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39. **Mitsuo Matsumoto and Masaru Aoki**: Application of Surface Active Agents in Pharmaceutical Preparations. X.[*1] Inactivation of *p*-Hydroxybenzoic Acid Esters by Nonionic Surfactants.

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Studies have so far been made of the effect of surface-active agents upon the activities of drugs, with special reference to a practical application regarding antimicrobial agents. During the past decade, numerous reports have reported the inactivation of various preservatives, especially phenolic substances in the presence of several nonionic surfactants. The related literature shows that a marked inactivation of phenolic preservatives is evidenced in the presence of Tween-type surfactants which are polyethers derived from ethylene oxide.

Several investigators[1)] have suggested the formation of a complex between phenolic preservatives and surfactants based on ethylene oxide condensate. Complex formation of these two compounds was experimentally suggested by Higuchi, *et al.*[2)] and referred to in many reports. However, this seems impossible to explain deNavarre and others' failure[1)] in experiments for inactivation by Carbowax, which is a polymer of ethylene oxide. This apparent failure suggests that the inactivation of phenolic substances cannot be attributed simply to a complex formation with polyoxyethylene of nonionic surfactant.

In a previous work, Aoki and others discussed the mechanism of inactivation in relation to affinity by which a preservative may be partitioned between the micellar and nonmicellar phases of a solution containing nonionic surfactant. The relationship between the degree of hydrophilic or lipophilic properties of preservatives and surfactants was considered to be a significant factor because of the following evidences.

In the presence of Tween 20, antifungal activity of *p*-hydroxybenzoic acid esters (Parabens) decreases with an increasing concentration of Tween 20. In this case, as the carbon number of ester group in Paraben increases (i. e. methyl → ethyl → propyl → butyl), the effect of Tween 20 on the antifungal activity is serially enhanced. It is understood that higher esters such as propyl and butyl ester are effective in lower concentrations than the methyl ester in water. However, this relationship is reversed in the presence of Tween 20. The same concentration of Tween 20 and Tween 80 obviously shows different effect on the same Paraben. That is also the case with similar surfactants and it is considered that surfactants having different chemical structures are very likely to be different in the inactivating effect.[3)]

[*1] Part III. M. Aoki, *et al.*: Yakugaku Zasshi, **77**, 1071 (1957).

[*2] Hotarugaike, Toyonaka-shi, Osaka-fu (松本光雄, 青木 大).

1) A. Bolle, A. Mirimanoff: J. Pharm. Pharmcol., **2**, 685 (1950); M.G. deNavarre, *et al.*: J. Soc. Cosmetic Chem., **7**, 427 (1956); 8, 68, 371 (1957); M.D. Barr, L.F. Tice: J. Am. Pharm. Assoc., Sci. Ed., **46**, 445 (1957).

2) T. Higuchi, J.L. Lach: J. Am. Pharm. Assoc., Sci. Ed., **43**, 465 (1954).

3) M. Aoki, *et al.*: Yakugaku Zasshi, **76**, 939 (1956).

A comparative study[4] was made, of using eight kinds of structurally different nonionic surfactants, on their effect on the activity of sparingly soluble preservatives, such as Hexachlorophen and Bithinol. As a result, it was concluded that a chemical structure of surfactants, the difference of which lies in hydrocarbon moiety or the balance of hydrophilic to lipophilic part, is responsible for varying inactivating effect on the potency of preservatives.

In the present investigation, further attempt was made to investigate the mechanism of the effect of surfactants which differ in the chemical structure of their lipophilic group and in the balance between hydrophilic and lipophilic affinity. For this purpose, three types of nonionic surfactants, TO-type (polyoxyethylene sorbitan monooleate), BO-type (polyoxyethylene oleyl ether), and NP-type (polyoxyethylene nonyl phenyl ether) were selected. Molar number of their ethylene oxide group was fixed synthetically within a range of 6 to 40 moles.[*3] The proportion of polyoxyethylene part to the whole molecule of the surfactants used is shown in Table I, where −x indicates the molar number of condensed ethylene oxide. OP-type[*3] (polyoxyethylene octyl phenyl ether) was also tabulated, which was used for an estimation of its solubilizing power on Parabens.

