in a supersaturated state, because the free FA concentration was greater than the FA solubility, 39 μg/ml, in purified water. In fact, when the cream containing 0.1% FA was observed microscopically at one month after the experiments, FA crystal formation was detected.

Vasoconstrictor Activity

The human vasoconstrictor activity of creams with various FA concentrations was examined and the results are shown in Fig. 10. The vasoconstrictor activity of these creams reached a maximum at 8 h after application and then decreased at 24 h. The vasoconstrictor activity increased as the FA concentration in the cream was increased.

The relationship between the vasoconstrictor activity at 8 h after application and free FA concentration in the aqueous phase of various creams is shown in Fig. 11. The creams containing various concentrations of polysorbate 80 showed a similar relationship to creams containing various concentrations of FA. Accordingly, in order to enhance the efficacy of the drug, it is necessary to select the correct amount and kind of surfactants and to increase the free FA concentration in the aqueous phase.

Acknowledgement The authors wish to thank Professor S. Tsuchiya, Tokyo College of Pharmacy, for valuable discussions during the preparation of this manuscript.

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