Benzocaine Diffusion from Polyethylene Glycol through Human Stratum Corneum

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Abstract □ The diffusion, penetration, and surface effects of benzocaine incorporated in various polyethylene glycol ointment bases through human stratum corneum were studied. Benzocaine diffusion was measured by following the benzocaine concentration in the receiving compartment of a diffusion cell. The ointment was placed in the other cell compartment and was separated from the receiving compartment by sheets of human stratum corneum. Surface effects were monitored by scanning electron micrographs of the stratum corneum. Results showed a decrease in drug diffusion in the presence of relatively high amounts of the lower molecular weight portions of polyethylene glycol. Scanning electron microscope studies showed that both benzocaine and polyethylene glycol affect the surface structure of the stratum corneum. Thermal analysis indicated that benzocaine dissolves in polyethylene glycol.

Keyphrases □ Benzocaine—diffusion from various polyethylene glycol ointment bases through human stratum corneum □ Diffusion—benzocaine from various polyethylene glycol ointment bases through human stratum corneum □ Surface effects—benzocaine diffusion from various polyethylene glycol ointment bases through human stratum corneum □ Ointment bases—various polyethylene glycols, benzocaine diffusion through human stratum corneum □ Anesthetics, topical—benzocaine, diffusion from various polyethylene glycol ointment bases through human stratum corneum

Benzocaine (ethyl *p*-aminobenzoate) is used extensively as a local anesthetic. Its relatively slow absorption renders it especially useful in treating skin ulcers, wounds, and mucous surfaces. The anesthesia is usually not as complete as that induced by the soluble anesthetics but is longer lasting.

BACKGROUND

The rate of benzocaine release is greater from water-soluble bases than from other bases (1, 2). Physical and chemical relationships between the ointment base and the medicament are important (3–7). While many studies have been directed toward traditional ointment bases, only a limited amount of work has been done using water-soluble ointment bases including polyethylene glycols.

Water-soluble bases are anhydrous but are capable of absorbing small amounts of water, are soluble in water, and are water removable. Although some water-dispersible ointment bases are often classified as water soluble, polyethylene glycols are the only widely used water-soluble ointment bases. The polyethylene glycols range from liquids to waxy solids, depending on molecular weight; by blending various molecular weight fractions, different base consistencies are possible.

Polyethylene glycols are important in pharmaceuticals as anhydrous, stable, bland bases for external preparations. They demonstrate low oral and dermal toxicity and low irritating properties (8). The ability of polyethylene glycols to form inert, emollient bases has enhanced their use as washable vehicles (9).

Penetration of drug through the skin and subsequent systemic absorption have been of particular interest recently. A primary consideration of transepidermal penetration is the stratum corneum, since the impermeability of skin largely resides in the stratum corneum, which is mechanically strong and capable of resisting chemical attack (10). The main barriers of the stratum corneum are the dead cells that restrict the inward and outward movement of chemical substances (11). Analysis of penetration and electron microscope studies support the concept that the penetration barrier consists of a keratin–phospholipid complex in the dead and relatively dry cells (12, 13). The thickness of the stratum corneum varies between subjects and depends on body location.

EXPERIMENTAL

Reagents and Equipment—The chemicals used included benzocaine[1]; polyethylene glycols 200, 600, 4000, 6000, and 20,000[2]; sodium bicarbonate[3]; monobasic sodium phosphate[3]; dibasic sodium phosphate[3]; sodium hydroxide USP[4]; lyophilized trypsin[5]; formaldehyde USP[6]; amyl acetate (mixed isomers)[2]; and alcohol USP[7]. The purity of benzocaine and polyethylene glycols 4000, 6000, and 20,000 was checked using differential thermal analysis[8]. Unless otherwise noted, reagents were analytical grade, except the lyophilized trypsin, and were used as received.

