[Chem. Pharm. Bull. 18(8)1563—1568(1970)] UDC 577.161.1 : 615.356.076.9

Mechanism of Drug Absorption from Micellar Solution. I. Absorption of Solubilized Vitamin A from the Rat Intestine[1]

Kiichiro Kakemi, Hitoshi Sezaki, Shozo Muranishi and Akemi Yano

Faculty of Pharmaceutical Sciences, Kyoto University[2]

(Received February 7, 1970)

Mechanism of drug absorption from micellar solution was studied through use of drugs which exist predominantly in micellar phase. Particular systems chosen were vitamin A acetate and vitamin A alcohol–Polysorbate-80.

Contrary to the notion that the only form in which the drug penetrate the membrane is free drug, both vitamins, entrapped almost completely in micelles, were absorbed fairly well, but not as such, form the rat large intenstine. Disappearance of the vitamins from the perfusate proceeded by two phases, the rapid (initial 15 minutes perfusion period) and the slow phase (follwing 60 minutes period). In the former, extent of the absorption of the vitamins were dependent on the initial concentration of surface–active agent, whereas in the latter, no such tendency was observed. It was demonstrated that in the very early stage of absorption, vitamins solubilized in micelles are absorved onto the membrane, which being favored by the surface–active agent and in the slow phase, absorption now becomes rate–limited by the partition or release of the vitamins from adsorbed micelles to the mucosal layer of the membrane. Despite the large effective surface area, disappearance in the slow absorptive phase of the vitamins from the solution circulated through the small intestine was smaller compared with the large intestine.

Surface–active agents have been widely used as emulsifiers, solubilizers, and other pharmaceutical formulation adjuncts. Numerous studies have shown that these agents may alter the transfer of drugs across biological membranes. Previous report from this laboratory on the rectal absorption of sulfonamides[3] revealed that it is the free drug which is utilized predominantly. However, contrary to the notion that the only form in which the drug penetrate the membrane is free drug and not drug in micelles, the latter was also absorbed to some extent.

It was the purpose of this study to make clear the mode of action of surface–active agents on the intestinal absorption of drugs entrapped in micelles. The approach was through use of drugs which exist predominantly in the micellar phase. In view of such considerations, vitamin A acetate (VAA) and vitamin A alcohol (VAOH)–polysorbate-80 (PS-80) were chosen for use as the particular systems in this study.

Experimental

Materials and Drug Solutions——VAA and PS-80 (Tokyo Kasei Kogyo Co., Ltd.); VAOH (Nutritional Biochemicals Corp., U.S.A.). All other chemicals used were reagent grade quality. VAA or VAOH was dissolved in isotonic buffered solution ($Na_2HPO_4 \cdot 12H_2O$ 49.4 mM KH_2PO_4 130.1 mM, pH 7.4) of PS-80. The solutions were freshly prepared for each experiment. Thin–layer chromatography and ultraviolet absorption spectrum revealed that PS–80 solutions of VAA and VAOH were stable at least for 8 and 6 hours respectively.

1) This paper constitutes the 44th report in a series of "Absorption and Excretion of Drugs" XLIII: K. Kakemi, H. Sezaki, R. Konishi, T. Kimura, and A. Okita, *Chem. Pharm. Bull.* (Tokyo), **18**, 1034 (1970).
2) Location: *Yoshidashimoadachi-cho, Sakyo-ku, Kyoto.*
3) K. Kakemi, T. Arita, and S. Muranishi, *Chem. Pharm. Bull.* (Tokyo), **13**, 976 (1965).

Dialysis——Equilibrium dialysis was carried out at 37° using Visking cellophane membrane (8/32 inch). Ultraviolet absorption spectrum of VAA or VAOH was not observed from these external phases.

Absorption Experiment——Male rats weighing 180 to 220 g were anesthetized with sodium pentobarbital by intraperitoneal injection in 4.0 to 4.5 mg per 100 g.

