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Effect of Crystallinity on the Percutaneous Absorption of Corticosteroid. II.[1] Chemical Activity and Biological Activity

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The effects of the crystallinity of hydrocortisone acetate and betamethasone dipropionate on their percutaneous absorption were examined. The potency of vasoconstriction of the amorphous corticosteroid was higher than that of the crystalline form and lower than that of the dissolved form. The amorphous state of hydrocortisone acetate in white petrolatum ointment was stabilized by coprecipitation of the drug with polyvinylpyrrolidone. The chemical activity was correlated with the biological activity.

Keywords——crystallinity; percutaneous absorption; hydrocortisone acetate; betamethasone dipropionate; ground mixture; coprecipitate; vasoconstriction; chemical activity; biological activity

The thermodynamic properties of topically applied drugs, especially their solubilities[2] and water–lipid partition coefficients,[3] are important physicochemical factors in relation to percutaneous absorption. Absorption is also affected by vehicles, concentration, diffusivity, surfactants, and so on.[4] Higuchi[2] has theoretically expressed the percutaneous absorption rate from a suspension-type ointment by means of the following equation,

$$\frac{dQ}{dt}=\sqrt{\frac{ADC_s}{2t}} \tag{1}$$

where Q is the amount absorbed in time t per unit area of exposure, A is the concentration of the drug, C_s is the solubility of the drug in the external phase of the ointment, and D is the diffusion constant of the drug molecule in the external phase. The drug permeates from the ointment base *via* the stratum corneum and the epidermis to the dermis, forming an activity gradient.[2]

It was shown in a previous paper[1] that samples of hydrocortisone acetate (HA) with different crystallinities could be obtained by grinding the drug with crystalline cellulose (CC) in various ratios. It is well known that the activity of the solid is affected by specific surface area, surface energy, lattice defects, lattice distortion, crystallinity, *etc.* These are all affected by the grinding process. The difference of crystallinity, as well as of the other factors controlling the solid-state activity, is quite possibly related to the total lattice energy, and so we may expect that the lower the crystallinity, the smaller the energy of solution. The absorption rates of slightly soluble drugs are, therefore, expected to increase as the degree of crystallinity decreases, that is, the activity of the solid state related to A and C_s in Eq. 1 increases.

In this paper, the dependence of percutaneous absorption of HA and betamethasone dipropionate (BD) on crystallinity was examined by the technique developed by McKenzie and Stoughton utilizing the vasoconstriction of human skin.[5]

Experimental

Materials——HA, CC, propylene glycol, liquid paraffin, and white petrolatum (WP) were of JP grade. BD was extracted from a commercial BD ointment. Polyvinylpyrrolidone (PVP) with a mean molecular weight of about 10000, PVP-K15 (Kishida Chem. Co., Ltd.), was used. Indium (99.99% pure) was used as a reference standard for thermal analysis. All other chemicals were of reagent grade.

Preparation of Ground Mixture——The steroid was ground with CC as reported in a previous paper.[1)]

Preparation of Coprecipitate——The steroid and PVP, in weight ratios of 2 : 1, 1 : 1, and 1 : 2, were dissolved in absolute ethanol and coprecipitates were prepared by removing the solvent *in vacuo*. The residues were then dried *in vacuo* at 37 °C to constant weight.

Preparation of Physical Mixture——The steroid and PVP, or the steroid and CC, were mixed uniformly in a mortar with care to avoid any grinding action.

Preparation of Ointments for *in Vivo* Assay——5% HA Ointments: Intact HA, HA–CC ground mixture, or HA–PVP coprecipitate was dispersed into WP (100 g scale).

0.064% BD Ointments: Intact BD, BD–CC ground mixture, or BD–PVP coprecipitate was dispersed into WP (100 g scale). BD ointment containing 10% propylene glycol was prepared as follows; a 0.064 g sample of BD was dissolved in 9.936 g of propylene glycol, and then 90 g of WP was heated until it melted and was added to the solution with vigorous agitation. The resulting substance was cooled to ambient temperature while being kneaded continuously in a mortar. BD remained as a propylene glycol solution homogeneously dispersed in the WP mass.