Table I. Proportion of Polyoxyethylene Part against the Whole Molecule of a Surfactant (%)

Types of surfactant	Condensed molar number of ethylene oxide								
	−6	−8	−10	−12	−15	−20	−25	−30	−40
TO	37 (21)	44 (19)	50 (18)	54 (16)	60 (14)	66 (12)		75 (9)	
BO	48.5	55	61	65	70	76	80	82	86
NP	53	60	66	70	74	79		85	
OP					75	80		86	

( ) indicates % of Sorbitan part in TO-type.
TO-type : Polyoxyethylene (POE) sorbitan monoöleate.
BO-type : POE oleyl ether. NP-type : POE nonyl phenyl ether.
OP-type : POE octyl phenyl ether.

For the sake of comparison, Carbowax and the polyoxyethylene additives[*3] of benzyl alcohol and *p*-cresol were also adopted. Because the latter polyoxyethylene condensates had a poor lipophilic moiety, they were expected to show an intermediate behavior between the surfactants so far mentioned and Carbowaxes which constitute a hydrophilic part of the nonionic surfactants.

In this study, particular attention was paid to the following : A standardized index was established on effective concentration of Parabens against *Aspergillus niger* and this index was used as an indicator to elucidate varying activities of methyl-, ethyl-, propyl-, and butyl-paraben in the presence of Carbowaxes, nonionic surfactants, or polyoxyethylene additive compounds as described above. Effective concentration was specifically determined not by an ordinary serial dilution method but by the authors' own method. Care was taken to check up all important factors that would have been overlooked in routine serial dilution method.

Materials

***p*-Hydroxybenzoic Acid Esters**——Rutine recrystallization from benzene was made. Methyl *p*-hydroxybenzoate, m.p. 127°; ethyl *p*-hydroxybenzoate, m.p. 118°; propyl *p*-hydroxybenzoate, m.p. 98°; butyl *p*-hydroxybenzoate, m.p. 71.5°.

Surfactants——The surfactants employed were synthesized by Nippon Surfactant Co., A condensation of ethylene oxide with lipophilic moiety, such as sorbitan monooleate and oleyl alcohol,

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[*3] Synthesized and kindly supplied by Nippon Surfactant Co. Ltd. (Tokyo).

4) M. Aoki, *et al.* : Yakugaku Zasshi, **77**, 1071 (1957).

was performed by polymerization with ethylene oxide gas to obtain a certain definite molar number. It was technically difficult to separate the product of a desired chain length, but as far as the present experiments are concerned, a surfactant having a certain number of ethylene oxide exhibits a definite hydrophilic property proportional to its number of ethylene oxide.

Carbowax 4000, Carbowax 1000, and Polyoxyethylene Glycol 300——These were the products of the Carbide and Carbon Chemicals Corporation.

Test organism——*Aspergillus niger*, a laboratory strain of the Research Institute for Microbial Diseases, Osaka University.

Media——The medium used in this experiment was of the same composition as that employed in J.P. Ⅵ (a fluid Subouraud medium containing 1% of peptone and 2% of hydrous glucose). In preparing cultur media for antifungal activity tests, a five-fold concentration of the medium was used as a basic working broth. The final test culture medium showed a pH range of 5.4∼5.8.

Experimental Technique

Concentrated working solutions were sterilized and all procedures thereafter were performed aseptically.

Concentrated working solutions of Carbowaxes (10%) and polyethylene glycol 300 (25%) and of surfactants (5%) were prepared. Concentrated working solutions of preservatives were also prepared by dissolving an appropriate proportion of *p*-hydroxybenzoic acid esters into distilled water and working solutions described above. These solutions could de mixed with other working solutions or broths without causing any precipitation of crystals. All working solutions were sterilized at 100° for 30 min. and cooled. The esters remained stable under the above condition, as reported in a previous paper.[4] After preparing various concentrations of *p*-hydroxybenzoic acid ester, a final test culture medium was obtained, which assured a reproducible result.

To prepare 10 cc. of a final test cultere medium containing the *p*-hydroxybenzoic acid ester and Carbowax (0.5∼20%) or surfactant (1%), an appropriate amount of the working solution was added so as to make a volume of below 8 cc. This mixture was diluted with 2 cc. of the working broth, followed by an addition of sterilized water to make 10 cc. of a final culture medium and kept at 25° before inoculation. The final culture medium was inoculated with one platinum loop of a standardized spore suspension of *Aspergillus niger*, which was prepared from 10 cc. of sterilized solution of Tween 80 (0.005%) and 5 platinum loops of spore of *Asp. niger* cultivated in a fluid Sabouraud medium for 2 weeks at 25°.

After an incubation for 10 days at 25°, growth of the mold was observed and decreased activity of *p*-hydroxybenzoic acid ester was judged by a response of the growth observed. The observation was carried out with two tubes and when both these tubes did not show the same result, the test was repeated until the same result was obtained. The effective concentration of *p*-hydroxybenzoic acid ester in a pure culture medium was checked to confirm normalized sprouting activity.

