Skin Permeation of Parabens in Excised Guinea Pig Dorsal Skin, Its Modification by Penetration Enhancers and Their Relationship with *n*-Octanol/Water Partition Coefficients

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Skin penetration of methyl, ethyl, propyl and butyl parabens through excised guinea pig dorsal skin was examined, and effects of the penetration enhancers, *l*-menthol plus ethanol, ethanol itself and *N*-dodecyl-2-pyrrolidone, were observed. Permeability coefficients of the parabens correlated with *n*-octanol/water partition coefficients. Addition of 1% *l*-menthol in 15% ethanol about sixteen times increased the permeability coefficient of methyl paraben, whereas this enhancer decreased that of butyl paraben to about one fifth of the control value. A similar, though weaker, tendency was observed for the effects of 15% ethanol itself. 0.025% suspension of *N*-dodecyl-2-pyrrolidone increased the permeability coefficient of methyl paraben about seven times, whereas it did not change that of butyl paraben significantly. Therefore, dependency of the permeability coefficients of the parabens on *n*-octanol/water partition coefficients almost disappeared in the presence of this compound. A spin label study with stratum corneum lipid liposomes revealed that increase of fluidity of the lipid bilayer by these penetration enhancers corresponded with their enhancement effects on skin penetration of methyl paraben. Perturbation of stratum corneum lipid lamella thus seems to be related with their enhancement of the absorption of the hydrophilic paraben.

Key words paraben; skin penetration; penetration enhancer; *l*-menthol; *N*-dodecyl-2-pyrrolidone; fluidity

Skin is a promising route of drug administration and there are several benefits in administering a drug transdermally. For example, hepatic first pass effect can be avoided, constant blood drug levels can be maintained and good compliance can be anticipated. The rigid lipid lamella structure of the stratum corneum works as a barrier, however, especially for the absorption of hydrophilic drugs.[1] This makes the addition of penetration enhancers or conversion to hydrophobic prodrugs necessary to apply hydrophilic drugs in practical transdermal preparations. However, the effects of these enhancers on skin penetration of drugs with different hydrophobicity are still not clear, and the precise mechanisms of the effects of enhancers are also unknown.

In this work we observed the *in vitro* skin penetration of four alkyl parabens, methyl, ethyl, propyl and butyl parabens, as model drugs and examined the effects of penetration enhancers, *l*-menthol in ethanol–phosphate buffered saline (PBS) solution, ethanol itself, and an Azone® (1-dodecylazacycloheptan-2-one) analog *N*-dodecyl-2-pyrrolidone.[2] We examined the change in relationship between the permeability and *n*-octanol/water partition coefficients of the parabens. Although these penetration enhancers differ in their structures and physicochemical properties, they have been suggested to improve skin permeation of various drugs by perturbation of the lipid lamella in stratum corneum.[3,4] Therefore, we also examined whether there is a common relationship for these enhancers between their enhancement of the absorption of the parabens and the effects on fluidity of stratum corneum lipid lamella by observing the fluidity change in the lipid bilayer of stratum corneum lipid liposomes.

Experimental

Materials Methyl, ethyl, propyl and butyl parabens were purchased from Wako Pure Chemical Industries (Osaka, Japan). *N*-Dodecyl-2-pyrrolidone, 5-doxylstearic acid methyl ester (5-DSME) and 16-doxylstearic acid methyl ester (16-DSME) were obtained from Aldrich Chemical Co. Inc. (Milwaukee, WI). Ceramide (type IV), cholesterol, and cholesteryl sulfate were purchased from Sigma Chemical Co. (St. Louis, MO). All other reagents were purchased from Wako Pure Chemical Industries.

