ENHANCING EFFECT OF ABSORPTION PROMOTERS ON PERCUTANEOUS ABSORPTION OF A MODEL DYE (6-CARBOXYFLUORESCEIN) AS POORLY ABSORBABLE DRUGS. I. COMPARISON OF PLASMA LEVELS AFTER ADDITION OF VARIOUS ABSORPTION PROMOTERS IN RAT

TAEYUKI OHSHIMA, HIROSHI YOSHIKAWA, KANJI TAKADA AND SHOZO MURANISHI*

Department of Biopharmaceutics, Kyoto Pharmaceutical University, Misasagi, Yamashina-ku, Kyoto, 607, Japan

(Received February 27, 1984)

We have investigated the promotive effect of various agents on percutaneous absorption of 6-carboxyfluorescein (CF), a water-soluble fluorescent dye, as poorly absorbable drugs. The absorption of CF was determined by measuring rat plasma CF levels. As an absorption promoter, several reagents such as surface-active agents, protein solubilizers and permeation promoters were used. The used concentration of the reagents was determined so as not to make a trauma on the skin. As results, plasma CF levels following the co-administration of 0.05 w/v% sodium dodecyl sulfate and 0.1 v/v% 2-mercaptoethanol showed the highest values. Plasma CF level was increased 40 times as compared to that of control experiment and was increased 9 times as compared to that of pretreatment with 4 w/v% calcium thioglycolate which was reported previously as a strong absorption promoter for theophylline by us. When the stratum corneum, having a barrier function for percutaneous absorption of many compounds, was removed mechanically, plasma CF levels of control experiment and pretreatment with 4 w/v% calcium thioglycolate were increased remarkably. However, plasma CF level after the co-administration of 0.05 w/v% sodium dodecyl sulfate and 0.1 v/v% 2-mercaptoethanol did not show a considerable difference as compared to that of the case with the presence of the stratum corneum.

Keywords—percutaneous absorption; 6-carboxyfluorescein; absorption promoter; sodium dodecyl sulfate; 2-mercaptoethanol; calcium thioglycolate; dimethylsulfoxide; urea; lipid-surfactant mixed micelle; rat

INTRODUCTION

Recently, many studies concerning the permeation of drugs after topical administration have been carrying out.¹⁻³⁾ Topical administration was useful for drugs which may be destroyed by enzymes in the digestive tract or trapped by the liver before entering the systemic circulation (first-pass effect),⁴⁾ and also useful to obtain long lasting effects.⁵⁾ But until now, the percutaneous route has been used only a little to obtain a systemic effect, because drugs of low partition coefficient and of large molecular weight are very

poorly permeable of the skin.^{6,7)}

On the other hand, many studies concerning the effects of absorption promoters, including dimethylsulfoxide (DMSO) which is the most widely known absorption promoter⁸⁾ and Azone[®] which is developed recently,⁹⁾ on the topical administration of drugs were carried out.^{10,11)} The purpose for the development of these absorption promoters is mainly to increase the permeability of drugs through the skin.

Histologically, the skin consists of the epidermis, the dermis and the hydrodermis. The stratum

* To whom correspondence should be addressed.

corneum on the epidermis, which has generally a principal barrier function of the skin, contains most of α -keratin filaments, and the fibrous protein is embedded in a proteic amorphous matrix rich in disulphide bonds.¹²⁾ Because of this lipophilic-structure characteristics of the stratum corneum, the percutaneous absorption of hydrophilic substance is generally considered to be very low. The study on the permeability of the skin deprived of the stratum corneum was carried out *in vitro*.¹³⁾ As a result, the isolated stratum corneum is almost as impermeable as the entire skin. From this report, in order to increase the permeability of the skin, the concept that should be attacked to the stratum corneum by the various method is derived.

In this study, 6-carboxyfluorescein (CF) was selected as a model of drugs having low partition coefficient, that is, a model dye of poorly absorbable drugs. In addition, we used surface-active agents (sodium dodecyl sulfate (SDS)), protein solubilizer (2-mercaptoethanol (MER), urea) and permeation promoter (mixed micelle and DMSO), calcium thioglycolate (Ca-TGA)) as an absorption promoter which is considered to act on the stratum corneum, and we carried out a fundamental experiment using rats *in vivo* system for percutaneous absorption. The percutaneous absorption efficiency of CF was determined by measuring CF levels in the rat plasma.