Results

Inactivation of Parabens by Carbowaxes——Effective concentrations of parabens against *Asp. niger* in the presence of Carbowaxes are shown in Table Ⅱ. Below 2%, Carbowaxes do not show any inactivation. They begin to show interference at 5% and a marked inactivation was observed at 20%. About the same degree of inhibition is

TABLE Ⅱ. Effective Concentration of Parabens against *Aspergillus niger* in the Presence of Polyethylene Glycols

Concn.	Methylparaben (%)			Ethylparaben (%)		Propylparaben (%)	
	300	1000	4000	300	4000	300	4000
0		0.070		0.040		0.022	
0.5	0.078	0.078	0.080	0.041	0.042	0.024	0.024
1.0	0.080	0.078	0.084	0.042	0.044	—	0.025
2.0	0.082	0.082	0.084	0.044	0.048	—	0.027
5.0	0.100	0.100	0.106	0.058	0.063	—	0.034
10.0	0.135	0.135	0.145	0.085	0.080	0.045	0.050
20.0	0.200	0.200	0.260	0.155	0.150	0.075	0.095
	Polyethylene glycol 300			Carbowax 1000		Carbowax 4000	

found in Methyl-paraben with 10% and Propyl-paraben with 20% of Carbowax, as demonstrated by only 1% of Tween 20.[3] Therefore, it may be said that Carbowaxes show less inactivation than nonionic surfactants. It is seen that the ratio of effective concentration with and without varying amounts of Carbowax is similar for each Paraben (Table III). However, the case is different with nonionic surfactants which disclose varying ratios for the respective Parabens employed.

Table III. Ratio of Effective Concentration of Parabens against *Aspergillus niger*, with and without Carbowax 4000

Parabens	Concentration of Carbowax 4000 (%)					
	0.5	1	2	5	10	20
Methyl-	1.15	1.20	1.23	1.54	2.2	3.6
Ethyl-	1.05	1.10	1.15	1.57	2.0	3.6
Propyl-	1.09	1.15	1.25	1.54	2.2	4.3

Inactivation of Parabens by Nonionic Surfactants——Figs. 1, 2, and 3 show the minimum inhibitory concentration of Parabens for *Asp. niger* in a medium containing 1% of one of three types of surfactants. In the presence of surfactants of a TO-type, the effective concentration of Methyl-paraben is not decreased so much, and is found almost equal in all TO-types with various numbers of ethylene oxide. In the case of Ethyl- or Propyl-parabens, some inactivation is observed but it is still weak in the presence of a large number of ethylene oxide, such as TO-20 mole and TO-30 mole. This inactivating effect is more definite in Propyl- than Ethyl-paraben (Fig. 1.). As for BO-types,

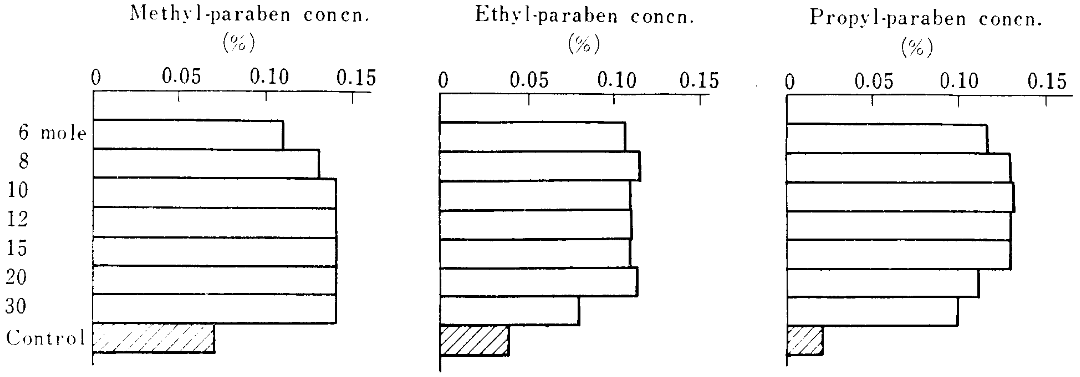

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Fig. 1. Effective Concentration of Parabens against *Aspergillus niger* in 1% TO-type Solutions