Particle Size Measurement——Particle size was microscopically measured by a Nihon Regulator Luzex 450 particle analyzer. Samples were dispersed in liquid paraffin. The particle size distribution was determined by repeated counting of approximately 5000 particles.

Differential Scanning Calorimetry——A differential scanning calorimeter (DSC), Perkin-Elmer Model DSC-1B, was used. Indium was used for calibrating the instrument. The area under the endothermic peak near 225 °C was measured with approximately 3 mg of a sample at a heating rae of 5 °C/min. The degree of crystallinity of a sample was determined in a manner similar to that of Fischer *et al.*[6)]

Measurement of X-Ray Diffraction——A Geiger Flex 2012 diffractometer (Rigaku Denki Co., Ltd.) was used. The measurement conditions were the same as reported in a previous paper.[7)]

Calculation of the Degree of Crystallinity——The degree of crystallinity, X_{cr}, and the disorder parameter, k, were determined with a Burroughs 7800 computer by the automated computing procedure reported in a previous paper.[7)]

Dissolution Method——The measurement conditions were the same as reported in a previous paper.[1)]

Viscosity Measurement——A cone and plate rheometer (Ishida Giken Model IGK-120) was used at shear rates of up to $100\,s^{-1}$ and at 25 ± 0.5 °C. The cone with a 2.15 cm radius made an angle of 0.005818 rad with the plate.

Physical Stability Test——The physical stability of the amorphous state of HA in the ointment was investigated by the X-ray diffraction method. The compositions of the ointments were as follows:

1. HA : PVP : WP = 10 : 10 : 80 (a coprecipitate)
2. HA : CC : WP = 10 : 40 : 50 (a ground mixture)

These two ointments were each divided into two parts, each of which was stored either for 40 d in 4 d cycles consisting of 2 d at 3 °C and 2 d at 40 °C, or for 40 d entirely at 5 °C.

***In Vivo* Percutaneous Absorption Test**——The percutaneous absorption of steroid was examined in double-blind tests in a similar manner to McKenzie and Stoughton's technique, that is, by assessment of the bleaching due to vasoconstriction of the human skin. Ten male volunteers, 23—28 years old took part in the test. On each volunteer's forearm was placed a cotton disk (1 cm in diameter) for patch test use, on which 100 mg of ointment had been spread. The disk was covered with a flexible plastic film with an adhesive border. The disks and the adhesive flexible film designed for patch tests were purchased from Torii Yakuhin Co., Ltd. After a suitable occlusion time (5 h for HA and 4 h for BD), the disk was removed, then the relevant portion of the forearm was wiped with soft tissue (twice) and left uncovered until the assessments of the bleaching due to vasoconstriction (carried out 30 min and 1 h later for HA, and 2 and 4 h later for BD). The bleaching was scored as follows: (0), no bleaching; (0.5), slight; (1), obvious; (2), very obvious; (3), pronounced; (4), very pronounced.

Results and Discussion

Physicochemical Properties of Ground Mixtures and of Coprecipitates

It was reported that the percutaneous absorption of fluocinolone acetonide was improved when the micronized grade drug was used.[8)] The chemical potential of a drug increases as its crystallinity decreases and as the surface area is increased by micronization. The biological potential of the drug was accordingly expected to increase with decreasing degree of crystallinity, and so the effect of crystallinity on the percutaneous absorption of

steroids was quantitatively investigated here.

As shown in Fig. 1, the particle sizes of both the HA and the BD samples used were below 10 μm. The arithmetic mean diameter of the HA particles was calculated to be 2 μm, and their mean volume diameter to be 4 μm. Figure 2 shows the X-ray diffraction patterns of both HA and BD. The X_{cr} and k of intact HA were calculated by an X-ray diffraction method to be about 90% and 3.5 Å^2, and those of intact BD were about 80% and 5 Å^2. In this experiment, the samples of ground mixtures of HA and CC were the same as those used in a previous study.[1] The X_{cr} and k of these samples are shown in Table I.

