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Permeation of Zidovudine and Probenecid from Oily Bases Containing Alcohols Through Rat Skin

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ABSTRACT

Permeation of zidovudine (3'-azido-3'-deoxythymidine, AZT) and probenecid from oily bases containing an alcohol through rat skin was examined. Isopropyl myristate (IPM), as an oily vehicle, showed a penetration enhancing effect for AZT and probenecid. Ethanol, n-propanol, and n-butanol were used as additives in IPM and were examined for their own permeation and the enhancing effect on the permeation of AZT and probenecid. The skin permeation of AZT and probenecid from IPM was enhanced by addition of the alcohol in IPM. The degree of the enhancement was decreased with increasing lipophilicity of the alcohol used. The permeation rate of the drug from those systems was shown to be governed by penetration-enhancing effects of the oily base and alcohol, and the penetration of the alcohol itself through the skin.

INTRODUCTION

Zidovudine (3'-azido-3'deoxythymidine, AZT), an inhibitor of the reverse transcriptase of the human immunodeficiency virus, has primarily been administered orally (1-3). In our previous studies (4-6), possibilities of transdermal application of AZT, as a new administration route, were discussed. The transdermal delivery system of AZT would necessitate less frequent dosing so that high patient acceptability and compliance could be obtained.

In the present study, permeation of AZT and probenecid from oily bases through rat skin is reported. Probenecid is useful to improve pharmacokinetics and distribution property of AZT (7-10). Probenecid transdermally coadministered with AZT may increase the plasma concentration and cerebrospinal fluid/plasma concentration ratio of AZT. The effective transdermal

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coadministration system of AZT and probenecid could be designed based on a good understanding on the mode of permeation of both the drugs through the skin. The oily vehicles used were silicone fluid, as an inactive base for penetration enhancement of drugs, and isopropyl myristate (IPM), as an active base for the enhancement (11). Alcohols such as ethanol have been shown to affect the skin lipid and also to serve as carrier for drugs, and hence, to enhance permeation of drugs through skin (12–14). In addition to ethanol, either *n*-propanol or *n*-butanol was also used as a coenhancer in order to further clarify the role of alcohol on the permeation of AZT and probenecid.

EXPERIMENTAL

Materials

AZT and probenecid were purchased from Yamasa Shoyu Co. (Chiba, Japan) and Sigma Chemical (St. Louis, MO), respectively. All other chemicals were of reagent grade and were used as received.

Permeation Studies

Male Wistar rats weighing 200–290 g were anesthetized with intraperitoneal injection of pentobarbital (50 mg/kg) and the abdominal hair was removed by clippers. The skin was excised and mounted in a two-chamber diffusion cell (15). This cell has an available diffusion area of 0.95 cm^2 and a half-cell volume of 2.3 ml. Cell sets were kept at 37°C in a water bath; the dermal and epidermal sides of the skin were exposed to saline as a receptor medium and test medium containing the drug, respectively. The test medium consisted of 20 mg/ml of AZT and 40 mg/ml of probenecid, both as suspensions, and one of the alcohols (0–20%) in IPM or silicone fluid. The medium was prepared by adding weighed amount of AZT and probenecid to the silicone fluid–alcohol or IPM–alcohol mixture. The medium was kept at 37°C for at least 12 hr before application to ensure saturation. A sample (1 ml) was withdrawn from the receptor hourly for up to 18 hr after application of the test solution, and the same volume of fresh saline was added to the receptor to keep the volume constant.

Determination of AZT, Probenecid, and Alcohols in Receptor Medium

A high performance liquid chromatography (HPLC) system used for the determination of AZT and probenecid in receptor medium was composed of a Shimadzu LC-6A pump, a Shimadzu SPD-6A UV detector, a Rheodyne 7125 injector, and a reversed-phase column (LiChrospher® RP-18e, 250 × 4 mm). The samples were mixed with the same volume of acetonitrile containing two internal standards (7-β-hydroxypropyltheophylline for AZT and *n*-butyl *p*-hydroxybenzoic acid for probenecid, each at a concentration of 5 μg/ml). After centrifugation at 14,000 rpm, the supernatant (20 μl) was applied to the HPLC twice. For AZT analysis, the ultraviolet (UV) detector was operated at 265 nm, and a mobile phase containing 84.9% water, 15% acetonitrile, and 0.1% acetic acid was flowed at 1 ml/min. For probenecid analysis, the UV detector was operated at 244 nm, and a mobile phase containing 49.9% water, 50% acetonitrile, and 0.1% acetic acid was flowed at 1 ml/min.