A. Recirculation Technique: Procedure of the absorption experiment have been described previously[3] except for the following modifications. Volume of the perfusing solution was 50 ml; concentration of the drug solution before the start of perfusion was set as zero time drug concentration; sulfaguanidine and methyl orange were used as volume change indicators. Similar experiments were performed at the large intestine. In this case, the cannulae were inserted into the beginning of the upper colon and into the anus. In the case of surface-active agents pre-treatment, an isotonic buffered solution containing PS-80 only was first perfused through the intestine. After ten minute perfusion, the entire solution in the intestine was forced out with the aid of a syringe and a small amount of the buffer solution, and solubilized VAA solution was perfused as usual absorption experiment.

B. Single Perfusion Technique: Similar experiments were performed at the large intestine. The solution containing drug was perfused and then ten 1 minute samples were collected from the rectal outflow. The concentration of the collected samples and the relative rate of adsorption was calculated from the difference in the concentration entering and leaving the intestine.

Preparation of the Enzymes and the Enzymatic Reaction——The separation of the contents, mucosae, and muscles of the large intestine was carried out according to Gangly, *et al.*[4] The mucosae were ground with a pestle and motar in acetone at −15°. The mixture was centrifuged and the precipitate was washed three times with fresh acetone at −15°. Finally the residual acetone was removed under suction. One gram of the acetone-dried powder was kept suspended in 25 ml of cold water for one hour at 0°, the solid then removed by centrifuging at 10000 g for 15 minutes and the supernatant was used as enzyme solution. The reaction mixtures consisted of 4 ml 0.1 M veronal buffer (pH 8.6) containing 2 mg of VAA and 1 ml of the enzyme solution. All incubations were carried out at 37° for 30 minutes. The reactions were quenched by the addition of KCN solution. Amount of free and esterified vitamin A were determined separately by the method of Anmo, *et al.*[5]

Analytical——A. VAA and VAOH: One milliliter sample was diluted with 9 ml of distilled water and extracted with 10 ml of benzene. Benzene extract was determined spectrophotometrically at 350 mμ. When VAA or VAOH was extracted from micellar solution, PS-80 was partially extracted. Since the interference by the latter compound was negligibly small at the concentration range used, the net absorbance of VAA or VAOH was calculated by Morton-Stubbs-Oster's correction method.[6]

Corrected absorbance $= 7 \times A_{330m\mu} - 3.64 \times A_{318m\mu} - 3.43 \times A_{343m\mu}$ Where A denotes the absorbance at the respective wave-length.

B. PS-80: Dermined by the modified method of Brown, *et al.*[7] Twenty milliliter of ammonium cobalt thiocyanate solution was added to 2 ml of sample solution. A blue complex formed was extracted with 15 ml of chloroform and measured spectrophotometrically at 620 mμ.

Result and Discussion

It was found in the preliminary experiments that VAA and VAOH solubilized in PS-80 were predominantly in micellar phase and chemically stable for the period of absorption experiment.

The disappearance of solubilized VAA and PS-80 from the rat large intestine is shown in Fig. 1.

It should be noted that contrary to VAA, disappearance of PS-80 was hardly observed at all in the last 60 minutes of perfusion, which indicates that VAA entrapped in micelles of PS-80 is not absorbed as such. Disappearance of VAA from the intestinal perfusate proceeded by two phases. VAA disappeared very rapidly and then declined at an approximately 0-order rate, and considerable amount of VAA disappeared in 75 minutes. Although the rapid phase, initial 15 minutes of perfusion, may partly result from the known characteristics inherent to the perfusion procedure, disappearance in this phase was definitely dependent on the initial concentration of the surface-active agent. This effect is well demonstrated in Fig. 2.

4) J. Gangly, S. Krishnamurthy, and S. Mahadevan, *Biochem. J.*, **71**, 756 (1959).
5) H. Anmo, M. Washitake, Y. Takashima, and S. Sato, *Bitamin*, **37**, 21 (1968).
6) R. A. Morton and A.L. Stubbs, *Analyst*, **71**, 348 (1946).
7) E.G. Brown and T.J. Hayes, *Analyst*, **80**, 755 (1955).