MATERIALS AND METHODS

Materials — A model drug, CF, was obtained from Eastman Kodak Co. Ltd. Ca-TGA was obtained from Tokyo Chemical Industry Co. Ltd. Polyoxyethylenelaurylether (BL-9EX) was supplied by Nikko Chemicals Co. Ltd. All other chemicals were reagent grade products obtained commercially.

Preparation of Test Solution — A 0.02 w/v% CF solution was prepared by dissolving in pH 7.4 phosphate buffer. DMSO was diluted to 80 v/v% by the addition of distilled water. A 4 w/v% Ca-TGA solution was prepared by dissolving in distilled water. All other solutions were prepared by dissolving all other chemicals in 0.02 w/v% CF

solution.

Analytical Method — To a 10 ml centrifuge tube containing 100 μ l of rat plasma, 3 ml of 1 N HCl and 6 ml of iso-amyl alcohol were added. The mixture was shaken on a reciprocating shaker for 15 min. The aqueous and organic phases were separated by centrifugation (3 000 rpm, 5 min). Then, 5 ml of the organic phase was placed in a 10 ml centrifuge tube with a Pasteur pipette and added 4 ml of pH 10 carbonate buffer. Then the mixture was shaken on a reciprocating shaker for 15 min. The aqueous and organic phases were separated by centrifugation (3 000 rpm, 5 min). Then, the organic phase was removed with a disposable pipette and the water phase was measured spectrofluorometrically. Excitation wavelength was 490 nm, emission wavelength was 520 nm, respectively.

Procedure for Animal Experiment — Male Wistar rats weighing 180 to 200 g were used. The hair of the abdominal region was carefully shaved with an electric hair clipper and an electric razor, with care being taken to prevent damage to the skin, one day before the experiment. Rats were anesthetized by an intraperitoneal injection of sodium pentobarbital (32 mg/kg). The exposed skin was first wiped clean cautiously with absorbent cotton soaked in 70 v/v% ethanol and then with one soaked in distilled water. As shown in Fig. 1, a glass chamber (inside diameter = 3.2 cm) was applied to the surface of the hairless abdomen using a cyanoacrylate adhesive (Aron Alpha®, Toa Gosei Chemical Co., Ltd., Tokyo, Japan). The body temperature of rats was maintained at $37 \pm 1^\circ\text{C}$.

FIG. 1. Apparatus for *in Vivo* Percutaneous Absorption Experiment in Rat

1) *Percutaneous Absorption of CF (Control Experiment)* — After 10 ml of 0.02 w/v% CF solution was placed in the glass chamber, 0.3 ml blood samples were collected at 1, 2, 3, 4, 5 and 6 h through polyethylene tubing cannulated into the carotid artery. The blood samples were centrifuged at 12 000 rpm for 3 min, and 100 μ l of plasma was used for the measurement of CF content.

2) *Effect of Pretreatment with 80 v/v% DMSO or 4 w/v% Ca-TGA on Percutaneous Absorption of CF* — A 10 ml specimen of 80 v/v% DMSO or 4 w/v% Ca-TGA solution was placed in the glass chamber. After 30 min, DMSO or Ca-TGA solution was removed and the inside of the glass chamber was washed several times with distilled water. Then 10 ml of 0.02 w/v% CF solution was placed in the glass chamber and blood samples were collected for 6 h as described in the control experiment.

3) *Effect of Mixed Micelle on Percutaneous Absorption of CF* — Mixed micelle solution containing CF was prepared by dissolving linoleic acid (2.5 v/v%) and polyoxyethylenelaurylether [BL-9EX] (5 w/v%) in 0.02 w/v% CF solution followed by sonication at 37 °C with a sonicator, Ohtake model 5202 (150 watt, 8 min). A 10 ml of mixed micellar solution was placed in the glass chamber and blood samples were collected for 6 h as described in the control experiment.

4) *Effect of the Addition of Other Absorption Promoters on Percutaneous Absorption of CF* — A 10 ml of 0.02 w/v% CF solution containing other absorption promoters (10 w/v% urea, 0.4 v/v% MER-9.6 w/v% urea, 0.04 w/v% SDS-0.1 v/v% MER, 0.05 w/v% SDS-0.1 v/v% MER, 0.1 v/v% MER and 0.05 w/v% SDS, respectively) was placed in the glass chamber and blood samples were collected for 6 h as described in the control experiment.