A peak due to the fusion of HA in the ground mixture appeared near 225 °C on the DSC thermogram. The DSC thermograms of intact HA and amorphous HA (HA–CC ground

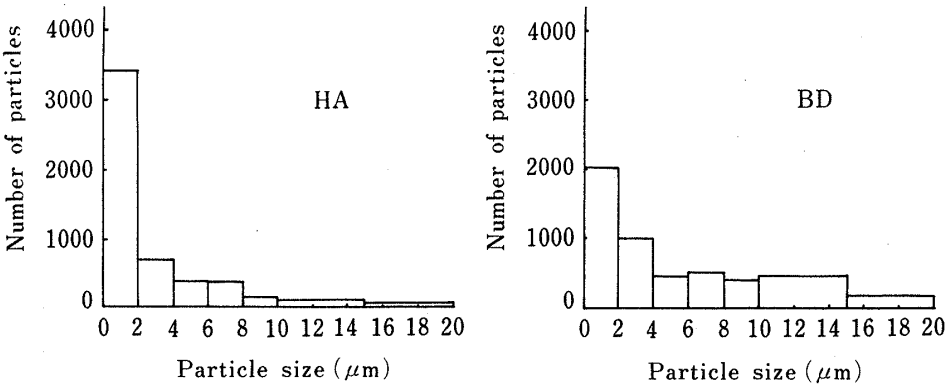

Fig. 1. Particle Size Distributions of HA and BD

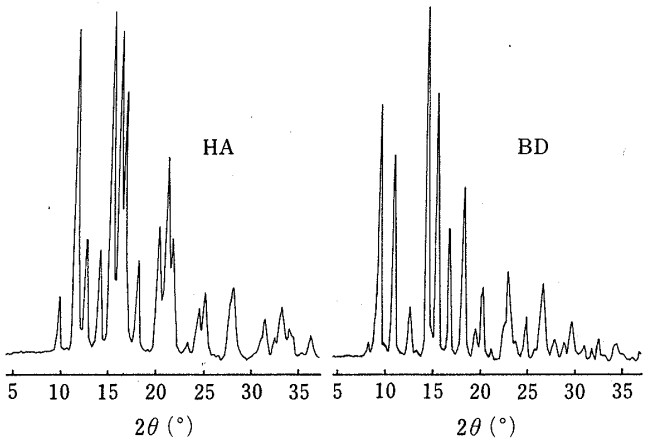

Fig. 2. X-Ray Diffraction Patterns of Intact HA and Intact BD

TABLE I. Effects of Grinding with CC on the Enthalpy Change of Apparent Heat of Fusion (ΔH_f) of HA, and Calculated Crystallinity and Disorder Parameter Based on Calorimetry and X-Ray Diffractometry

Sample	Ratio HA : CC	ΔH_f kcal/mol	DSC	X-Ray method	
			X_{cr}	X_{cr}	k
HA (intact)	—	8.0	1.00	0.90	3.5
Ground mixture	8 : 2	3.4	0.43	0.65	5.1
	6 : 4	4.3	0.54	0.60	7.5
	4 : 6	3.8	0.48	0.45	4.0
	2 : 8	3.9	0.49	0.00	—
	1 : 9	0.0	0.00	0.00	—

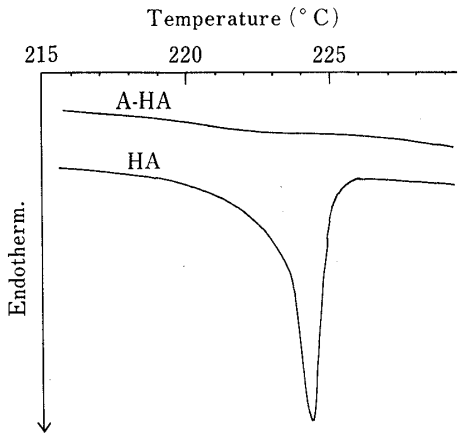

Fig. 3. DSC Thermograms of Intact HA and Amorphous HA in a Ground Mixture with CC in the Ratio of 1:9 (A-HA)