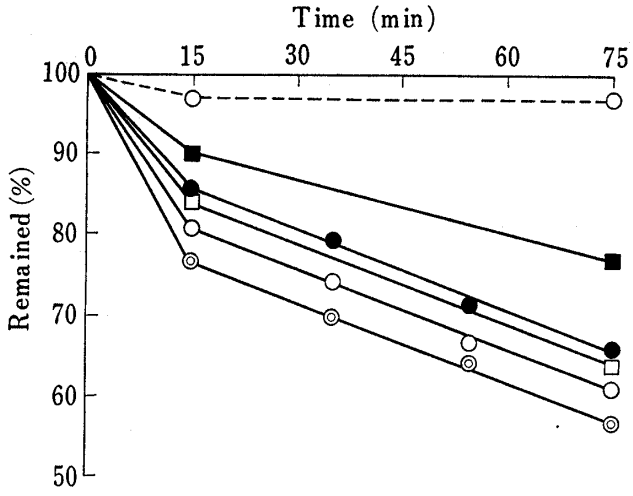

Fig. 1. Disappearance of VAA and PS-80 from the Large Intestine

concentration of VAA=0.15 mM
● : with PS-80 0.05%
○ : with PS-80 0.2%
◎ : with PS-80 1.0%
□ : with PS-80 3.0%
■ : with PS-80 5.0%
— : disappearance of VAA
--- : disappearance of PS-80

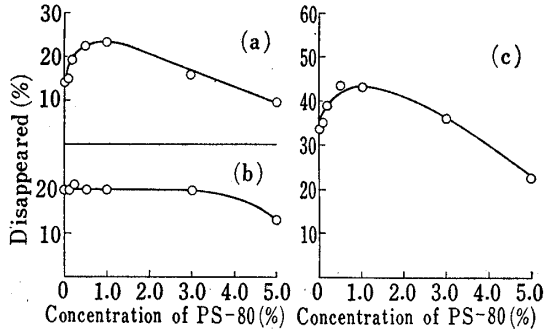

Fig. 2. Effect of PS-80Concentration on the Absorption of VAA from the Large Intestine

(a) for the initial 15 min (b) for the subsequent 60 min (c) for total 75 min

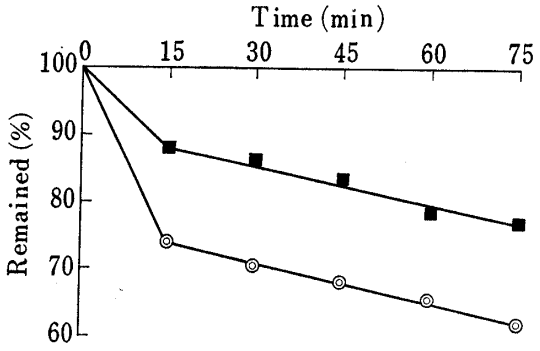

Fig. 3. Disappearance of VAA from the Small Intestine

concentration of VAA=0.15 mM
◎ : with PS-80 1.0%
■ : with PS-80 5.0%

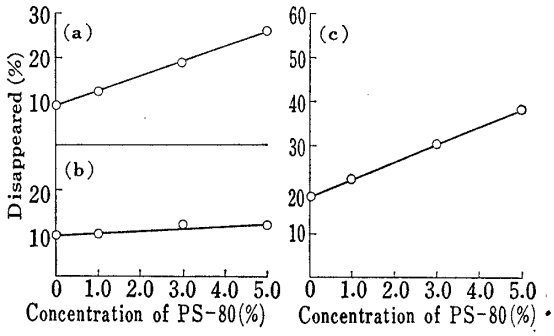

Fig. 4. Effect of PS-80 Concentration on the Absorption of VAA from the Small Intestine

(a) for the initial 15 min (b) for the subsequent 60 min (c) for the total 75 min

It is clear from curves (a) and (b) that disappearance of VAA was more dependent on the concentration of PS-80 in the first 15 minutes, the rapid phase, than the subsequent 60 minutes period, the slow phase. In the latter, disappearance of VAA from the perfusate was almost independent on the initial concentration of PS-80. Pre-treatment with 5 or 0.01% PS-80 solution prior to the usual absorption experiment with VAA in 0.05% PS-80 solution demonstrated no significant effect on the disappearance of VAA, which ruled out the possibility that the surface-active agent may affect the integrity of absorptive membrane.