Solutions were prepared from the reagents listed and included 6.6×10^{-2} *M* monobasic sodium phosphate, 6.6×10^{-2} *M* dibasic sodium phosphate, 0.1 *N* NaOH, 10% formaldehyde, 0.5% sodium bicarbonate, and 0.0001% trypsin. Two buffers, pH 5.9 and 4.9, were prepared from the phosphate solutions.

The equipment included a spectrophotometer[9] for the benzocaine assay, a constant-temperature shaker bath[10], and custom-made polymethacrylate diffusion cells[11] similar to those described by Patel and Foss (14). The scanning electron microscope studies were carried out on a laboratory scope[12] utilizing a critical point drying apparatus[13] and vacuum evaporator[14].

Ointment Formulation—Ointment bases were prepared using varying amounts of different molecular weight polyethylene glycols. Ointments were prepared by the use of heat and mechanical incorporation. The polyethylene glycols were placed in a vessel and heated on a steam bath until liquefied. The ointment was removed from the heat and stirred until congealed. Powdered benzocaine was incorporated by spatulation.

Approximately 25 ointment base formulations composed of combinations of polyethylene glycol 200, 600, 4000, 6000, and 20,000 were prepared. Four formulations were especially suitable pharmaceutically: 50% polyethylene glycol 4000 plus 50% polyethylene glycol 600 (I), 25% polyethylene glycol 4000 plus 75% polyethylene glycol 600 (II), 50% polyethylene glycol 6000 plus 50% polyethylene glycol 600 (III), and 25% polyethylene glycol 6000 plus 75% polyethylene glycol 600 (IV). Ointments were prepared by incorporation of 5% (w/w) benzocaine in each base.

Stratum Corneum Preparation—Human stratum corneum was obtained by treating human cadaver skin taken at autopsy. Full thickness samples of adult abdominal skin proximal to the umbilicus were taken at autopsy and separated by scalpel from underlying subcutaneous fat. The epidermal sheet was removed from the dermis after immersion of the whole skin in 60° water for 30 sec (15).

Epidermal sheets were placed dermal side down on several sheets of filter paper saturated with 0.0001% trypsin containing 0.5% sodium bicarbonate; the pH was adjusted to between 8.0 and 8.6. The filter paper was covered with proteolytic solution in a small petri dish, which was incubated overnight at 37°. After incubation, the tissue was placed on a flat surface. Any remaining epidermis was removed by gentle massage with a moistened cotton-tipped applicator.

[1] Eastman Kodak, Rochester, NY 14650.
[2] J. T. Baker, Phillipsburg, NJ 08865.
[3] Fisher Scientific, Fair Lawn, NJ 07410.
[4] Mallinckrodt, St. Louis, MO 63160.
[5] Worthington Biochemicals, Freehold, NJ 07728.
[6] Amend Drug & Chemical, Irvington, NJ 07111.
[7] U.S. Industrial Chemicals, New York, NY 10001.
[8] DuPont 990 thermal analyzer, DuPont Instruments, Wilmington, DE 19898.
[9] Beckman DB-G with recorder, Beckman Instruments, Fullerton, CA 92634.
[10] Model 50, Precision Scientific, Chicago, IL 60647.
[11] Davis Machine, Opelika, AL 36801.
[12] Advanced Metals Research model 1000, Burlington, MA 01803.
[13] Denton, Cherry Hill, NJ 08003.
[14] Denton model DV-502, Cherry Hill, NJ 08003.

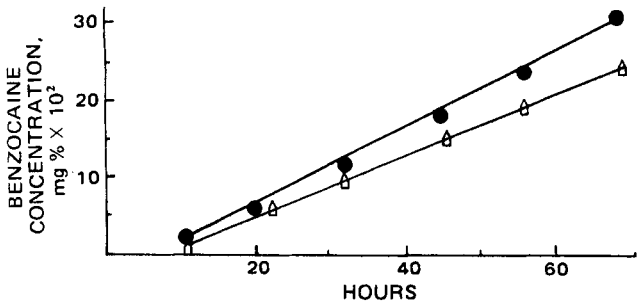

Figure 1—*Benzocaine diffusion through human stratum corneum at pH 5.9 from Ointments I (●) and II (△).*

The resultant stratum corneum was a smooth, transparent, resilient sheet. It was stored by floating on water and placed on aluminum foil. The resultant product has been shown to be stable even at ambient conditions for extended periods (16). Sample intactness was checked by optical microscopic examination prior to use.