5) *Role of the Stratum Corneum on Percutaneous Absorption of CF* — The stratum corneum was removed slowly twice by a cellophane tape without a trauma on the skin and the removal of the stratum corneum was confirmed microscopically. After that, a glass chamber was attached to the

skin. A 10 ml specimen of each reagent was placed in the glass chamber and blood samples were collected for 6 h as described in the control experiment.

RESULTS

As a control experiment, a 10 ml aliquot of 0.02 w/v% CF solution was applied into the glass chamber adapted on the rat abdomen, and the resultant plasma concentration-time curve is shown in Fig. 2.

In rat plasma, CF was detected even at 1 h after the percutaneous administration and plasma CF levels reached the plateau after that time. However, plasma CF concentration at 6 h after percutaneous dose was less than 3 ng/ml. We also investigated the promotive effect of various

FIG. 2. *Effect of Various Absorption Promoters on Plasma Concentration-Time Curves of CF Following percutaneous Administration in Rats*

Each point represents the mean $\pm$ S.E.

○ control ($n=9$), ● pretreatment with 4 w/v% Ca-TGA for 30 min ($n=7$), □ mixed micelle (2.5 v/v% linoleic acid and 5 w/v% BL-9EX) added to the chamber ($n=4$), △ 10 w/v% urea added to the chamber ($n=4$), ▲ 0.4 v/v% MER and 9.6 w/v% urea added to the chamber ($n=4$).

reagents. Each reagent was administered with CF solution. However, when pretreatment with Ca-TGA or DMSO was carried out, no trauma was observed on the abdominal skin after the percutaneous treatment of the reagent respectively. At first, urea which has been used as a denaturing agent of the stratum corneum¹⁴⁾ was examined. After 10 w/v% urea solution was added into the glass chamber attached to the rat abdomen, the constant plasma CF concentration appeared at least 1 h after percutaneous administration of CF and the plasma CF concentration at 6 h after the topical administration was higher by about 2.5 times at a control experiment. Next Ca-TGA, reported previously,¹⁵⁾ which was used in our study to promote the percutaneous absorption of theophylline, was examined. However, Ca-TGA was deposited in the pH 7.4 solution. In addition, a trauma appeared on the skin when Ca-TGA solution (4 w/v%) was placed for a long time (1–3 h) on the skin. Therefore, the rat skin was pretreated with Ca-TGA. After pretreatment with 4 w/v% Ca-TGA solution was carried out, the constant plasma CF concentration was obtained at 1 h after the percutaneous dose of CF. The plasma CF concentration at 6 h after administration was increased about 4 times as compared to the control experiment. Recently, we have succeeded to promote the poorly absorbable drug (interferon *etc.*) with mixed micelle^{16–21)} composed of 2.5 v/v% linoleic acid and 5 w/v% BL-9EX. Then, mixed micelle was examined as an absorption promoter. When this mixed micellar solution containing CF was topically applied, considerable amount of CF was detected in the rat plasma, and plasma CF level was gradually increased till 6 h. Plasma CF concentration at 6 h after the topical administration in mixed micelle was the same as that of 4 w/v% Ca-TGA pretreatment. At the end of the percutaneous absorption experiment, we observed visually that mixed micelle was adsorbed to the rat abdominal skin. Next, 0.4 v/v% MER and 9.6 w/v% urea co-administered as a protein solubilizer²²⁾ was examined as a percutaneous absorption promoter. After CF solution contain-

ing these two reagents was administered, plasma CF level was gradually increased till 6 h, and plasma CF concentration at 6 h after the topical administration was increased to 11 ng/ml.

DMSO is the most known absorption promoter for percutaneous administration of drugs.¹⁰⁾ Therefore, we examined the absorption promotive effect of DMSO in this study, and the result is shown in Fig. 3.

The plasma CF concentration–time curve obtained by pretreatment with 80 v/v% DMSO solution was higher than that obtained by all of the previously used reagents. Consequently, we also carried out the experiment to find a new percutaneous absorption promoter stronger than DMSO. As a candidate, SDS and MER were used, as these reagents have been used as a protein solubilizer.^{23,24)} The plasma CF levels following the co-administration of 0.05 w/v% SDS and 0.1

FIG. 3. Effect of DMSO and Protein Solubilizer on Plasma Concentration–Time Curves of CF Following Percutaneous Administration in Rats

Each point represents the mean $\pm$ S.E.