Disappearance of VAA from the small intestine was studied in the two concentration ranges of PS-80. Although overall disappearance curves are similar to those in the large intestine as shown in Fig. 3, a different picture is seen with PS-80 concentration dependency. Fig. 4 shows that disappearance of VAA increases with the increase of PS-80 concentration in the rapid phase, whereas that in the slow phase was apparently independent on the PS-80 concentration. It is worthy to note that despite the large effective surface area, disappearance of VAA from the small intestine in the slow phase, 60 minute period, was smaller compared with the large intestine.

A possible reason for the PS-80 concentration dependency in the disappearance of VAA can be related to the effect of the surface active agent on the de-esterification step in the absorptive process of VAA from the intestine. It has been well demonstrated[8,9] that, during absorption from the intestine, VAA is totally de-esterified to VAOH at the outer-surface of the brush border facing the lumen of the intestine, and the product VAOH crosses the brush border to enter into the mucosal cell, where it is re-esterified. With the above in mind, it became desirable to compare the absorption of VAOH with that of VAA in the large intestine. As can be seen in Fig. 5, essentially the same pattern was observed with VAOH.

Rapid and slow phases were distinct and similar PS-80 concentration dependent profiles were observed.

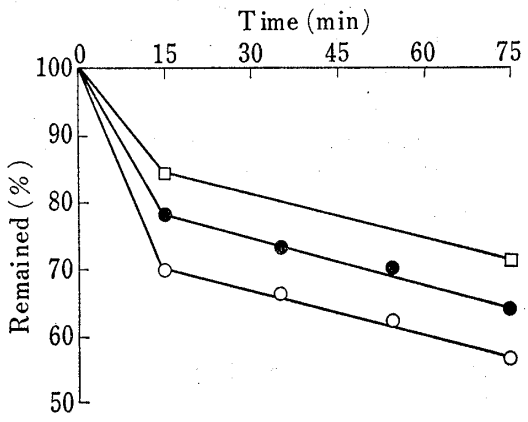

Fig. 5. Disappearance of VAOH from the Large Intestine

concentration of VAOH=0.15 mM
● : with PS-80 0.1%
○ : with PS-80 0.5%
□ : with PS-80 4.0%

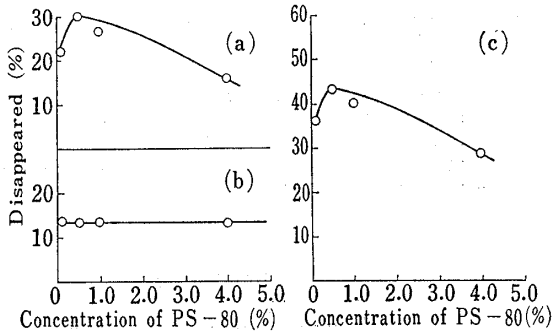

Fig. 6. Effect of PS-80 Concentration on the Absorption of VAOH from the Small Intestine

(a) for the initial 15 min (b) for the subsequent 60 min (c) for the total 75 min

That no concentration effect of PS-80 was observed in the VAA hydrolysis experiment *in vitro* with the mucosal enzyme solution further demonstrated the apparent lack of the effect of the surface-active agent on the step of de-esterification. Although significant difference was not observed in the rapid phase, disappearance of VAOH in 60 minute-slow-phase was about one half the value of VAA in the same phase. Since enzymatic de-esterification is not involved in the absorption of VAOH, it appears that adsorption to the surface, a step before hydrolysis on the surface, might be playing a significant role in the disappearance of these compounds from the perfusate in an early stage of absorption. Fig. 7 shows the results of another experiment indicating the importance of adsorption step in the effect of surface-active agent.

Solution of PS-80 alone were perfused for 75 minutes through the small or the large intestine and the amount disappeared (adsorbed) of the surface-active agent from the perfusate was determined. In the small intestine, adsorption of PS-80 seems incomplete even in the higher concentration ranges of the surface-active agent, whereas in the large intestine, amount adsorbed of PS-80 shows a tendency to level off. It appears that percentage disappearance PS-80 concentration profile in the rapid phase may be explained by the adsorption of VAA or VAOH entrapped in micelles and the difference observed in such profiles in Fig. 2 and 4 could be attributed to the difference of the amount of PS-80 at the surface of the absorptive