Diffusion Study—Diffusion measurements were carried out by packing one-half of the cell with ointment, attaching the stratum corneum between the two half-cells, and then securing both sides tightly together. After assembly, the other cell compartment was filled with 20 ml of buffer through an opening that was subsequently stoppered. The entire assembly was immersed in the constant-temperature shaker bath, which was held to 37 ± 1°. The shaker bath was agitated at 100 oscillations/min.

At specified time intervals, 1-ml samples of buffer were withdrawn from the receptacle side of the cell. All sampling was done with replacement, and all experiments were done in duplicate with fresh stratum corneum. Blank ointments (no medicament) were also run for comparison.

Benzocaine appearance in the buffer compartment was measured by treating the 1-ml sample with 0.1 *N* NaOH and measuring the hydrolyzed benzocaine spectrophotometrically at its maximum absorbance, 267 nm. A standard curve was previously prepared similarly such that a direct correlation was shown between concentration and absorbance.

Thermal Analysis—All polyethylene glycols, benzocaine, ointment bases, and ointments containing benzocaine were studied by thermal analysis techniques. Differential scanning calorimetry was performed by hermetically sealing samples in cups. An empty cup was sealed in a similar manner and used as a reference. Samples were run from 11 to 200°, and results were recorded using an *x–y* recorder.

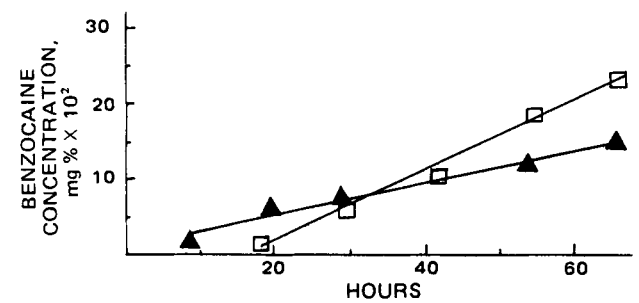

Figure 2—*Benzocaine diffusion through human stratum corneum at pH 5.9 from Ointments III (□) and IV (▲).*

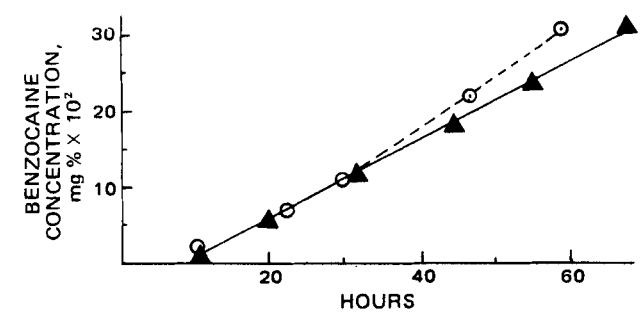

Figure 3—*Benzocaine diffusion through human stratum corneum from Ointment I at pH 4.9 (⊙) and 5.9 (▲).*

