14 and 4.9 min, respectively, at 37°. Catalysis in these solutions is probably the result of a combination of general base, nucleophilic, carbon dioxide, and even some enzyme catalysis. Therefore, it is expected that many amino acid esters of phenolic drugs would be rapidly tranformed to the drug under *in vivo* conditions.

4. Many amino acids are normal dietary constituents or are substances with little toxicity. Thus, the compound formed along with the drug when the prodrug is hydrolyzed is unlikely to cause toxic reactions.

Detailed results of a kinetic study of the reactions of II, III, and IV in aqueous solutions together with some mechanistic considerations will be published subsequently.

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Phenol Formation from Alkylparabens by Bacteria

Keyphrases ◻ Phenol—formation from alkylparabens by bacteria ◻ Parabens, alkyl—role in formation of phenol by bacteria ◻ Preservatives—phenol formation from alkylparabens by bacteria

To the Editor:

Alkylparabens are widely used as preservatives for various pharmaceutical preparations such as injections, solutions, emulsions, and suspensions. They are also used in cosmetic preparations containing various fats and oils susceptible to microbial attack. Alkylparabens are also involved in gelatin preparation from animal sources to prevent proliferation of contaminated bacteria.

When a paste of gelatin containing alkylparabens was incubated at room temperature, we found that phenol formation was followed by the liquefaction of gelatin.

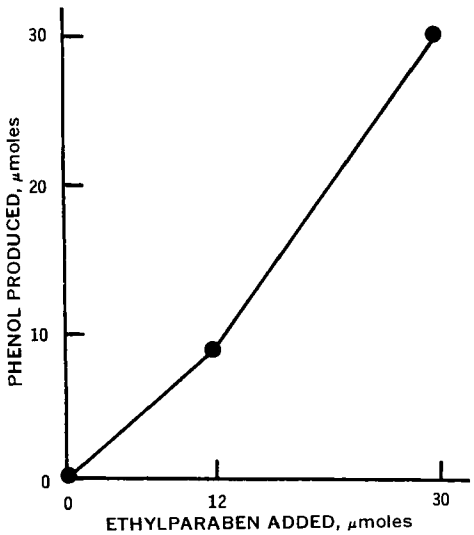


Figure 1—*Effects of various levels of ethylparaben on phenol formation, using resting cells as the enzyme source. Reaction mixtures contained ethylparaben and 7.6 × 10^{10} cells in a final volume of 12 ml. The mixed cell suspension containing strains 3, 4, and 5 was added. Reaction mixtures were incubated at 37° without shaking for about 110 hr. Viable cell counts in the reaction mixtures after incubation at the lowest and highest levels were estimated as 2.9 × 10^9 and 1.7 × 10^9, respectively. Each experiment was carried out with two flasks.*

With the advancement of the Good Manufacturing Practices, microbial contamination, even in nonsterile drugs in either final products or raw materials, has become of concern for the quality control of pharmaceutical manufacturing processes. This communication is concerned with the finding that alkylparabens are decomposed to phenol by the cooperative action of two different bacteria.

Three microorganisms were isolated from the pharmaceutical manufacturing processes using gelatin; they were identified as *Klebsiella aerogenes* (strain 3), *Pseudomonas aeruginosa* (strain 4), and *Pseudomonas aeruginosa* (strain 5). They are able to grow in a gelatin paste containing alkylparabens.

Each microorganism was grown on soybean casein digest agar slant medium (1) for 18 hr at 37° without shaking. The grown cells were harvested aseptically by centrifugation, washed once with sterile water, and suspended in sterile water. The cell suspensions thus prepared were kept in a refrigerator until use and were used for the following experiments.

Counting of viable cells was carried out by means of the plate method, using soybean casein digest agar as the test medium as described in USP XVIII (1). Various alkylparabens[1] and *p*-hydroxybenzoic acid[2] were obtained commercially. Other reagents used were of the best quality commercially available. TLC[3] was carried out according to the methods described by Sadamatsu *et al.* (2). Phenol was determined by literature methods (3, 4).

[1] Wako Pure Chemicals Co.
[2] Tokyo Kasei Co.
[3] DC Fertigplatten Kiesel gel G 60 F 254 from Merck was used.