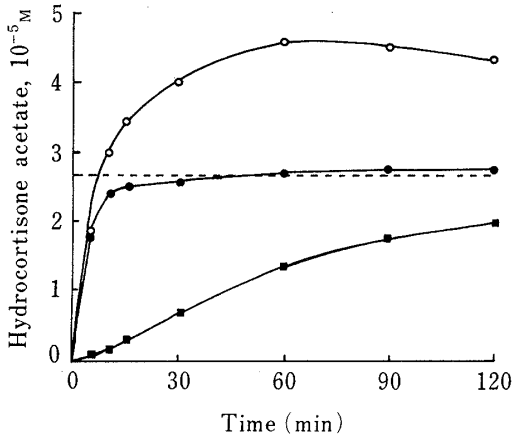

Fig. 4. Dissolution Profiles of HA from a Coprecipitate with PVP

Modified JP paddle method;[1] sample weight = corresponding to 30 mg of HA; test solution = 300 ml of saline solution; 100 rpm; $n=3$; ○, a coprecipitate (HA: PVP = 1:2); ●, a physical mixture (HA: PVP = 1:2); ■, drug alone; ----, solubility.

mixture in the ratio of 1:9) are shown in Fig. 3. A slight coloring of the sample in the pan, a sign of decomposition, was noted at the same time. However, the heat of decomposition was neglected in calculating the degree of crystallinity, X_{cr} (by DSC), because the decomposition that caused the coloration was thought to be only the initial stage of total decomposition, so that the heat liberated was only a small portion of the total decomposition heat as shown in Fig. 3, and the number of molecules participating in the decomposition was considered to be negligible from a stoichiometric viewpoint. The results of the calculations are shown in Table I. The chemical potential of HA in ground mixtures was higher than that of intact HA. The crystallinity decreased in the ground mixture and the thermodynamic crystallinity, X_{cr} (by DSC), showed that a 1:9 ratio of CC was necessary for complete destruction of the crystalline structure of HA, although the X-ray crystallographical structure had already disappeared at a CC ratio of 2:8 (halo pattern, data not shown).

All coprecipitates prepared were confirmed to be amorphous. As shown in Fig. 4, the dissolution rate of HA from PVP coprecipitate was observed to be faster than that of intact HA, as was the case for amorphous HA in ground mixtures.[1]

In Vivo Assay

Vasoconstriction studies were carried out with the HA ointments. The vasoconstriction was assessed soon after the removal of the patch, because no bleaching was observed after 2 h. The reason may be either the low potency of HA or the rapid disappearance of HA from the skin. The results are shown in Fig. 5. The data at 0.5 h after the removal of the patch indicate that the absorption increased with decreasing crystallinity. The tendency for the absorption at high CC ratios to decrease may be due to the resistance, due to viscosity, to the diffusion of HA through the ointment base.

The viscosities of the ointments used are shown in Table II. The viscosity of the HA ointment containing a ground mixture (1:9) was approximately 26 times that of the ointment containing intact HA. The absorption was improved by the reduction of HA crystallinity until this effect was overcome by the effect of the increase in viscosity. The responses at 1 h after removal of the patch were observed to decrease, although the tendency seemed similar to that at 0.5 h after the removal.

Figure 6 shows the results after the application of the ointments containing HA–PVP

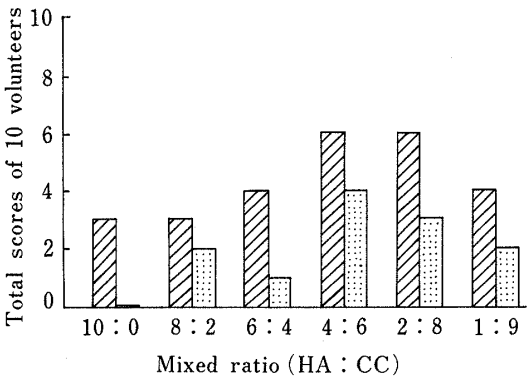

Fig. 5. Comparison of the Percutaneous Penetration of HA in Ground Mixtures with CC in 5% WP Ointment after 5 h of Occlusion

$n=10$; ▨, assessment after 0.5 h; ▩, assessment after 1 h. Degrees of bleaching: no bleaching=0, slight=0.5, obvious=1, very obvious=2, pronounced=3, very pronounced=4.