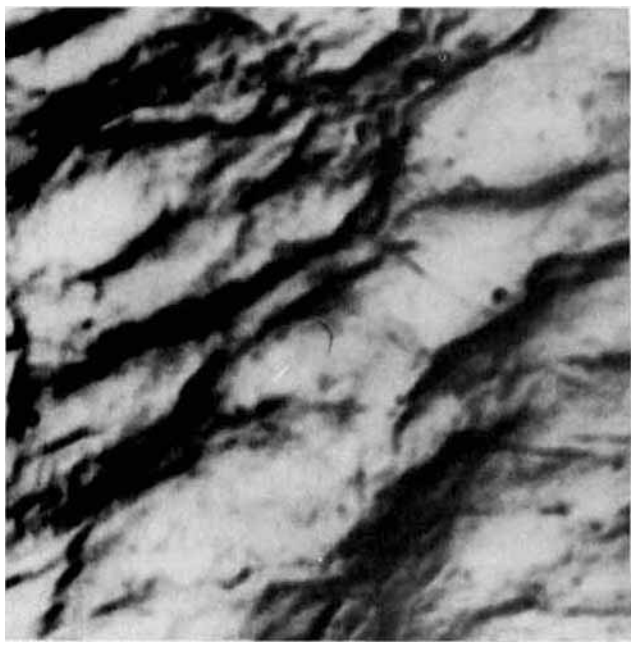

Figure 4—*Scanning electron micrograph of normal human stratum corneum (1000× magnification).*

Scanning Electron Microscopy—Stratum corneum samples before and after treatment were studied for surface structure changes. Samples were taken after exposure to blank ointment bases for comparison.

Specimens were washed with water after exposure to ointments and then soaked in 10% formaldehyde solution. Subsequently, they were dehydrated by exposure to increasing concentrations of ethanol and then exposed to amyl acetate for 5 min. Specimens were mounted on stubs and subjected to liquid carbon dioxide in the drying apparatus. After dehydration, sample coating was achieved using a 60% gold–40% palladium alloy in the vacuum evaporator.

RESULTS AND DISCUSSION

Figure 1 illustrates benzocaine diffusion from Ointments I and II through the stratum corneum into pH 5.9 buffer. Greater penetration

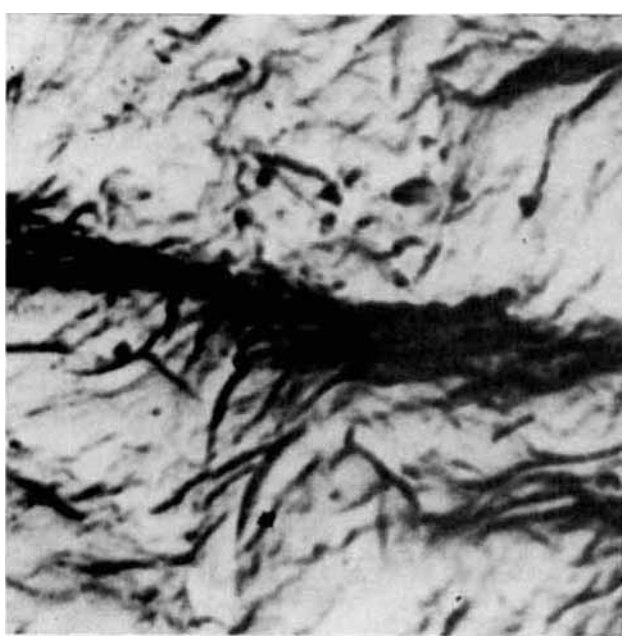

Figure 5—*Scanning electron micrograph of human stratum corneum after treatment with 5% benzocaine in Ointment I (1000× magnification).*

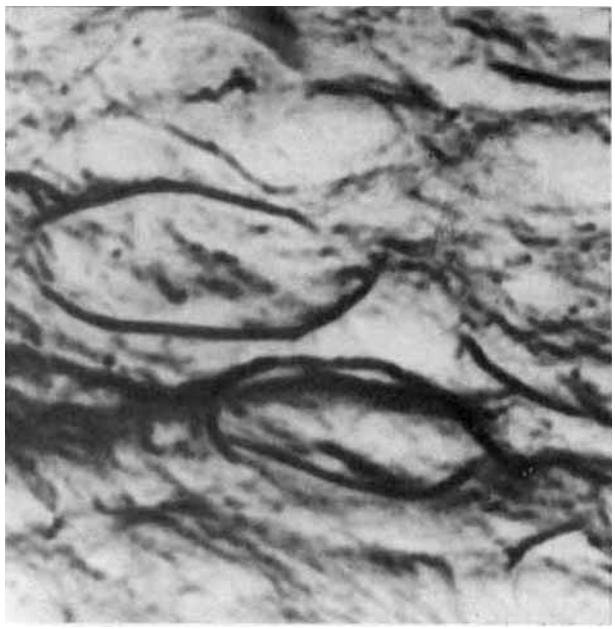

Figure 6—*Scanning electron micrograph of human stratum corneum after treatment with 5% benzocaine in Ointment II (1000× magnification).*