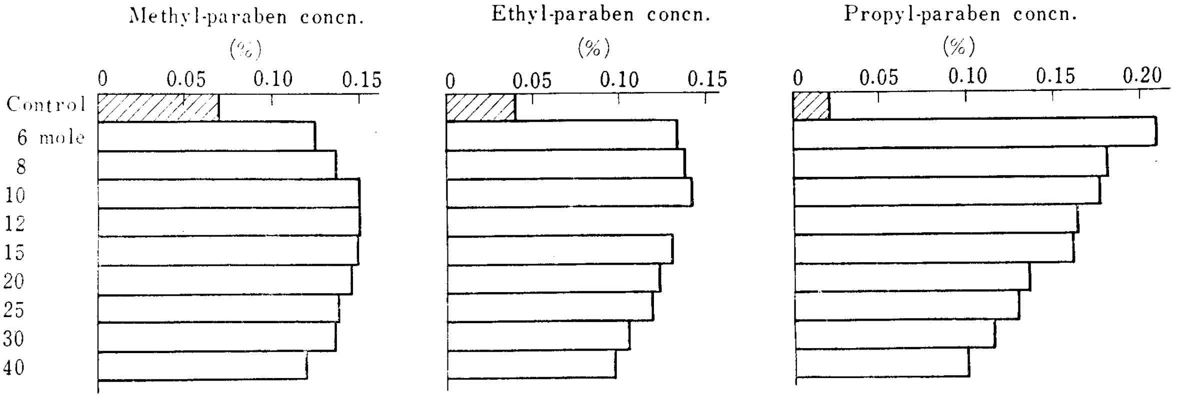

Fig. 2. Effective Concentration of Parabens against *Aspergillus niger* in 1% BO-type Solutions

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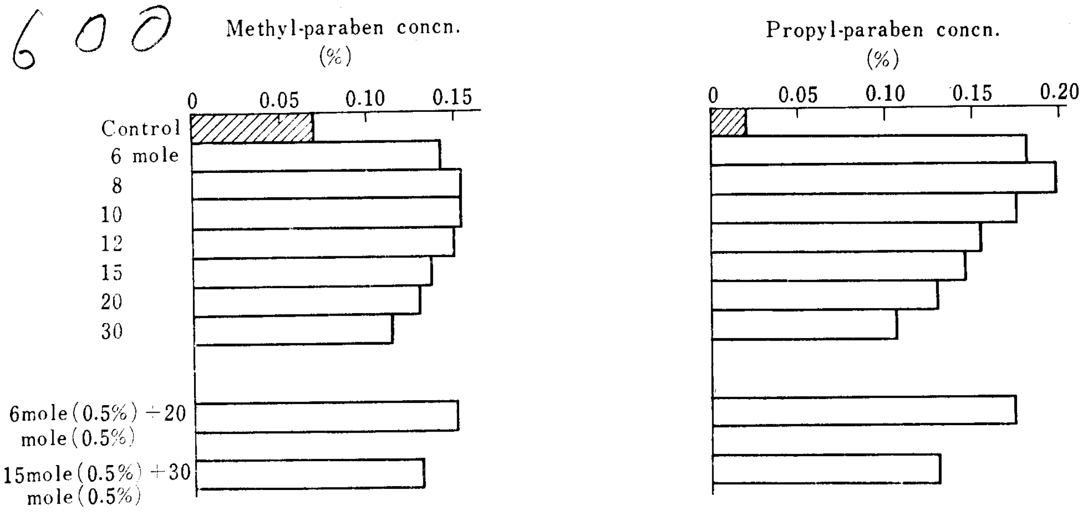

Fig. 3. Effective Concentration of Parabens against *Aspergillus niger* in 1% NP-type Solutions

BO-10 mole and -15 mole show the strongest inactivating effect upon Methyl-paraben. In the case of lower condensates (BO-6 mole, -8 mole) or higher condensates (BO-20 mole, -25 mole, -30 mole, and -40 mole), the inactivating effect is found to be decreased. In a similar manner the highest inactivating effect is observed with BO-10 mole against Ethyl-paraben and with BO-6 mole against Propyl-paraben. This inactivating effect is affected by the number of ethylene oxide, showing the highest in Propyl-paraben and the lowest in Methyl-paraben. It is understood that as the carbon number of the ester moiety of Parabens becomes greater, the number of ethylene oxide in surfactant decreases which, being lipophilic, indicates the maixmum inactivation. Similar results are obtained with NP-types as shown in Fig. 3. In this case, a mixture of NP-6 mole (0.5%) and NP-20 mole (0.5%) causes a stronger effect on Parabens than 1% of the surfactant used independently. The mixture exhibits an effect almost similar to that of NP-10 mole (1%). When NP-15 mole (0.5%) and NP-30 mole (0.5%) are mixed, a resultant effect is similar to an averaged effect of the two surfactants. It is nearly the same as that of NP-20 mole in effect. This is suggested that when surfactants are mixed, a ratio of hydrophilic to lipophilic affinity comes close to an averaged value of respective surfactants. The suggestion is reflected in a mixture of NP-6 mole and NP-20 mole and that of NP-15 mole and NP-30 mole which are similar in the inactivating effect to NP-10 mole and NP-20 mole, respectively. This seems to be explainable by hydrophile