8) B.D. Drujan, R. Castillon, and E. Guerrero, *Anal. Biochem.*, **23**, 44 (1968).
9) J. Gangly and S. Mahadevan, *Biochem. J.*, **81**, 53 (1961).

membrane caused by the difference of surface areas. Two lines in Fig. 7 well correlate with the 15 minute disappearance plots shown in Fig. 2 and 4. Gradual decrease in 15 minute absorption of VAA from the large intestine at higher concentration range of PS-80 could be interpreted in terms of the adsorption of PS-80 on the surface of the large intestine. As is evident from Fig. 7, adsorption is saturated with respect to PS-80 at a relatively low concentration of the surfactant. It is conceivable, therefore, that in the post-saturative concentration region, adsorption of VAA-free surfactant micelles gradually outweighs the adsorption of VAA-containing ones thus reducing the available concentration of VAA on the surface for absorption.

Results of the single perfusion experiment shown in Fig. 8 indicate that the largest amount of VAA is adsorbed for the first one-minute fraction of the perfusate regardless of the initial concentration of the surface-active agent and that PS-80 concentration dependency in the adsorption of VAA for ten minutes is quite similar to those appeared in Fig. 1.

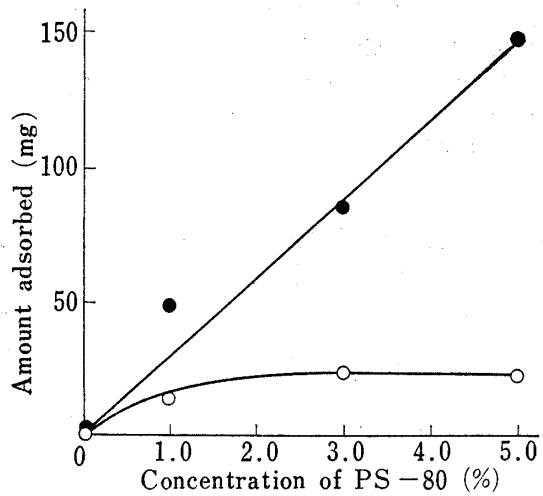

Fig. 7. Adsorption of PS-80 on the Intestinal Mucosa by the Recirculation Technique

○ : large intestine
● : small intestine

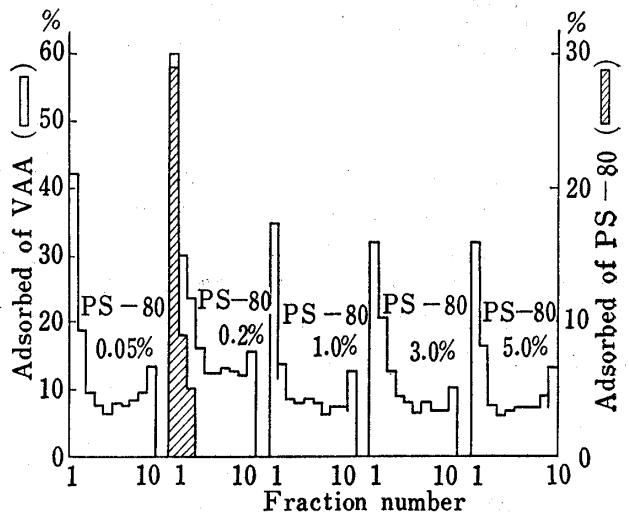

Fig. 8. Adsorption of VAA on the Intestinal Mucosa by the Single Perfusion Technique

Adsorption of PS-80 at the concentration of 0.2% seems to follow the similar pattern as shown in Fig. 8. That no adsorption of PS-80 was observed in the fraction No. 4 and thereafter strongly supports the foregoing finding that VAA entrapped in PS-80 micelles is not absorbed as such.

These data provide further evidence that the mechanism appears to involve a rapid micellar adsorption of VAA in a very early stage of perfusion and the surface-active agent mediated effect is most significant in such period.

Chart 1 is a shematic representation of the intestinal absorption of VAA.