○ control, ($n=9$), □ pretreatment with 80 v/v% DMSO for 30 min ($n=4$), △ 0.04 w/v% SDS and 0.1 v/v% MER added to the chamber ($n=7$), ▲ 0.05 w/v% SDS and 0.1 v/v% MER added to the chamber ($n=7$).

v/v% MER showed the highest values (Fig.3). Plasma CF concentration-time curve is 2.0 times higher as compared to that of 80 v/v% DMSO and is 40 times higher as compared to that of the control experiment. Furthermore, plasma CF concentration increased gradually and did not reach the plateau level even at 6 h after the administration.

To elucidate whether this stronger percutaneous absorption promotive effect of SDS and MER is due to the synergism of these two reagents, SDS or MER was individually added to CF solution and the same absorption experiment was carried out.

As shown in Fig. 4, the plasma CF concentration-time curve by the presence of SDS or MER was considerably lower as compared to that of the co-administration experiment. That is, the effect of the co-administration of SDS and MER is not an additive effect but a synergistic effect.

FIG. 4. Absorption Promotive Effect of SDS and MER on the Percutaneous Absorption of CF in Rats

Each point presents the mean $\pm$ S.E.

○ control, (n=9), ● 0.1 v/v% MER added to the chamber (n=5), △ 0.05 w/v% SDS added to the chamber (n=5), ▲ 0.05 w/v% SDS and 0.1 v/v% MER added to the chamber (n=7).

In addition, to elucidate the mechanism of the absorption promoter, we investigated the role of the stratum corneum on the percutaneous absorption of CF. For this purpose, the stratum corneum of rats was removed mechanically and we ascertained microscopically that the stratum corneum was well removed with our stripping method.

As shown in Fig. 5, the plasma CF concentration at 6 h after administration was increased about 60 times by the mechanical removal of the stratum corneum. In the case of both experiments, pretreatment with Ca-TGA and co-administration with SDS and MER, where the stratum corneum was mechanically removed, there was no

FIG. 5. Effect of Presence or Absence of the Stratum Corneum on Plasma CF Concentration-Time Curves Following Percutaneous Administration of CF in Rats

Each point represents the mean $\pm$ S.E.

Statistical comparison was done by Student's *t*-test.

a), b), c) not significant.

○ control, (n=9), ● stripped (n=5), △ pretreatment with 4 w/v% Ca-TGA for 30 min (n=7), ▲ pretreatment with 4 w/v% Ca-TGA for 30 min after stripping (n=5), □ 0.05 w/v% SDS and 0.1 v/v% MER added to the chamber (n=7), ■ 0.05 w/v% SDS and 0.1 v/v% MER added to the chamber after stripping (n=5).

more enhancing effects on the percutaneous absorption of CF. In all of the percutaneous absorption experiments carried out in this study, we confirmed that no trauma was observed on the rat abdominal skin at the end of the experiments.

DISCUSSION

We have investigated several percutaneous absorption promoters which are thought to attack to the stratum corneum having a barrier function for the percutaneous absorption of drugs. Alkalis, strong acid, and certain detergents are reported to increase the permeability of the skin by damaging the stratum corneum.²⁵⁾ Organic solvents are efficient delipidizing solvents and rapidly alter the barrier.²⁶⁾ Of organic solvents, DMSO showed the most excellent absorption promotive effect and many studies were carried out.²⁷⁾ However, the side effect (*i.e.* a lens change) of DMSO following topical and oral administration was observed primarily in dogs. The use of this reagent has been limited in humans for the treatment of interstitial cystitis by intravesicular instillation.²⁸⁾ Therefore, we have been investigating a new reagent that promotes the permeability of drugs through the skin without damaging the stratum corneum, the barrier for the percutaneous absorption of drugs. As shown in Fig. 2, the constant plasma CF level appeared at least 1 h after the percutaneous administration of CF when a weak protein solubilizer (co-administration with urea and MER) and a depilatory (pretreatment with Ca-TGA) was added into the glass chamber attached to the rat abdomen. However, after a denaturing agent of the stratum corneum (urea) and mixed micelle were added, the promotive effects of these agents were observed even at 6 h after percutaneous administration of CF and plasma CF level was increased gradually till 6 h. This result suggests that urea and mixed micelle attacked gradually to the stratum corneum (*i.e.* a barrier for percutaneous absorption of CF). The purpose for developing the percutaneous absorption promoter is (1) to increase the plasma concentration earlier and higher, (2) to obtain long lasting effect. In this paper, our study was focused