Measurement of *in Vitro Skin* Penetration *In vitro* skin penetration of drugs was examined as described.[5] Full thickness dorsal skin was excised from male guinea pigs and mounted in two-chamber diffusion cells with a water jacket (37 °C). Available diffusion area was about 0.65 cm^2, and each half-cell volume was about 5.4 ml. The donor and receiver compartments, which were stirred by a magnetic stirrer, were filled with phosphate buffered saline (PBS) (pH 7.4) in the presence or absence of penetration enhancer (donor compartments) and in its absence (receiver compartments). Twelve hours later, a suspension of parabens in PBS either in the presence or absence of penetration enhancer was added to the donor cells and the penetration experiment was started. *N*-Dodecyl-2-pyrrolidone was added as a suspension in PBS without any organic solvent. One hundred fifty μl of samples were taken from the receiver cells periodically and analyzed by measuring UV absorbance at 256 nm, and the permeability coefficients, K_p, were calculated according to Eq. 1 from the initial straight portion of the penetration curve, dC_R/dt, shown in Fig. 1 for propyl paraben as an example.

$$K_p = \frac{dC_R}{dt} \cdot \frac{V_R}{A} \cdot \frac{1}{C_d} \quad (1)$$

where C_R and V_R are the concentration of paraben in the receiver compartment and the volume there, respectively, C_d is the concentration of paraben in the donor compartment which is equal to the solubility of paraben, and A is the diffusion area. Solubility of each paraben was measured after incubation of it in an excess amount in PBS in the presence or absence of penetration enhancers at 37 °C for 24 h. Concentration of the filtrate through a nitrocellulose type membrane filter (pore size 0.45 μm) was calculated by measuring UV absorbance at 256 nm and the solubility of each paraben was obtained. Hydrolysis of parabens, which was ascertained by HPLC, was within 3.4% of the total parabens transferred into receiver fluids under this experimental condition.

Depletion of stratum corneum lipids was done by incubating the excised skin with a chloroform–methanol mixture (2:1 vol) for 12 h. After washing the skin with PBS, the lipid-depleted skin was mounted in the two-chamber diffusion cells and permeation of the parabens through lipid-depleted skin was observed by the procedure described above.

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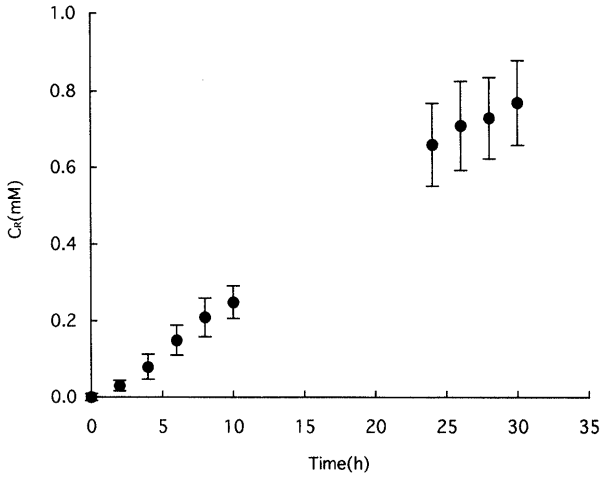

Fig. 1. Increase in Concentration of Propyl Paraben in Receiver Compartment, C_R, due to Transfer through Guinea Pig Dorsal Skin

Data are means±S.D. of three experiments.

Preparation of Liposomes Ceramide, cholesterol, cholesteryl sulfate and palmitic acid were dissolved in chloroform at a weight ratio of 4.0:2.5:1.0:2.5 (Molar ratio, 3.2:3.2:1.0:4.4), as described.[6] The solvent was evaporated, the lipids were suspended in PBS and multilammelar vesicles were prepared by vortex mixing. The total lipid concentration was 1.2 mM. Then, the vesicle suspension was sonicated with a probe-type sonicator at 70—80 °C for 10 min at an output power of 80W under a stream of nitrogen.

Measurement of Fluidity of Lipid Bilayer of Liposomes by Spin Labeling Method Fluidity of lipid bilayer of stratum corneum liposomes was measured by the spin labeling method. Aliquots of methanol solution of either 5-DSME or 16-DSME were dried in a plastic tube. Eighty μl of stratum corneum lipid liposome suspension was added to the tube and the mixture was incubated at 37 °C for 2 min with or without penetration enhancers. The spin-labeled liposome suspension was then transferred to duplicate 20 μl capillary tubes and one end of each tube was sealed with Hematoseal (Terumo, Tokyo, Japan). The final concentration of the spin label was 25μM. The tubes were inserted into a quartz cell and set in a holder in a thermostat-regulator at 37 °C. Electron spin resonance (ESR) spectra were measured with a JEOL TE-200 (X-band) spectrometer with 100 kHz field modulation frequency and 0.1 mT modulation amplitude at an output power of 8 mW. The apparent rotational correlation time, τ_0, was calculated as described previously.[7]