Alcohols in receptor medium were determined by gas–liquid chromatography (GLC). A Shimadzu GC-15 gas chromatograph equipped with a hydrogen flame ionization detector, an integrator and a 1-m glass column packed with PEG-20M 10% on Chromosorb WAW 60/80 (Japan Chromato Ind. Co.) was operated with the temperatures set at 160°C for injector and detector and 140°C for column. Nitrogen as a carrier gas was flowed at 50 ml/min. To 100 μl of sample solution, 100 μl of dioxane containing an internal standard (*n*-propanol for ethanol, ethanol for *n*-propanol and *n*-butanol) was added and mixed. A 2-μl portion of the solution was applied to the GLC.

Solubility Measurement

An excess amount of AZT or probenecid was added to water, one of the alcohols, IPM or IPM containing one of the alcohols, and the mixture was equilibrated for 12 hr at 37°C. The mixture was passed through a membrane filter of 0.45 μm in pore size; then an aliquot of the filtrate was adequately diluted with dixane for measurement of UV absorption at 265 nm or 244 nm for AZT or probenecid, respectively.

RESULTS AND DISCUSSION

Physicochemical Properties of AZT and Probenecid

AZT and probenecid are similar in the molecular weight (AZT, 267; probenecid, 285) but different in the lipophilicity. The melting points of AZT and probenecid are 124°C (6) and 199°C (16), respectively. The val-

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ues of lipophilic index of AZT and probenecid, which were determined from the retention times in the reversed-phase HPLC according to the method described by Yamana et al. (17), were 1.41 and 3.15, respectively, suggesting that AZT is more hydrophilic than probenecid. Table 1 shows the solubilities of AZT and probenecid in several solvent systems. In the hydrophilic solvent systems as water, ethanol, and *n*-propanol, AZT is more soluble than probenecid. On the other hand, AZT is less soluble than probenecid in the lipophilic solvent systems.

Effect of Ethanol on the Permeation of AZT and Probenecid from Silicone Fluid

Ethanol has been shown to exert enhancement on skin permeation of drugs. Possible effect of ethanol on the skin permeation of AZT and probenecid was investigated. Figure 1 shows the permeation profiles of AZT and probenecid through rat skin from silicone fluid. Cumulative amounts of both AZT and probenecid permeated in 8 hr were very low (less than 10^{-2}% of applied amount).

When ethanol was added in silicone fluid at 20% level, the permeation of AZT and probenecid was enhanced with lag times of 3 hr and 5 hr, respectively. Approximately tenfold increase in the cumulative amount of permeation in 8 hr was observed for each drug. The enhancing effect of ethanol for the skin permeation of AZT and probenecid was thus clarified.

Table 1

Solubility of AZT and Probenecid in Various Solvent Systems

Media	Solubility at 37°C, mM	
	AZT	Probenecid
Water	113	0.175
Ethanol	228	189
n-Propanol	187	165
n-Butanol	124	163
10% ethanol/IPM	19.8	47.0
20% ethanol/IPM	28.2	80.3
20% *n*-propanol/IPM	23.5	76.0
20% *n*-butanol/IPM	23.2	68.8
40% ethanol/IPM	116	130
IPM	1.5	6.2