Table IV. Ratio of Effective Concentration of Parabens against *Aspergillus niger* with and without 1% of Surfactant

Types of surfactant	Molar number of ethylene oxide								
	−6	−8	−10	−12	−15	−20	−25	−30	−40
				Methyl-yparaben					
TO	1.57	1.86	2.0	2.0	2.0	2.0		2.0	
BO	1.8	1.9	2.1	2.1	2.1	2.05	1.9	1.9	1.7
NP	2.05	2.2	2.2	2.15	1.95	1.86		1.64	
				Ethyl-paraben					
TO	2.7	2.9	2.7	2.7	2.7	2.8		2.0	
BO	3.35	3.4	3.5		3.2	3.0	2.9	2.6	2.4
				Propyl-paraben					
TO	5.4	5.9	5.9	5.9	5.9	5.0		4.5	
BO	9.5	8.2	7.9	7.35	7.3	6.2	5.9	5.2	4.5
NP	8.2	8.9	7.9	7.0	6.6	5.9		4.9	

lipophile balance (HLB), although a mere concept of hydrophile lipophile balance does not seem sufficient to give a full explanation of the above experiment results. The ratio of effective concentration of Parabens with and without a surfactant is shown in Table IV, where a striking contrast in the case of Carbowaxes (cf. Table III) is observed.

Solubility of Parabens in Aqueous Solution of Carbowax and of Nonionic Surfactants——A 20% aqueous solution of Carbowax 4,000 shows only 4 times greater solubility of Methyl-paraben and about 3 times greater solubility of Propyl-paraben than in pure water (Fig. 4).

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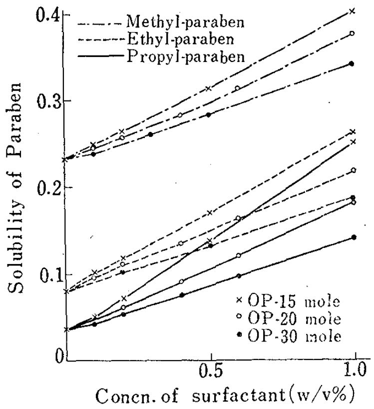

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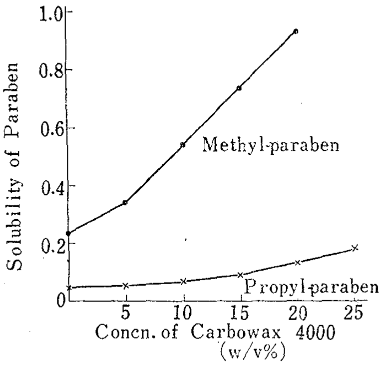

Fig. 4. Solubility of Parabens in Water at 25° as a Function of Carbowax 4000 Concentration

Fig. 5. Solubility of Parabens in Water at 25° as a Function of Concentration of OP-type Surfactants

Fig. 5 shows solubility curves of Parabens in aqueous solution of OP-type surfactants which have varying chain length of polyoxyethylene. Methyl-paraben has about 1.5 times greater solubility even in a 1% solution of the respective surfactrants than in pure water. In the case of Propyl-paraben, 3.5 times greater solubility is observed even in a 1% solution of OP-30 mole which shows the lowest action among the surfactants employed. OP-15 mole exhibits the most enhancing effect on the solubility of Parabens. The solubility of Parabens in a 1% solution of OP-type surfactans is compared with that in pure water (Table V).

Table V. Ratio of Solubility of Parabens with and without 1% of OP-type Surfactant

	OP-15 mole	OP-20 mole	OP-30 mole
Methyl-paraben	1.74	1.63	1.52
Ethyl-paraben	2.94	2.47	2.02
Propyl-paraben	6.86	5.15	3.86

Discussion

Before discussions are made on the ability of nonionic surfactant to form a complex with phenolic preservatives, it seems necessary to review the literature in which a micelle formation is postulated on the inactivation of preservatives by various surfactants.

The concept of micelle formation concerning inactivation was advanced first by Alexander and his collaborators.