Results

Permeability of Parabens We first examined the permeation of methyl, ethyl, propyl and butyl parabens through excised guinea pig dorsal skin. As shown in Fig. 1 for propyl paraben, permeation proceeded with little lag time. Permeabilities of these parabens in guinea pig dorsal skin (Table 1) were larger than those in shed snake skin.[8] As shown in Fig. 2 and Eq. 2, permeability increased with the increase of *n*-octanol/water partition coefficients of the parabens and logarithm values of permeability coefficients, K_p, correlated with logarithm values of the partition coefficients, P_{oct}, although there was a tendency for the dependency on the partition coefficients to decrease with increase in hydrophobicity and molecular weight.

$$\log K_p = 0.723 \log P_{oct} - 3.260 \quad (n=4,\ r=0.949) \qquad (2)$$

Penetration through stratum corneum lipid lamella has been suggested to be a rate limiting step for penetration of drugs,[1] especially of hydrophilic drugs. Therefore, to learn the barrier properties of the lipid lamella in the absorption process of the alkyl parabens, we observed the change of permeability resulting from depletion of stratum corneum lipids. As shown in Fig. 2, lipid depletion increased the skin permeability, especially for hydrophilic parabens. That is, the permeability coefficient of methyl paraben increased about twenty times, whereas no significant increase was observed for butyl paraben and differences of permeability among the four parabens almost disappeared.

Table 1. *n*-Octanol/Water Partition Coefficient (P_{oct}), Molecular Weight (MW), Solubility (C_d), and Permeability Coefficient of Parabens

Paraben	log P_{oct} [a]	MW	C_d [b] (mM)	K_p [b] ($\times 10^{-3}$ cm·h^{-1})
Methyl	1.66	152.15	26.70±0.68	6.51± 2.30
Ethyl	2.19	166.18	9.74±0.62	32.67±11.27
Propyl	2.71	180.20	3.67±0.12	66.26±12.43
Butyl	3.24	194.23	2.02±0.15	92.17±27.18

a) Values were cited from ref. 20. *b*) Data are means±S.D. of three experiments.

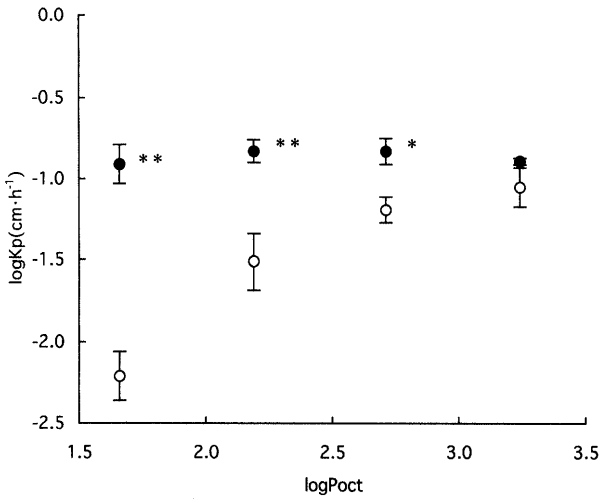

Fig. 2. Relationships between *n*-Octanol/Water Partition Coefficients, P_{oct}, of Parabens and Their Permeability Coefficients, K_p, through Guinea Pig Dorsal Skin and Its Lipid Depleted Skin

○, control skin; ●, lipid depleted skin. Data are means±S.D. of three experiments. The significance of difference between permeability coefficient of each paraben in control skin and that in lipid depleted skin was determined by Student's *t*-test: $*p<0.05$, $**p<0.01$.

Effects of *l*-Menthol and Ethanol on Skin Permeation Cyclic monoterpenes such as *l*-menthol have been shown to enhance transdermal drug penetration.[9,10] This enhancing effect is known to depend on ethanol concentration.[9] To avoid the delipidization of stratum corneum by a high percentage of ethanol,[11] in this work we examined the effect of 1% *l*-menthol in 15% ethanol on skin absorption of the parabens. As shown in Fig. 3, addition of 1% *l*-menthol in 15% ethanol increased the permeability coefficient of methyl paraben about sixteen times. However, permeability coefficients of ethyl and propyl parabens did not change significantly and that of butyl paraben decreased in their presence to about one fifth of the control value. A similar, though weaker, tendency was observed in the presence of 15% ethanol itself without *l*-menthol as also shown in Fig. 3.