on the former point. Then, all of the experiments were done in 6 h. With respect to the later point, more precise studies are now in progress. In our previous paper, the promotive effect of Ca-TGA on the percutaneous absorption of theophylline was reported.¹⁵⁾ However, Ca-TGA did not show an excellent absorption promotive effect on the percutaneous absorption of CF. Therefore, we consider that this disagreement is due to the difference of the character of drug itself. Theophylline is more lipophilic as compared to CF. Consequently, when CF was used as a model drug, less absorption promotive effect of Ca-TGA was detected as compared to the case when theophylline was used.

On the other hand, when co-administration of SDS and MER was carried out, plasma CF level was increased gradually till 6 h. However, from the viewpoint of the absorption, the addition of these reagents showed higher plasma CF level as compared to that of the addition of other reagents (Fig. 3). This result suggests that the permeability of drug was increased by solubilizing the protein-like compound in the barrier of the skin, because SDS has a solubilizing effect on proteins except proteins containing "sulfur" and because MER has a solubilizing effect on proteins containing "sulfur". Fig. 4 shows that more absorption promotive effect was obtained by the co-administration of SDS and MER than individual administration. That is, the mechanisms of the action of SDS and MER are considered to be different. By taking off the stratum corneum mechanically, the mechanisms of the action of these reagents were studied (Fig. 5). From these results, we suspect that co-administration of SDS and MER, and pretreatment with Ca-TGA may attack to the stratum corneum. To prove this hypothesis about the mechanism of the absorption promoters, more histological studies are required. If so, the mechanism of the action of the co-administration of SDS and MER will be explained more precisely.

As we have studied, the most absorption promotive effect was detected by the co-administration of SDS and MER and no trauma was

observed on the skin at the end of the experiment. Furthermore, it was predicted that the mechanism of the action is to attack onto the protein-like compound in the barrier (the stratum corneum). Therefore, it is speculated that the absorption of CF is enhanced by penetrating through the intracellular pathways, not through the parallel pathways. Further histological studies are now required to elucidate the mechanism of the absorption promotive effect of the co-administration of SDS and MER.