of benzocaine from the ointment containing 50% polyethylene glycol 600 (I) than from the 75% polyethylene glycol 600 ointment (II) is shown by the upward displacement and increases in slope for the Ointment I line. In Fig. 2, penetration again appears greater with the 50% polyethylene glycol 600 ointment (III) compared to the ointment containing 75% polyethylene glycol 600 (IV).

Figure 3 illustrates benzocaine transfer from Ointment I through the stratum corneum into pH 4.9 buffer solution. Similar results were obtained with Ointments II–IV. Alteration of pH in the receiving compartment did not significantly alter benzocaine diffusion in this system.

Figures 4–7 are scanning electron micrographs of human stratum corneum normally (Fig. 4) and after various treatments (Figs. 5–7). As shown in Fig. 5, there were noticeable changes in the surface structure of the stratum corneum after treatment with 5% benzocaine in 50% polyethylene glycol 4000 plus 50% polyethylene glycol 600 base (Ointment

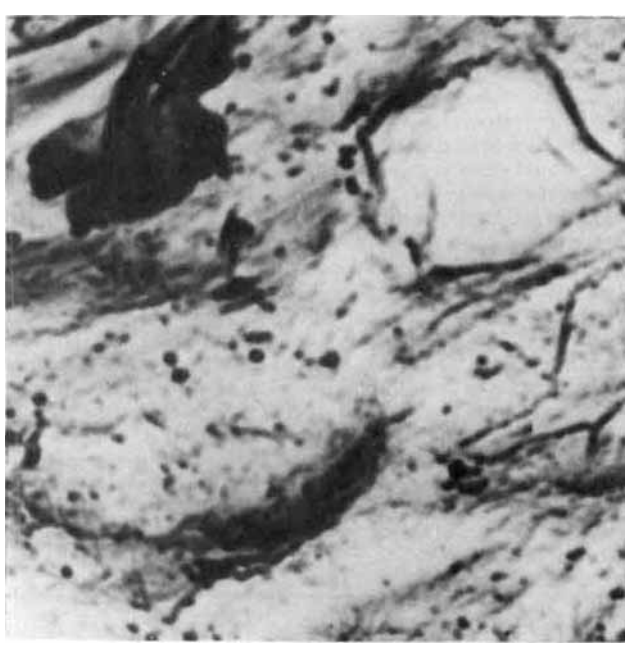

Figure 7—*Scanning electron micrograph of human stratum corneum after treatment with Ointment II (1000× magnification).*

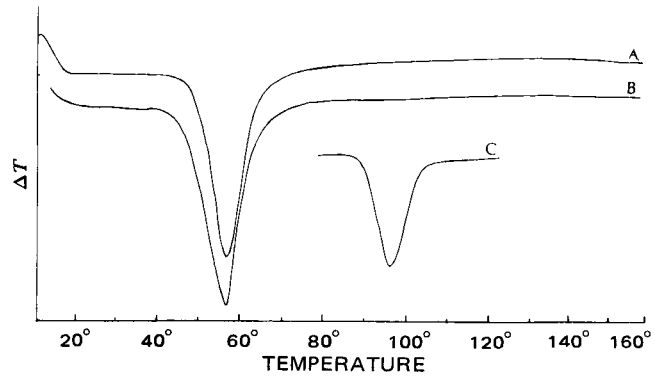

Figure 8—*Thermograms of Ointment III (A), 5% benzocaine in Ointment III (B), and pure benzocaine (C).*

I). A considerable increase in surface roughness was seen along with an increase in surface ridge sharpness.