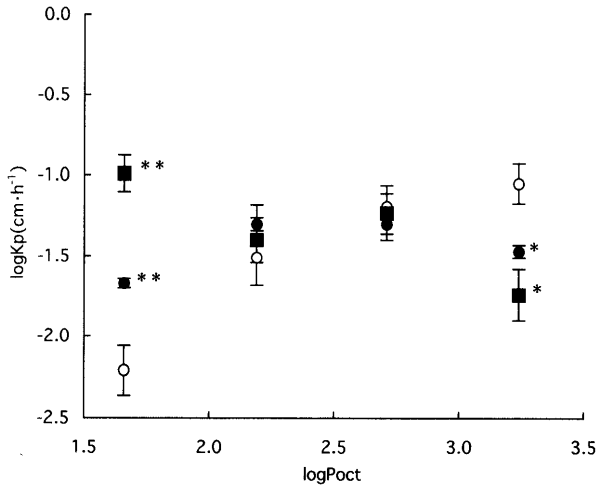

Fig. 3. Effects of 1% *l*-Menthol in 15% Ethanol and 15% Ethanol on Permeability Coefficients of Parabens through Guinea Pig Dorsal Skin

○, control; ■, with 1% *l*-menthol in 15% ethanol; ●, with 15% ethanol. Data are means±S.D. of three experiments. The significance of difference between permeability coefficient of each paraben without enhancer and that with either 1% *l*-menthol in 15% ethanol or 15% ethanol was determined by Student's *t*-test: $*p<0.05$, $**p<0.01$.

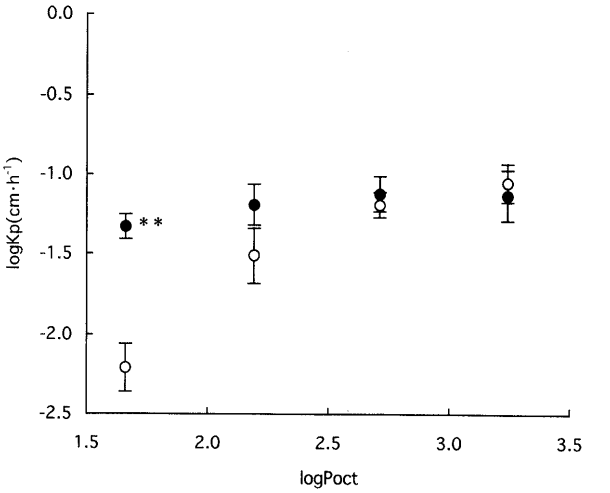

Fig. 4. Effects of 0.025% Suspension of *N*-Dodecyl-2-pyrrolidone on Permeability Coefficients of Parabens through Guinea Pig Dorsal Skin

○, control; ●, with *N*-dodecyl-2-pyrrolidone. Data are means±S.D. of three experiments. The significance of difference between permeability coefficient of each paraben and that in the presence of *N*-dodecyl-2-pyrrolidone was determined by Student's *t*-test: $**p<0.01$.

Effects of *N*-Dodecyl-2-pyrrolidone on Skin Permeation of Parabens Phillips and Michniak reported that *N*-dodecyl-2-pyrrolidone enhanced dermal drug penetration dependent on the hydrophilic nature of the drug,[2] as true of Azone®.[2,12] According to their report, this enhancer stimulates the penetration of hydrophilic drugs such as 5-fluorouracil, whose log P_{oct} values were smaller than 2, and its efficacy was greater than Azone®.[2] We also reported the marked enhancing effect of this compound on *in vitro* skin penetration of a hydrophilic derivative of 5-fluorouracil.[13] Therefore, we next examined the effects of this compound on skin permeation of the parabens. As shown in Fig. 4, *N*-dodecyl-2-pyrrolidone stimulated permeation of relatively hydrophilic parabens, especially methyl paraben whose log P_{oct} value was smaller than 2. Permeability coefficient of methyl paraben increased about seven times, whereas that of butyl paraben, whose log P_{oct} value was larger than 3, did not change significantly. These results are consistent with the previous findings of the effects of this compound mentioned above,[2,13] but are different from those on the effects of *l*-menthol in 15% ethanol or 15% ethanol itself described above.