Alexander and Tomlinson⁵⁾ measured the survival time of *Bacillus coli* when exposed

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5) A.E. Alexander, A.J.H. Tomlinson: "Surface Chemistry," 317 (1949). Interscience Publishers Inc., New York.

to aqueous solution of phenol and chlorinated phenols in the presence of anionic and cationic surfactants, and found that the survival time was minimal at a critical micellar concentration of the surfactant. A similar phenomenon was observed by Alexander and Trim⁶⁾ in the anthelmintic activity of hexylresorcinol-soap mixtures. These workers suggested that a soap concentration beyond the critical miceller concentration resulted in a passage of phenol into the micelle, leading to a reduction in bactericidal activity due to a decrease of phenol in aqueous phase.

Bean and Berry⁷⁾ observed in agreement with the findings of Alexander and Tomlinson, that the bactericidal activity of chlorocresol increased up to the critical micellar concentration of potassium laurate, but as the amount of soap increased beyond the critical concentration, the bactericidal activity showed a sharp decrease followed by an increase. In explaining the activities displayed in the presence of micellar concentrations of soap, Bean and Berry used a term of "saturation of micelle with phenol" while Alexander referred to it as "free phenol concentration in aqueous phase."

Despite the difference in these workers' terminology, they maintained that phenol may be partitioned between the micellar and nonmicellar phases. Allawala and Riegerman⁸⁾ emphasized that only the unsolubilized agents left free outside the micelle were biologically active and that this activity was evidenced beyond a certain "percentage saturation."

Recently, Kostenbauder and his co-workers⁹⁾ quantitatively studied interactions between Methyl- and Propyl-parabens and Tween 80, by means of a dialysis method employing a semi-permeable Nylon membrane. In agreement with the present authors' previous findings they stated that the preservative activity of Parabens in the presence of nonionic surfactants is primarily dependent upon the concentration of "unbound" preservatives. According to these workers, the dialysis method enabled them to estimate the degree of "binding." However, it was left unclarified whether the association was due to the postulated complex formation or to some other mechanism such as preferential solubility of preservatives within a micelle. A series of the present and previously reported experiments³⁾⁴⁾¹⁰⁾ concluded that the antifungal activity of Paraben in the presence of a surfactant may be due to that present in the aqueous phase outside the micelle. Here, the degree of partition between the micellar and nonmicellar phases is not only dependent upon a hydrophilic property of Paraben, but also upon the chemical structure of the surfactant. The chemical structure is the hydrocarbon moiety or the balance of hydrophilic to lipophilic affinity of a surfactant.

The present work on solubility of Parabens in an aqueous solution of either Carbowaxes or nonionic surfactants is important in advocating the present authors' concept with respect to micelle formation. In an aqueous solution of Carbowaxes, the solubility of Parabens did not increase so markedly as in the solution of surfactants (Figs. 4 and 5). As previously reported,³⁾¹⁰⁾ the solubility of Parabens in an aqueous solution of surfactants, such as Tween 20 or Tween 80, increases very rapidly with a slight increaese in the concentration of the surfactant used. It is seen that the higher the carbon number of the ester residue of Paraben becomes, the more rapidly the solubility incresses. In the present experiments on OP-type surfactants similar results were obtained even in a 1% solution of a surfactant (Fig. 5). A highly enhancing effect on the solubility of Parabens is displayed by OP-15 mole which is assumed to have a strong inactivating

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6) A. E. Alexander, A. R. Trim: Proc. Roy. Soc., (London), **113B**, 220 (1946).

7) H. S. Bean, H. Berry: J. Pharm. Pharmacol., **2**, 484 (1950); **3**, 639 (1951); **5**, 632 (1953); **6**, 649 (1954); **8**, 425 (1956); H. Berry, A. Briggs: *Ibid.*, **8**, 1143 (1956).

8) N. A. Allawala, S. Riegelman: J. Am. Pharm. Assoc., Sci. Ed., **42**, 267, 396 (1953).

9) H. B. Kostenbauder, *et al.*: *Ibid.*, **47**, 289 (1958); **48**, 310, 315 (1959).

10) M. Aoki, *et al.*: Yakuzaigaku, **18**, 174 (1958).

effect on Parabens. The ratios of solubility of Parabens with and without surfactants represented in Table V seem to be analogous in its implication to the ratio of effective concentrations shown in Table IV. This may give support to the assumption that in an aqueous solution of nonionic surfactants, Parabens are solubilized and pass into the micellar phase.