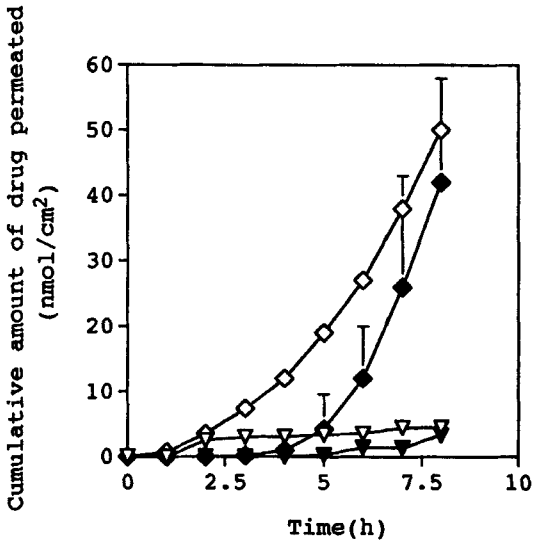

Figure 1. Permeation of AZT and probenecid through rat skin from silicone fluid containing ethanol in the donor medium. AZT (open) and probenecid (closed): ▽, ▼: 0% ethanol; ◇, ◆: 20% ethanol. Each point represents the mean ± *SD* of 3 determinations.

Effect of Ethanol on the Permeation of AZT and Probenecid from IPM

The permeation profiles of AZT and probenecid from IPM through rat skin are shown in Fig. 2. The permeation patterns of ethanol itself are also shown in Fig. 3. The permeation rates of both the drugs through the skin from IPM were greater than those from silicone fluid dipicted in Fig. 1. Mean flux values (F_{3-8}) of the drugs and ethanol itself, calculated from the permeation data between 3 to 8 hr, from IPM and silicone fluid systems are listed in Table 2. When compared the fluxes of AZT and probenecid from IPM and silicone fluid each containing 20% ethanol, the F_{3-8} values for AZT and probenecid in the IPM systems were 360 and 240 times higher, respectively, than those in the silicone fluid systems. In these cases, the flux of ethanol itself from IPM system was only 5 times higher than that from silicone system. These results clearly indicate that IPM acts as a penetration enhancer for AZT and probenecid.

The effect of the content of ethanol in IPM on the permeation of AZT and probenecid can easily be seen in Fig. 2 and Table 2. With increase in the initial content of ethanol, both cumulative amount permeated in 8 hr and the F_{3-8} values of AZT and probenecid were also increased. The F_{3-8} value of ethanol itself increased pro-

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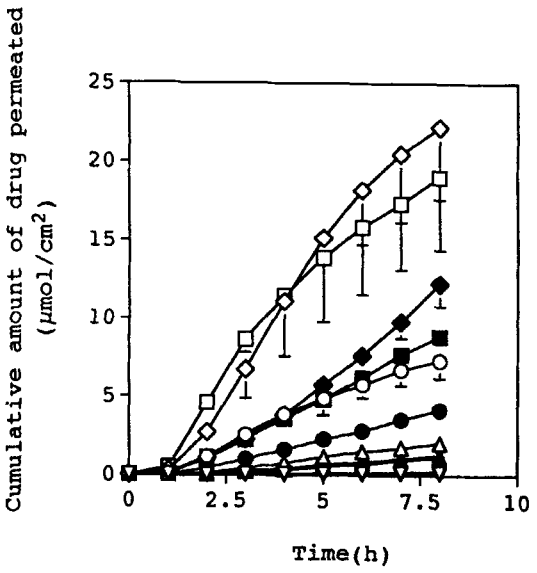

Figure 2. Permeation of AZT and probenecid through rat skin from IPM containing ethanol in the donor medium. AZT (open) and probenecid (closed): ∇, ▼: 0% ethanol; Δ, ▲: 5% ethanol; ○, ●: 10% ethanol; □, ■: 15% ethanol; ◇, ◆: 20% ethanol. Each point represents the mean ± *SD* of 3 (for ∇, ▼, Δ, ▲, ○, ●, □, ■) or 6 (for ◇, ◆) determinations.