Solubilized VAA molecules diffuse in the intestinal lumen to the surface of the intestine. Therefore, VAA entrapped in the surface-active agent are adsorbed as such followed by the release from the micelles and de-esterification. Step (d), hydrolysis reaction, takes place largely on the outer surface of the brush border facing the lumen of the intestine and is hardly affected by PS-80. Step (a) and (b) are influenced by the surface-active agent. The higher the concentration of PS-80, the slower the diffusion of the micelles to the adsorption surface or the slower the release of VAA or VAOH from the micelles. The latter possibility was demonstrated by the extraction experiment in which the rate of extraction of VAA into benzene from the micelles was measured as a function of surface-active agent concentration. Rate of extraction was inversely proportional to the amount of PS-80 in the solution.

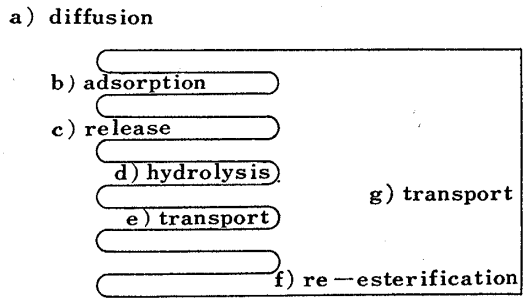

Chart 1. Schematic Representation of the Absorption Process of VAA

a) diffusion	lumen
b) adsorption	surface
c) release	
d) hydrolysis	
e) transport	membrane
f) re-esterification	cell
g) transport	basement-membrane
	lymph

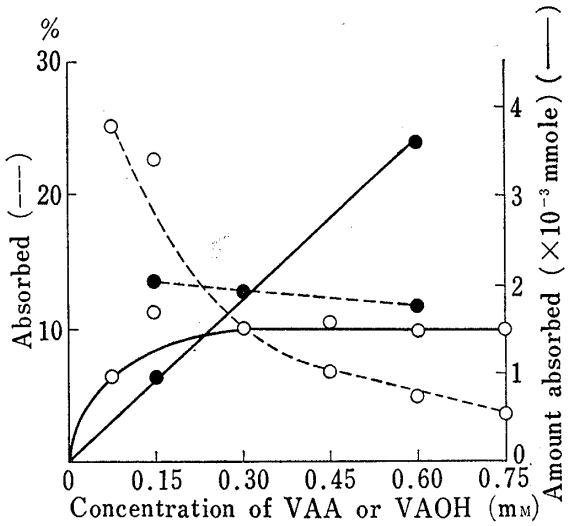

Fig. 9. Absorption of VAA and VAOH from the Solution of Various Concentrations

○ : VAA
● : VAOH

In the very early stage of perfusion (rapid phase), VAA or VAOH molecules solubilized in micelles are adsorbed onto the membrane, adsorption being favored by the surface-active agent and the local concentration of the drugs are built up there, an effect which is not observed by the PS-80 pretreatment. In the slow phase, disappearance now becomes rate-limited by the partition or release of VAA or VAOH from adsorbed micelles to the mucosal layer of the membrane. In this phase, VAA disappears faster than VAOH probably due to its physicochemical characteristics. Difference of PS-80 concentration dependency in two sites of absorption, the small and the large intestine, is probably not due to the localization of hydrolytic enzymes at the large intestinal surface as reported by Dawson, *et al.*[10] but rather to the difference of the surface area for PS-80 adsorption. VAA released from micelles at the outer surface of the brush border is then subjected to de-esterification, a saturable enzymatic process. This step is well demonstrated in Fig. 9, in which percentage and amount disappeared of VAA and VAOH are plotted against the initial concentration of the drugs. At a constant PS-80 concentration, percentage disappearance of VAA rapidly falls to very low values, whereas that of VAOH, remains nearly constant. Metabolic inhibitors such as 2,4-dinitrophenol (0.5 mM) and monoiodoacetamide (0.5 mM) almost completely inhibits the disappearance of VAA in the slow phase without interfering that in the rapid phase.

Although one probably cannot generalize on the effect of micellar agents, it is interesting that apart from its solubilization potential, PS-80 also plays a vital role in the early stage of the absorption of the compounds almost completely entrapped in the micelles.

Further mechanism of the absorption of the drugs in micellar solutions is being carried out in this laboratory.

10) Ian. M. P. Dawson and J. Pryse-Davies, *Gastroenterology*, 44, 745 (1964).