Further support may be given from the following discussion on the solubilizing mechanism of several surfactants studied in relation to their inactivating effect on Parabens. As mentioned above with regard to the mechanism of inactivation of phenolic preservatives by nonionic surfactants, several researchers have suggested a complex formation through hydrogen bonding. It may be assumed from their suggestion that Carbowaxes show similar or higher inactivation than nonionic surfactants used in the same concentration, but only a markedly less inactivating effect has been observed during the present experiments.

Moreover, it may be considered that Parabens with -O- group in polyoxyethylene chain is less capable for hydrogen bonding than those with OH group at the end of their chain. Carbowax 4,000 and PEG 300 exhibited a similar inactivating effect upon Parabens (Table II), although the latter has a smaller molecular weight than the former and contains about 10 times more OH group in the same concentration. These phenomena seem to suggest that no relationship exists between the inactivating effect of polyoxyethylene chain and the complex formation.

According to a generally accepted interpretation of complex formation, within the same type of surfactants, a surfactant having higher ratio of polyoxyethylene group may exhibit a higher inactivating effect than those with a lower ratio. However, the highest inactivating effect is observed in the presence of a surfactant having a certain number of ethylene oxide, as shown in Figs. 1, 2, and 3. Considerations from a stand point of a balance between hydrophilic and lipophilic affinity of the surfactant seem to throw a light upon interpreting the results described above.

The ratio of hydrophilic to lipophilic affinity of a surfactant is always subject to effective concentration of Parabens. It seems that varying influences of similar type of surfactants in the same series upon Parabens are dependent upon their ratio of hydrophilic to lipophilic affinity. A certain ratio has been found to be most effective for inactivating Parabens. This maximum ratio is different for each Paraben used. With an increase of carbon number of the ester group in Parabens, a surfactant, which is more lipophilic, demonstrates the maximum inactivating effect.

Furthermore, the ratio of the effective concentration of each Paraben with and without a surfactant was found to be different (Table IV). The ratio increases as the number of carbon atoms of the ester group increases. In the presence of Carbowaxes a similar ratio was obtained for each Paraben, unlike in the case with surfactants.

Hence, stress must be laid on the differences caused by their chemical structure in the balance between hydrophilic and lipophilic affinity of the surfactants. The chemical structure in question refers to the difference in the length of polyoxyethylene chain as the hydrophilic part and in the chemical structure of the lipophilic part. This supposition may be further supported by the following experimental results.

The inactivating effect on Parabens can only be observed following the addition of the polyoxyethylene condensate of *p*-cresol and benzyl alcohol, to a concentration as high as 4% or more (Table VI). The inactivating effect of these condensates is slightly stronger than that of Carbowaxes but still much weaker than that of nonionic surfactants so far mentioned. Referring to chemical structure, the concept of the ratio of hydrophilic to lipophilic affinity of nonionic surfactants seemed to be analogously applicable to the above condensates. However, in the range of concentration tested, it seemed

Table VI. Effective Concentration of Propyl-paraben against *Aspergillus niger* in the Presence of Polyoxyethylene Condensate

POE Condensate	Concn. of the Condensate (%)			
	1	2	4	8
PC–10.5 mole	0.023	0.027	0.050	0.150
PC–4 mole	0.020	—	0.044	0.100
Benzyl–5.5 mole	0.020	—	—	0.050

PC: Polyoxyethylene *p*-cresyl ether
Benzyl: POE benzyl ether

difficult for these condensates to form a micellar structure in water phase because of their poor lipophilic affinity.

It has been reported that in a series of the same type of ionic surfactants a critical micellar concentration increases with a decrease of the carbon atoms in the hydrocarbon chain of a surfactant,[11] i.e. a decrease in lipophilic affinity. Furthermore, it is known[12] that the ability of solubilization varies with the change of the carbon number in ionic surfactants. Yet the carbon-chain length must be defined for the optimal efficacy.

Nonionic surfactants do not seem to act in the same manner as ionic surfactants. However, as a surface-active agent, a nonionic surfactant shows a property similar to an ionic surfactant in that it gives the varying influence according to the varying degree of its lipophilic affinity. It seems reasonable to consider that the condensates described above possess a Paraben solubilizing ability ranging between those of Carbowaxes and nonionic surfactants. Therefore, the fact that these condensates and Carbowaxes exert a weak influence upon the activity of Parabens suggests that the inactivation is not subject to the mere interaction between phenols and polyoxyethylene chain.