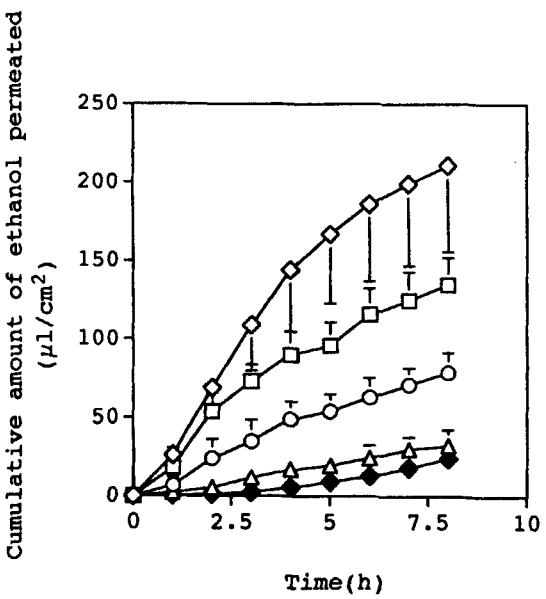

Figure 3. Permeation of ethanol through rat skin from IPM or silicone fluid containing ethanol in the donor medium. Δ: 5% ethanol/IPM; ○: 10% ethanol/IPM; □: 15% ethanol/IPM; ◇: 20% ethanol/IPM; ◆: 20% ethanol/silicone fluid. Each point represents the mean ± *SD* of 3 (for Δ, ○, □, ◆) or 6 (for ◇) determinations.

portionally to its initial content in IPM. On the other hand, the F_{3-8} values of AZT and probenecid increased rather exponentially with increase in the initial content of ethanol. Thus the concentration-dependent coenhancing effect of ethanol on the skin permeation of AZT and probenecid with IPM was indicated.

Comparison of Effect of *n*-Propanol and *n*-Butanol with Ethanol

In order to further clarify the effect of coadded alcohol in IPM on the skin permeation of AZT and probenecid, *n*-propanol and *n*-butanol were also employed in addition to ethanol. The permeation profiles of AZT and probenecid from IPM containing 20% of ethanol, *n*-propanol, or *n*-butanol, and the permeation of each alcohol itself in those systems are shown in Fig. 4 and Fig. 5, respectively. Calculated values of flux (F_{3-8}) of AZT, probenecid, and each alcohol in these systems are shown in Table 2. The permeation rate of alcohol itself decreased with increase in the number of carbon atoms in the alcohol molecule. Lower permeation rate of alcohol with longer alkyl chain may be due to lower escaping tendency in the lipophilic base (IPM) and, to some extent, larger molecular volume of the alcohol. The permeation rate of AZT was lower when more lipophilic alcohol was added. The same tendency was observed for probenecid.

The manner in which chemical solvents, especially ethanol, enhance the skin permeability of many drugs has been studied. The enhancement has been shown to be correlated with physical perturbation of the lipoidal barrier, delipidization (18), or solvent flow (14) in the stratum corneum. Now focusing only on the contribution of alcohol flow to the net flux of drugs, solubility of the drug in the penetrating alcohol as well as the flux of the alcohol should be the main determinants. In IPM system containing one of the alcohols, F_{3-8} value of AZT was always larger than that of probenecid except for 20% *n*-butanol/IPM system. In this case, larger solubility of probenecid in *n*-butanol than AZT was assumed to contribute significantly to the net flux. Affinity of the drug with penetrating alcohol through skin can thus affect the permeation rate of the drugs.

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Table 2

Mean Flux of Drug and Alcohol from Various Solvent Systems

Media		Mean Flux (F_{3-8})[a]	$F_{drug}/F_{alcohol}$[b]
Silicone fluid	AZT	$4.0 \times 10^{-4} \pm 0.3 \times 10^{-4}$	
	Probenecid	$6.6 \times 10^{-4} \pm 0.4 \times 10^{-4}$	
20% ethanol/silicone fluid	AZT	$8.5 \times 10^{-3} \pm 1.5 \times 10^{-3}$	2.0×10^{-3}
	Probenecid	$8.4 \times 10^{-3} \pm 2.2 \times 10^{-3}$	1.9×10^{-3}
	Ethanol	4.34 ± 0.79	
IPM	AZT	$3.0 \times 10^{-2} \pm 0.8 \times 10^{-2}$[c]	
	Probenecid	$2.0 \times 10^{-2} \pm 0.7 \times 10^{-2}$[c]	
5% ethanol/IPM	AZT	0.33 ± 0.11	0.080
	Probenecid	0.20 ± 0.07	0.050
	Ethanol	4.11 ± 1.61	
10% ethanol/IPM	AZT	0.94 ± 0.14	0.107
	Probenecid	0.63 ± 0.02	0.071
	Ethanol	8.82 ± 0.91	
15% ethanol/IPM	AZT	2.08 ± 0.12	0.165
	Probenecid	1.32 ± 0.10	0.105
	Ethanol	12.6 ± 0.71	
20% ethanol/IPM	AZT	3.09 ± 1.16[c]	0.151
	Probenecid	2.00 ± 0.13[c]	0.098
	Ethanol	20.5 ± 6.10[c]	
20% *n*-propanol/IPM	AZT	2.42 ± 0.96[c]	0.164
	Probenecid	2.15 ± 0.76[c]	0.145
	n-Propanol	14.8 ± 1.05[c]	
20% *n*-butanol/IPM	AZT	1.35 ± 0.07	0.208
	Probenecid	1.71 ± 0.31	0.263
	n-Butanol	6.50 ± 0.93	