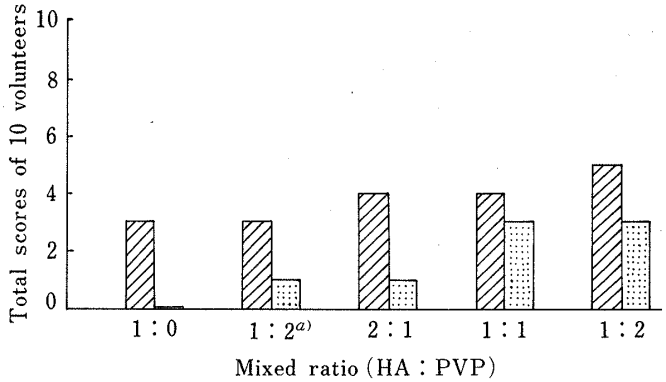

Fig. 6. Comparison of the Percutaneous Penetration of HA in Coprecipitates with PVP in 5% WP Ointment after 5 h of Occlusion

$n=10$. Symbols and bleaching scores are the same as those in Fig. 5.
a) A physical mixture.

TABLE II. Viscosities of 5% HA Ointments Used for *in Vivo* Assay at a Shear Rate of $100\,s^{-1}$

Composition ratio of ointments	Viscosity (P)
HA (intact) : WP = 5 : 95	11.6
HA : CC : WP = 5 : 7.5 : 87.5	56.0
HA : CC : WP = 5 : 20 : 75	93.0
HA : CC : WP = 5 : 45 : 50	300.0
HA : PVP : WP = 5 : 10 : 85	37.0

coprecipitates. It seems possible that an HA–PVP complex may be formed in the presence of moisture (from sweat) under an occlusion dressing with a resulting decrease of the chemical potential of HA. However, Fig. 6 indicates that the absorption was still increased by coprecipitation. The increased solubility arising from the reduced crystallinity may have predominated over the inactivation by complex formation. The vasoconstriction by HA was so weak that a score of (2) was never attained at an HA concentration of 5%.

Further investigation was carried out to clarify the effects of the amorphous state on the percutaneous absorption of the other corticosteroid, BD, which is 700—800 times more potent as a vasoconstrictor than HA. The results after 2 h of occlusion are shown in Fig. 7. A ground mixture of BD (1 : 9) was observed to be more potent than the crystalline form, but less potent than the dissolved form.

As shown in Fig. 8, similar results were obtained after 4 h of occlusion. The difference between the crystalline and dissolved forms was significant at the confidence limit of variance, $1-\alpha=0.95$; that between the crystalline and amorphous forms was significant at $1-\alpha=0.90$; and that between the amorphous and dissolved forms was significant at $1-\alpha=0.80$. Thus, in relation to vasoconstriction, the amorphous form is intermediate between the crystalline and dissolved forms.

These corticosteroids can be dissolved in an ointment, but the absorption rate of a drug can also be improved by reducing its crystallinity, which affects apparent C_s in Eq. 1, even if the drug is not dissolved in an ointment. The results obtained indicate that the chemical activity is correlated with the biological activity.

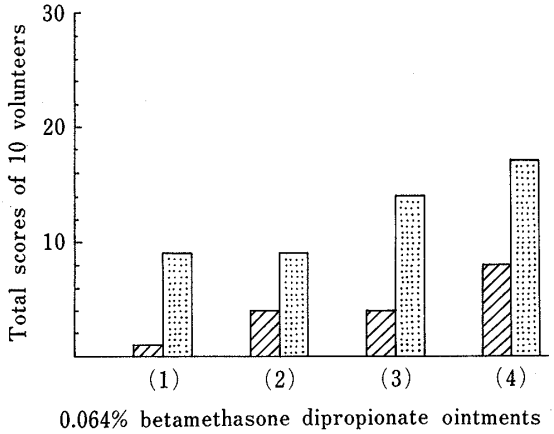

Fig. 7. Comparison of the Percutaneous Penetration of BD in 0.064% WP Ointment after 2 h of Occlusion

(1) crystals, (2) ground mixture with CC (1 : 9), (3) coprecipitate with PVP (1 : 1), (4) dissolved in 10% propylene glycol; $n=10$; ▨, assessment after 2 h; ▩, assessment after 4 h. Bleaching scores are the same as those in Fig. 5.