Recently, Becket and his collaborators[13] made an interesting report on the interaction of phenolic compounds with bacteria. Of particular interest were the results on inactivation of hexylresorcinol by Cetomacrogol. These workers reported that hexylresorcinol may become bound to the bacteria in the presence of excess Cetomacrogol as a monomolecular layer of the phenol-nonionic complex with the nonionic surfactants associated with the other surface of the film, thus blocking the building of multilayers of the phenol and also preventing the phenolic molecules from penetrating the bacteria. They further stated that, if the hexylresorcinol molecules penetrate the bacteria in association with Cetomacrogol molecules, the tendency to form hydrogen bond with the oxygen atoms in the ether chains of the nonionic surfactants may be greater than the tendency to disrupt and replace the natural hydrogen-bonding structure of the nucleic acid. It seems possible to make another interpretation of these workers' concept from the present view that an affinity is assumed to be present, between Paraben-surfactant and Paraben-nucleic acid.

Summary

Data have been presented to prove that high solubilization of *p*-hydroxybenzoic acid ester is demonstrated by the nonionic surfactant which has a suitable ratio of hydrophilic to lipophilic affinity in regard to the property of the ester. The condensed molar number of ethylene oxide in a surfactant which exhibits a maximum inactivation against *p*-hydroxybenzoic acid ester decreases with an increase in the carbon number of the

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[11] Mary E.L. McBain, E. Hutchinson: "Solubilization and Related Phenomena," 41 (1955); H.V. Tartar: J. Colloid Sci., **14**, 115 (1959).

[12] H.B. Klevens: Chem. Revs., **47**, 20 (1950).

[13] A.H. Becket, *et al.*: Nature, **180**, 1206 (1957); **181**, 712 (1958); J. Pharm. Pharmcol., 8, 1072 (1956); **11**, 360, 367 (1959).

ester group, i.e. methyl, ethyl, propyl, and butyl. Moreover, it is understood that the ability of solubilization is dependent upon the property of the surfactant, particularly upon the chemical structure of its lipophilic group. Therefore, degree of inactivating effect on Parabens differs according to the type of surfactants. e.g., TO-, BO-, and NP-type.

The results obtained in the present series of experiments give a reasonable support to the conclusion that the antifungal activity of *p*-hydroxybenzoic acid ester in the presence of a surfactant is represented only by what is present in aqueous phase outside the micelle.

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40. **Mitsuo Matsumoto and Masaru Aoki :** Application of Surface Active Agents in Pharmaceutical Preparations. XI.[1] Inactivation of *p*-Hydroxybenzoic Acid Esters in the Solubilized Solution of Oily Substances.

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The preceding paper[1] presented the data demonstrating that the antifungal activity of *p*-hydroxybenzoic acid esters in the presence of a nonionic surfactant is caused only by what is present in aqueous phase outside the micelle. The degree of inactivation of *p*-hydroxybenzoic acid esters in the presence of polyethylene glycols or different series of nonionic surfactants having different molar numbers of condensed ethylene oxide was compared and discussed with reference to solubilizing ability. It was suggested that the degree of partition of *p*-hydroxybenzoic acid ester between micellar and nonmicellar phases was not only dependent upon the hydrophilic property of the ester but also upon the chemical structure and balance of hydrophilic and lipophilic affinity of the surfactant.

In regard to the change of effective preservative levels of *p*-hydroxybenzoic acid esters according to the type of preparations, e.g. cream, lotion, etc., Sokol[2] cited a tabulation prepared by Suess.[3] On the basis of this, Sokol suggested that the greater the molecular weight of the ester in the alkyl series is, the smaller becomes the quantity recommended for preservation of any of these types of pharmaceutical preparations. At the same time, however, it was understood that the amount of *p*-hydroxybenzoic acid ester to be incorporated for preservation increased with an increase of oil content in a preparation.

So far, few discussions have been made on individual effect of surfactants and the amount of oils upon the effect of incorporated preservatives.

In the experiments employing Hydrophilic Ointment (J.P. Ⅵ and U.S.P. XV) and similar emulsions,[4] it was found that the amount of *p*-hydroxybenzoic acid ester required for preservation was dependent not only on the nature of the ester used but also on the nature of components in oil and water phase.

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[1] Part X. M. Matsumoto, M. Aoki : This Bulletin, **9**, 251 (1961).

[2] H. Sokol : Drug Standard, **20**, 89 (1952).

[3] A. Suess : Am. Perfumer Essent. Oil Rev., **32**, 55 (1936).

[4] M. Aoki, M. Matsumoto, I. Yoshika, Y. Isa : Yakuzaigaku, **17**, 231 (1957).