REFERENCES

- 1) J. M. Franz, A. Gaillard, H. I. Maibach and A. Schweizer: Percutaneous absorption of griseofulvin and proquazone in the rat and in isolated human skin, *Arch. Dermatol. Res.*, **271**, 275–282 (1981).
- 2) R. L. Bronaugh, R. F. Stewart, E. R. Congdon and A. L. Giles, Jr.: Methods for *in vitro* percutaneous absorption studies. I. Comparison with *in vivo* results, *Toxicol. Appl. Pharmacol.*, **62**, 474–480 (1982).
- 3) U. G. Dalvi and J. L. Zatz: Effect of skin binding on percutaneous transport of benzocaine from aqueous suspensions and solutions, *J. Pharm. Sci.*, **71**, 824–826 (1982).
- 4) A. H. Beckett, J. W. Gorrod and D. C. Taylor: Comparison of oral and percutaneous routes in man for the systemic administration of 'ephedrine', *J. Pharm. Pharmacol.*, **24**, Suppl. 65P–70P (1972).
- 5) S. T. Horhota and H.-L. Fung: Percutaneous nitroglycerin absorption in rats, *J. Pharm. Sci.*, **68**, 608–612 (1979).
- 6) M. Katz and Z. I. Shaikh: Percutaneous corticosteroid absorption correlated to partition coefficient, *J. Pharm. Sci.*, **54**, 591–594 (1965).
- 7) R. J. Scheuplein and I. H. Blank: Permeability of the skin, *Physiol. Rev.*, **51**, 702–747 (1971).
- 8) H. Baker: The effect of dimethylsulfoxide, dimethylformamide and dimethylacetamide on the cutaneous barrier to water in human skin, *J. Invest. Dermatol.*, **50**, 283–288 (1968).
- 9) R. B. Stoughton: Enhanced percutaneous penetration with 1-dodecylazacycloheptan-2-one, *Arch. Dermatol.*, **118**, 474–477 (1982).
- 10) M. A. Ball, J. L. McCullough and G. D. Weinstein: Percutaneous absorption of methotrexate: Effect on epidermal DNA synthesis in hairless mice, *J. Invest. Dermatol.*, **79**, 7–10 (1982).
- 11) J. J. Windheuser, J. L. Haslam, L. Caldwell and R. D. Shaffer: The use of *N,N*-dimethyl-*m*-toluamide to enhance dermal and transdermal delivery of drugs, *J. Pharm. Sci.*, **71**, 1211–1213 (1982).
- 12) A. G. Matoltsy: Keratinization, *J. Invest. Dermatol.*, **67**, 20–25 (1976).
- 13) H. D. Onken and C. A. Moyer: The water barrier in human epidermis, *Arch. Dermatol.*, **87**, 584–590 (1963).
- 14) G. Swanbeck: A new treatment of ichthyosis and other hyperkeratotic conditions, *Acta Derm.-Venereol.*, **48**, 123–127 (1968).
- 15) K. Kushida, K. Masaki, M. Matsumura, T. Ohshima, H. Yoshikawa, K. Takada and S. Muranishi: Application of calcium thioglycolate to improve transdermal delivery of theophylline in rats, *Chem. Pharm. Bull.*, **32**, 268–274 (1984).
- 16) S. Muranishi, Y. Tokunaga, K. Taniguchi and H. Sezaki: Potential absorption of heparin from the small and the large intestine in the presence of monoolein mixed micelles, *Chem. Pharm. Bull.*, **25**, 1159–1161 (1977).
- 17) Y. Tokunaga, S. Muranishi and H. Sezaki: Enhanced permeability to macromolecules. I. Effect of monoolein-bile salts mixed micelles on the small intestinal absorption of heparin, *J. Pharm. Dyn.*, **1**, 28–38 (1978).
- 18) S. Muranishi, N. Muranushi and H. Sezaki: Improvement of absolute bioavailability of normally poorly absorbed drugs: Inducement of the intestinal absorption of streptomycin and gentamicin by lipid-bile salt mixed micelles in rat and rabbit, *Int. J. Pharm.*, **2**, 101–111 (1979).
- 19) S. Muranishi, H. Yoshikawa and H. Sezaki: Absorption of 5-fluorouracil from various regions of gastrointestinal tract in rat. Effect of mixed micelles, *J. Pharm. Dyn.*, **2**, 286–294 (1979).
- 20) K. Taniguchi, S. Muranishi and H. Sezaki: Enhanced intestinal permeability to macromolecules. II. Improvement of the large intestinal absorption of heparin by lipid-surfactant mixed micelles in rat, *Int. J. Pharm.*, **4**, 219–228 (1980).
- 21) H. Yoshikawa, K. Takada, S. Muranishi, Y. Satoh and N. Naruse: A method to potentiate enteral absorption of interferon and selective delivery into lymphatics, *J. Pharm. Dyn.*, **7**, 59–62 (1984).
- 22) F. H. White, Jr.: Reduction and reoxidation at disulfide bonds, "Methods in Enzymology," ed. by C. H. W. Hirs and S. N. Timasheff, Academic Press, Inc., New York, 25-B, 1972, pp. 387–392.
- 23) A. Helenius and K. Simons: Solubilization of membranes by detergents, *Biochim. Biophys. Acta*, **415**, 29–79 (1975).
- 24) A. K. Dunker and R. R. Rueckert: Observations on molecular weight determinations on polyacrylamide gel, *J. Biol. Chem.*, **244**, 5074–5080 (1969).
- 25) I. Bernard: Vehicle effects in percutaneous absorption, *Drug Metab. Rev.*, **14**, 207–222 (1983).
- 26) R. J. Scheuplein and I. H. Blank: Mechanism of per-

- cutaneous absorption. IV. Penetration of non-electrolytes (alcohols) from aqueous solutions and from pure liquids, *J. Invest. Dermatol.*, **60**, 286–296 (1973).
- 27) D. D. Munro and R. B. Stoughton: Dimethylacetamide (DMAC) and dimethylformamide (DMFA) effect on percutaneous absorption, *Arch. Dermatol.*, **92**, 585–586 (1965).
- 28) R. D. Brobyn: The human toxicology of dimethylsulfoxide, *Ann. N. Y. Acad. Sci.*, **243**, 497–506 (1975).