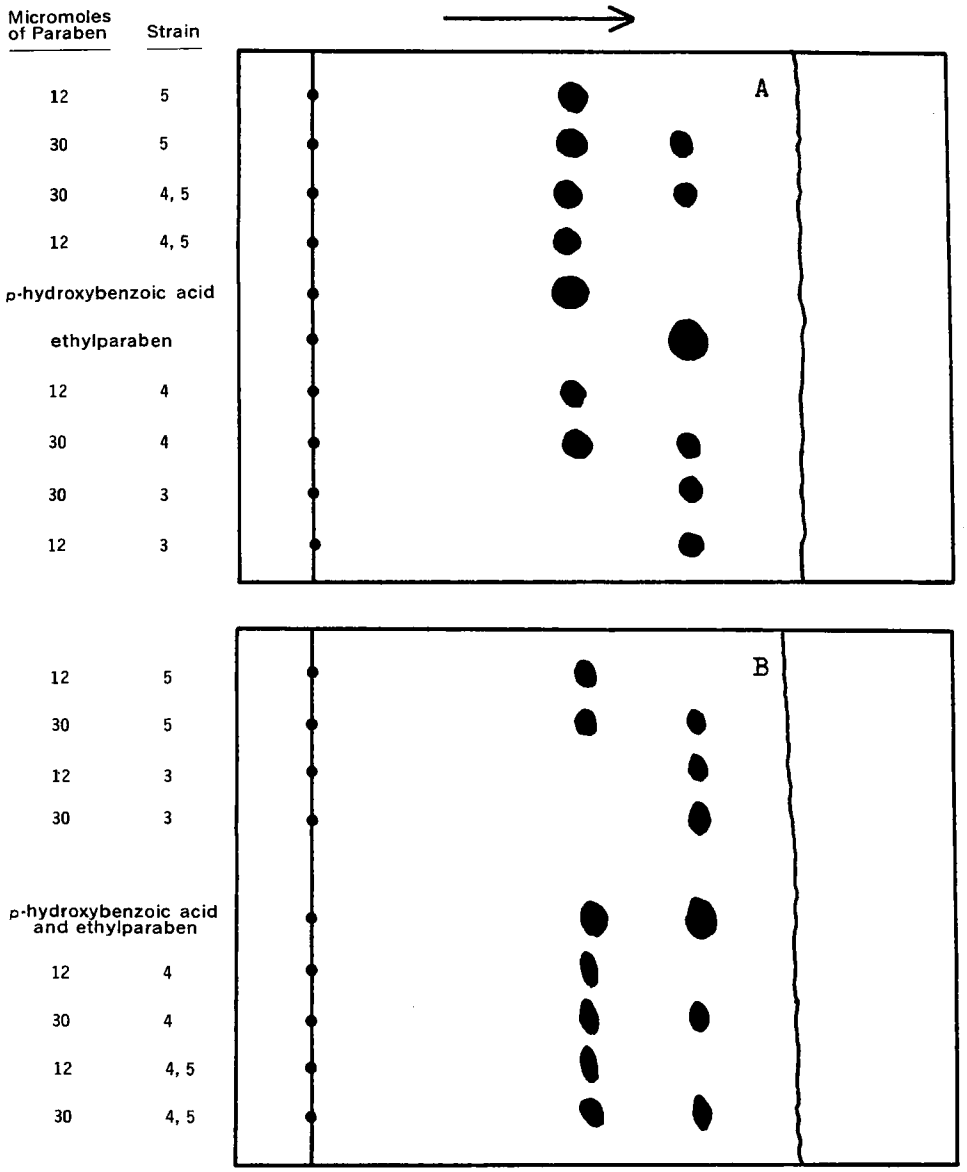


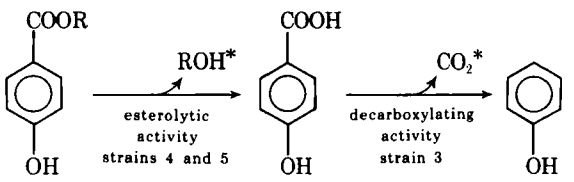
Figure 2—*TLC identification of esterolytic activity formed by strains 3 and 5. Reactions were carried out in the same manner as described in Table I. Developing solvents used were: A, chloroform–propionic acid (1:4); and B, benzene–dioxane–acetic acid (90:25:4). Spots were detected with a UV lamp.*

When the mixed cell suspensions of strains 3, 4, and 5 were added to the sterilized ethylparaben solution and incubated at 37° for about 110 hr, phenol was formed, depending on the amount of ethylparaben added (Fig. 1). Table I shows that neither strain 3, 4, nor 5 was capable of forming phenol from ethylparaben, but strain 3 was able to form phenol from *p*-hydroxybenzoic acid. It was also able to produce phenol from ethylparaben in combination with strains 4 and/or 5. Phenol was also formed from methyl-, propyl-, and butylparabens under the same conditions.