Table 2. Effects of Penetration Enhancers on Apparent Rotational Correlation Time, τ_0, of 5-Doxylstearic Acid Methyl Ester (5-DSME) and 16-Doxylstearic Acid Methyl Ester (16-DSME) in Stratum Corneum Lipid Liposomes at 37 °C

Enhancer	τ_0 ($\times 10^{-9}$ s)[a]	
	5-DSME	16-DSME
None	2.609±0.079 (5)	1.880±0.043 (8)
15% ethanol	2.372±0.021 (7)***	1.815±0.048 (6)*
15% ethanol +1% *l*-menthol	1.315±0.020 (4)***	1.190±0.027 (4)***
0.025% *N*-dodecyl-2-pyrrolidone	2.103±0.153 (6)***	1.616±0.048 (6)***

a) Data represent means±S.D. for the numbers of replicate experiments shown in parentheses. Significance of difference between values with or without enhancer was determined by Student's *t*-test: $*p<0.05$, $***p<0.001$.

Effects of Penetration Enhancers on Fluidity of Stratum Corneum Lipid Bilayers It has been suggested that absorption enhancement of drugs by cyclic monoterpenes is due to the perturbation of the stratum corneum lipid lamella.[14] Since *N*-dodecyl-2-pyrrolidone is an Azone® analog, it also seems to enhance drug permeation by perturbation of lipid lamella as suggested for the effect of Azone®.[4] Therefore, we attempted to determine the relation between the effects of the enhancers and perturbation of the bilayer by observing the change of fluidity of lipid bilayer of stratum corneum lipid liposomes. For that purpose we used two spin labels, 5-DSME and 16-DSME. Due to the difference in the position of the nitroxide group, 5-DSME seems to reflect the fluidity in the interfacial region of the lipid bilayer, while 16-DSME seems to reflect that deep inside. As shown in Table 2, 15% ethanol, 1% *l*-menthol in 15% ethanol and 0.025% *N*-dodecyl-2-pyrrolidone all decreased the apparent rotational correlation time of 5-DSME and 16-DSME, which indicated an increase in the fluidity of lipid bilayer by these compounds. Especially, the addition of *l*-menthol markedly decreased the apparent rotational correlation time by abolishing the anisotropy of these spin probes. The order of the decrease in apparent rotational correlation time of each spin label corresponded with the order of the enhancing effects on skin penetration of methyl paraben shown in Figs. 3 and 4, although the change in apparent rotational correlation time of 5-DSME was more marked than that of 16-DSME.

Discussion

The parabens examined in this work are thought to penetrate *via* non-polar stratum corneum lipid lamella in both human and animal skin as a rate limiting step for skin permeation. Dependence of their permeability coefficients on their *n*-octanol/water partition coefficients shown in Fig. 2 is consistent with this. Increase of permeability coefficients and abolition of their dependence on *n*-octanol/water partition coefficients by depletion of

stratum corneum lipids also support this speculation. The free energy of transfer of the methylene group from the aqueous phase to guinea pig dorsal skin, which was calculated from the slope of log K_p *vs.* carbon number plot, was about -2.26 kJ/mol. Therefore, the free energy of transfer was larger than that in shed snake skin (-1.82 kJ/mol)[8] and that in human skin (-1.84 kJ/mol).[15] This finding suggests that lipid lamella in guinea pig dorsal skin is slightly more hydrophobic in nature than that in human skin or shed snake skin.

ESR study in liposomes suggests that ethanol, *l*-menthol and *N*-dodecyl-2-pyrrolidone stimulate skin penetration of relatively hydrophilic paraben at least partly by perturbation of the stratum corneum lipid lamella. Disruption of lipid packing by these compounds seems to permit easier diffusion through the skin, which will promote the penetration of polar molecules.[2] The almost complete disappearance of anisotropy in ESR spectra of the spin probes by the addition of *l*-menthol observed here may result from the destruction of the bilayer structure of the lipids by the marked perturbation. These results indicate that the enhancers tested in this study increase skin penetration of hydrophilic paraben by increasing fluidity of stratum corneum lipid lamella, which seems to lead to the increase of diffusion coefficients of the drug.