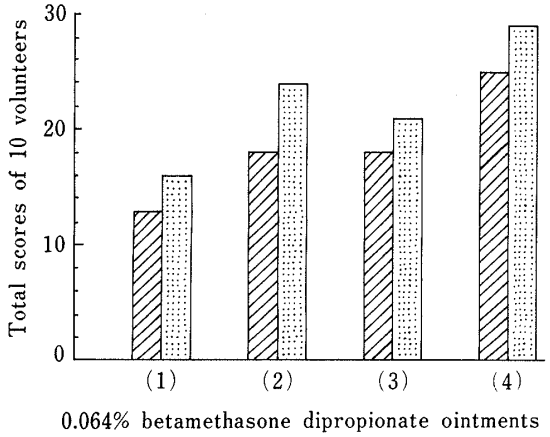

Fig. 8. Comparison of the Percutaneous Penetration of BD in 0.064% WP Ointment after 4 h of Occlusion

$n=10$. Samples, symbols, and bleaching scores are the same as those in Fig. 7.

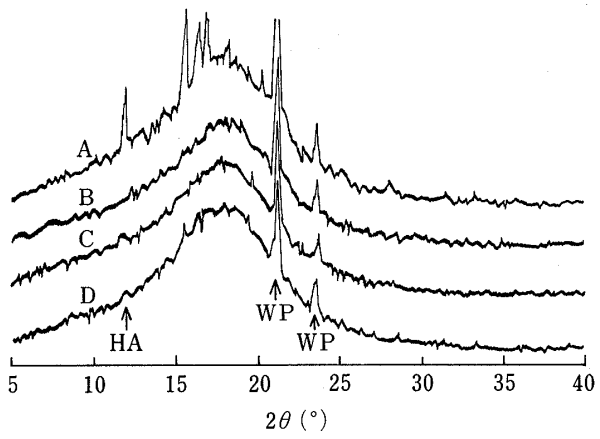

Fig. 9. Effects of Accelerated Storage Conditions on the Physical Stability of HA in a Coprecipitate with PVP in WP as Revealed by X-Ray Diffraction Patterns

A, a physical mixture; B, intact coprecipitate; C, coprecipitate (40 d cycles consisting of 2 d at 3 °C and 2 d at 40 °C); D, coprecipitate (5 °C for 40 d); composition, HA : PVP : WP = 10 : 10 : 80.

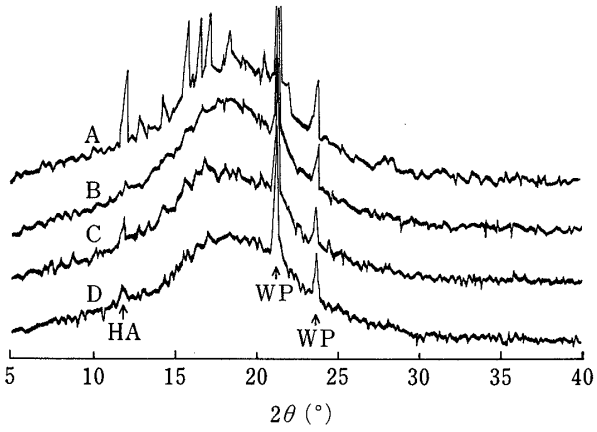

Fig. 10. Effects of Accelerated Storage Conditions on the Physical Stability of HA in a Ground Mixture with CC in WP as Revealed by X-Ray Diffraction Patterns

A, a physical mixture; B, intact ground mixture; C, ground mixture (40 d cycles consisting of 2 d at 3 °C and 2 d at 40 °C); D, ground mixture (5 °C for 40 d); composition, HA : CC : WP = 10 : 40 : 50.

Physical Stability of the Amorphous State of HA in a WP Ointment

The physical stability of the amorphous state of HA in both a ground mixture and a coprecipitate in WP was investigated. The results are shown in Figs. 9 and 10. The sharp diffraction peak at $2\theta=12°$ characteristic of HA disappeared as a result of coprecipitation and the halo pattern remained after 40 d of temperature cycles between 3 and 40 °C or 40 d at a constant temperature of 5 °C. The same peak of HA also disappeared with a ground mixture, but reappeared after 40 d under both conditions. The amorphous state of HA in a coprecipitate in WP was thus more stable than that of HA in a ground mixture. However, these experiments are insufficient to indicate whether the difference of physical stability between these two amorphous forms results from a thermodynamic difference or a kinetic

difference. Nevertheless, it can be said that physical stabilization of the metastable state can be attained by such modifications of crystalline forms.

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References and Notes

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