[a]Mean flux calculated from the permeation data from 3 to 8 hr. Each datum presents the mean $\pm$ *SD* ($n = 3$). Unit: drug, μmol/cm²/hr; alcohol, μl/cm²/hr.
[b]F_{3-8} of drug/F_{3-8} of alcohol.
[c]$n = 6$.

In the skin permeation experiments, the higher permeation rates of AZT and probenecid were observed with the higher penetrations of alcohol coadded in IPM which could be accomplished by adding larger amount of an alcohol of less lipophilicity. From the standpoint of safety and efficiency, a system which gives greater enhancement for drug permeation with less assumption of alcohol would be desired. In Table 2, flux ratios of drug and alcohol ($F_{drug}/F_{alcohol}$) calculated from the permeation data from 3 to 8 hr, were also shown. The higher values of $F_{drug}/F_{alcohol}$ indicate efficient delivery for the drugs. When ethanol was used as an coadditive in IPM, the system containing 15% of ethanol was the most efficient base for the delivery of both AZT and probenecid. Among the three alcohols examined, *n*-butanol was the most efficient additive. Since probenecid is a lipophilic drug, the $F_{drug}/F_{alcohol}$ value markedly increased with increasing lipophilicity of alcohol used. Ratio of permeation rate (flux) of AZT and probenecid also changed among the alcohols used.

In conclusion, we reported the permeation of AZT and probenecid from oily bases containing an alcohol through rat skin. By understanding the mode of permeation of the drugs, an effective transdermal coadministration will be designed. Ratio of permeation rate of AZT and probenecid may be controlled by selecting a coenhancer, an alcohol, or other solvent with proper lipophilicity. Our finding can be useful as a basic un-

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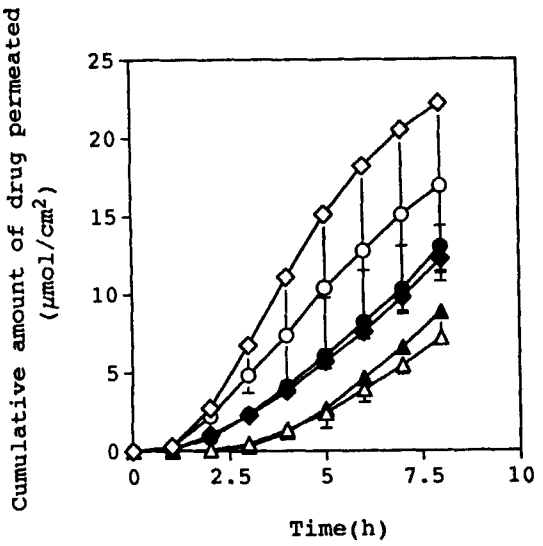

Figure 4. Permeation of AZT and probenecid through rat skin from IPM containing ethanol, *n*-propanol, or *n*-butanol in the donor medium. AZT (open) and probenecid (closed): ◇, ◆: 20% ethanol; ○, ●: 20% *n*-propanol; △, ▲: 20% *n*-butanol. Each point represents the mean ± *SD* of 6 (for ◇, ◆, ○, ●) or 3 (for △, ▲) determinations.