l-Menthol in 15% ethanol or 15% ethanol itself, on the other hand, significantly decreased the permeability coefficient of relatively hydrophobic butyl paraben. The reduction of permeability coefficients by the enhancers tested here did not correlate with their perturbation effect on the lipid bilayer observed by ESR analysis. The decrease in the permeability coefficient of butyl paraben by *l*-menthol in ethanol or ethanol itself may be due to a reduction in partitioning of butyl paraben between skin and vehicle due to increase in the solubility of butyl paraben in the vehicle (solubility of butyl paraben in the presence of 15% ethanol increased about 2.5 times compared with that in its absence). This speculation corresponds with previous findings on the effects of ethanol. That is, the permeability coefficients of lipophilic drugs such as β-estradiol and nonionized diclofenac were lowered by the addition of ethanol,[16,17] possibly due to the decrease of partitioning between skin and vehicle.[17,18] *d*-Limonene is also suggested to reduce partitioning of butyl paraben between the non-polar route and vehicle.[19] Therefore, *l*-menthol in addition to ethanol may decrease the partitioning of butyl paraben to the non-polar region of the skin more markedly than ethanol itself, and lower its skin permeability coefficient.

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References

1) Walters K. A., "Transdermal Drug Delivery," ed. by Hadgraft J., Guy R. H., Marcel Dekker, New York, 1989, pp. 197—246.
2) Phillips C. A., Michniak B. B., *J. Pharm. Sci.*, **84**, 1427—1433 (1995).
3) Williams A. C., Barry B. W., *Pharm. Res.*, **8**, 17—24 (1991).
4) Walker R. B., Smith E. W., *Advanced Drug Delivery Reviews*, **18**, 295—301 (1996).
5) Kitagawa S., Yokochi N., Murooka N., *Int. J. Pharm.*, **126**, 49—56 (1995).
6) Kitagawa S., Sawada M., Hirata H., *Int. J. Pharm.*, **98**, 203—208 (1993).
7) Kitagawa S., Kametani F., Tsuchiya K., Sakurai H., *Biochim. Biophys. Acta*, **1027**, 123—129 (1990).
8) Itoh T., Xia J., Magavi R., Nishihata T., Rytting J. H., *Pharm. Res.*, **7**, 1042—1047 (1990).
9) Obata Y., Takayama K., Machida Y., Nagai T., *Drug Des. and Discovery*, **8**, 137—144 (1991).
10) Sugibayashi K., Kobayashi D., Nakagaki E., Hatanaka T., Inoue N., Kusumi S., Kobayashi M., Kimura M., Morimoto Y., *Int. J. Pharm.*, **113**, 189—197 (1995).
11) Hatanaka T., Shimoyama M., Sugibayashi K., Morimoto Y., *Yakuzaigaku*, **52**, 215—223 (1992).
12) Baker E. J., Hadgraft J., *Pharm. Res.*, **12**, 993—997 (1995).
13) Sato S., Hirotani Y., Ogura N., Kitagawa S., *Chem. Pharm. Bull.*, submitted.
14) Hashida M., Yamashita F., "Percutaneous Penetration Enhancers," ed. by Smith E. W., Maibach H. I., CRC Press, Boca Raton, 1995, pp. 309—321.
15) Anderson B. D., Higuchi W. I., Raykar P. V., *Pharm. Res.*, **5**, 566—573 (1988).
16) Ghanem A. H., Mahmoud H., Higuchi W. I., Rohr U. D., Borsadia S., Liu P., Fox J. L., Good W. R., *J. Controlled Release*, **6**, 75—83 (1987).
17) Obata Y., Takayama K., Maitani Y., Machida Y., Nagai T., *Int. J. Pharm.*, **89**, 191—198 (1993).
18) Megrab N. A., Williams A. C., Barry B. W., *Int. J. Pharm.*, **116**, 101—112 (1995).
19) Koyama Y., Bando H., Yamashita F., Takakura Y., Sezaki H., Hashida M., *Pharm. Res.*, **11**, 377—383 (1994).
20) Valvani S. C., Yalkowski S. H., "Physical Chemical Properties of Drugs," ed. by Yalkowski S. H., Sinkula A. A., Valvani S. C., Marcel Dekker, New York, 1980, pp. 201—229.