TLC patterns of the reaction mixture (Fig. 2) showed that *p*-hydroxybenzoic acid was formed from ethylparaben with strains 4 and/or 5, whereas ethylparaben remained unchanged with strain 3.

These findings can be explained if it is assumed (Scheme I) that both strains 4 and 5 have esterolytic activity and that strain 3 contains a decarboxylating system. It is also suggested that the rather rapid hydrolysis of alkylparabens by esterolytic activity of either strain 4 or 5 favors the survival of strain 3, which plays the leading role in the phenol formation from alkylparabens *via* *p*-hydroxybenzoic acid.

Previous studies (5) showed that *K. aerogenes* can form phenol from *p*-hydroxybenzoic acid, suggesting the presence of a decarboxylase system in the microorganisms. On the other hand, tyrosine phenol lyase



*Scheme I—Speculated mechanisms of phenol formation from alkylparabens (R = methyl, ethyl, propyl, and butyl; * = speculated)*

Table I—Effects of Various Parabens and *p*-Hydroxybenzoic Acid on Phenol Formation in the Presence of Single or Combined Cell Suspension as Enzyme Source[a]

Microorganisms Added (Strain)	Substrate, 12 μmoles (1.2 m*M*)					
	No Substrate	*p*-Hydroxybenzoic Acid	Methylparaben	Ethylparaben	Propylparaben	Butylparaben
3	0	11.4 12.2	—	0	—	—
4	0	0	—	0	—	—
5	0	0	—	0	—	—
3 and 4	0	7.4 10.0	—	9.4 9.0	—	—
3 and 5	0	9.9 9.7	—	9.4 9.3	—	—
4 and 5	0	0	—	0	—	—
3, 4, and 5	0	8.3 9.8	8.5 9.7	9.1 9.9	8.8 9.6	8.9 9.3

[a] Each figure in the table shows the amount of phenol formed (micromoles). Reaction conditions were those given in Fig. 1, except that each viable cell count of strains 3, 4, and 5 added was 5.9×10^{10}, 1.3×10^{10}, and 2.1×10^{10}, respectively, in a final volume of 10 ml.

has also been studied intensively by many investigators (6–10), who found that it catalyzes direct splitting of alanine from tyrosine to form phenol. In our studies with tyrosine as the substrate in place of alkylparabens, however, phenol was not produced.

Various alkylparabens are used or prescribed to prevent proliferation of contaminated microorganisms in pharmaceutical preparations. However, *P. aeruginosa* (NCTC 7244) was found to utilize alkylparabens as a carbon source and to grow well in an eye drop solution containing them (11). Furthermore, as described in the present communication, microorganisms such as strain 3 *(K. aerogenes)* metabolize *p*-hydroxybenzoic acid into phenol under restricted nutritional conditions.

Pseudomonas or *Klebsiella* species and other organisms have been isolated from various nonsterile pharmaceutical or cosmetic preparations and the production environment (12). It also has been shown that serious spoilage of drugs is brought about by these microorganisms. Although the mechanism of deterioration has not been elucidated from the standpoint of bacteriological metabolism, this communication presents a typical example concerning drug deterioration by microorganisms.

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Antiviral Activity of Gossypol and Apogossypol

Keyphrases □ Gossypol—screened for antiviral activity □ Apogossypol—screened for antiviral activity □ Antiviral activity—gossypol and apogossypol

To the Editor:

The pigment gland of the cotton seed contains an array of organic compounds including gossypol (I), which has been demonstrated to have antibacterial (1), antiviral (2–4), and antitumor (5) activities as well as mammalian toxicity (6, 7). Apogossypol (II), which has lower mammalian toxicity, is formed by deformylation of gossypol with base (8). The following report of preliminary findings establishes that apogossypol retains the potent antiviral activity of gossypol.

Gossypol can inactivate influenza virus infectivity *in vitro*, and influenza virus inactivated by treatment with gossypol was given to mice with a resultant 96–100% protection rate (2–4). Since little is known about either the mechanism of action or the activity spectrum of gossypol, an investigation of the *in vitro* inactivation of viruses by this compound and the less toxic apogossypol was initiated.