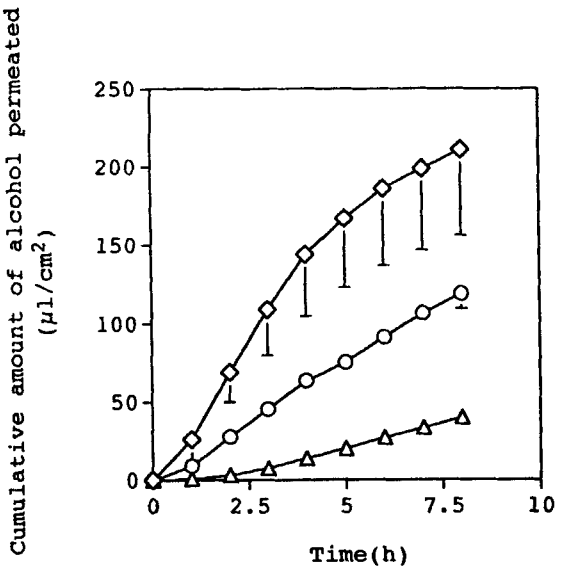

Figure 5. Permeation of alcohol through rat skin from IPM containing 20% alcohol. ◇: ethanol; ○: *n*-propanol; △: *n*-butanol. Each point represents the mean ± *SD* of 6 (for ◇, ○) or 3 (for △) determinations.

derstanding for the transdermal coadministration of AZT and probenecid. Further studies on the flux of each drug and formulation design needed for effective performance in vivo are under way in our laboratories.

REFERENCES

1. H. Mitsuya, K. J. Weinhold, P. A. Furman, M. H. St. Clair, S. N. Lehrman, R. C. Gallo, D. Bolognesi, D. W. Barry, and S. Broder, Proc. Natl. Acad. Sci. U.S.A., 82, 7096 (1985).
2. R. Yarchoan, G. Berg, P. Brouwers, M. A. Fischl, A. R. Spitzer, A. Wichman, J. Grafman, R. V. Thomas, B. Safai, A. Brunetti, C. F. Perno, P. J. Schmidt, S. M. Larson, C. E. Myers, and S. Broder, Lancet, i, 132 (1987).
3. R. W. Klecker, J. M. Collins, R. Yarchoan, R. V. Thomas, J. F. Jenkins, S. Broder, and C. E. Myers, Clin. Pharmacol. Ther., 41, 407 (1987).
4. T. Seki, T. Kawaguchi, K. Sugibayashi, K. Juni, and Y. Morimoto, Int. J. Pharmaceut., 57, 73 (1989).
5. T. Seki, C. Toeda, T. Kawaguchi, K. Juni, K. Sugibayashi, and Y. Morimoto, Chem. Pharm. Bull., 38, 3086 (1990).
6. T. Seki, T. Kawaguchi, and K. Juni, Pharm. Res., 7, 948 (1990).
7. M. A. Hedaya and R. J. Sawchak, J. Pharm. Sci., 78, 716 (1989).
8. R. J. Sawchak and M. A. Hedaya, Pharm. Res., 7, 332 (1990).
9. M. A. Hedaya, W. F. Elmquist, and R. J. Sawchak, Pharm. Res., 7, 411 (1990).
10. M. Qian, T. S. Finco, M. Mehta, C. T. Viswanathan, and J. M. Gallo, J. Pharm. Sci., 80, 1007 (1991).
11. K. Sato, K. Sugibayashi, and Y. Morimoto, Int. J. Pharmaceut., 43, 31 (1988).
12. K. Sugibayashi, M. Nemoto, and Y. Morimoto, Chem. Pharm. Bull., 36, 1519 (1988).
13. T. Seki, K. Sugibayashi, and Y.Morimoto, Chem. Pharm. Bull., 35, 3054 (1989).
14. D. R. Friend and S. I. Smeley, Int. J. Pharmaceut., 97, 39 (1993).
15. K. Sugibayashi, K. Hosoya, Y. Morimoto, and W. I. Higuchi, J. Pharm. Pharmacol., 37, 578 (1985).
16. Determined on a Yanagimoto MPS-3 Micro Melting Apparatus (Japan).
17. T. Yamana, A. Tsuji, E. Miyamoto, and O. Kubo, J. Pharm. Sci., 66, 747 (1977).
18. T. Hatanaka, K. Katayama, T. Koizumi, K. Sugibayashi, and Y. Morimoto, J. Controlled Release, 33, 423 (1995).

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