Figure 6 shows a more prominent change in the physical structure from treatment with 5% benzocaine in 25% polyethylene glycol 4000 and 75% polyethylene glycol 600 (Ointment II). Considerable changes in physical structure appeared in the form of circumscribed hexagonal- and pentagonal-shaped areas. These circumscribed areas also appeared in blank (no benzocaine) ointments (Fig. 7). These results indicate that the ointment base affects the physical surface structure of human stratum corneum. In addition, ointments containing benzocaine have a similar but somewhat less pronounced effect. The principal effect appears to be a function of the polyethylene glycol 600 content.

Thermal analysis studies showed benzocaine dissolved in the polyethylene glycol ointment bases. Figure 8 shows thermograms of Ointment III with 5% benzocaine (curve B). Pure benzocaine exhibited an endotherm at 90–100° (curve C). The blank ointment (III without benzocaine) is shown as curve A. Similar curves were obtained with all other ointments.

CONCLUSIONS

Benzocaine release from various polyethylene glycol ointments appeared to depend on the polyethylene glycol composition. While various molecular weight fractions of polyethylene glycol could be blended for the desired consistency, ointments containing relatively large portions of lower molecular weight polyethylene glycols may retard benzocaine release. This finding may have implication for ointment formulations containing water-soluble ointment bases since polyethylene glycols are major constituents of many water-soluble bases currently used.

Alteration of pH in the receiving compartment of the diffusion cell did not influence drug release. However, considering the narrow pH range used, perhaps a greater pH differential should be chosen.

Thermal analysis showed that benzocaine dissolved in all polyethylene glycol ointment bases used. The solubility is, of course, dependent on the drug–polyethylene glycol interaction and does not occur with all drugs incorporated in polyethylene glycol bases. However, if a drug dissolves in polyethylene glycol, it may suggest release alteration if the polyethylene glycol composition is altered.

Scanning electron microscope results indicate that polyethylene glycol has a significant effect on the surface structure of the stratum corneum. Benzocaine may, in addition, have a surface effect; however, it appears to be a lesser effect compared to the polyethylene glycol ointment bases.

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Effect of Acylated Amino Acids and Acylated Amino Acid Analogs on Microbial Antitumor Screen

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Abstract □ A series of *N*-acetyl, *N*-propionyl, and *N*-chloroacetyl derivatives of amino acids and amino acid analogs was tested for growth-inhibitory activity using a *Lactobacillus casei* system as a prescreen for possible antitumor activity. While none of the acetyl or propionyl derivatives of these amino acids and amino acid analogs caused any remarkable inhibition, certain chloroacetyl derivatives exhibited significant activity. The chloroacetyl derivatives, especially those of essential amino acids and of analogs of essential amino acids, showed modest, but pharmacologically significant, inhibition; those of nonessential amino acids exhibited no activity. When two such inhibitory acyl derivatives were combined in a single assay, the extent of inhibition was neither additive nor synergistic but was that of the more active of the two test components.

Keyphrases □ Amino acids, various acylated—evaluated in microbial antitumor screen □ Acylated amino acids, various—evaluated in microbial antitumor screen □ Antitumor activity—various acylated amino acids evaluated in microbial screen □ Structure–activity relationships—various acylated amino acids evaluated in microbial antitumor screen

In a search for antineoplastic activities among a series of β-hydroxy-α-amino acids, some unsubstituted acids exhibited no growth-inhibitory action in a *Lactobacillus casei* test system, but their chloroacetyl derivatives showed pharmacologically significant activity in this system (1, 2). As a result of that observation, an investigation was initiated to note whether the behavior of other analogs of natural amino acids known to be antimetabolites might not be affected similarly by acylation.

The *N*-acetyl, *N*-chloroacetyl, and *N*-propionyl derivatives of a series of amino acids and amino acid analogs were prepared. These compounds were tested against an *L. casei* system (3) to determine the presence of growth-inhibitory capacity. This microbial system was selected as a prescreen for antitumor activity because of its capability of detecting, very quickly, a high percentage of compounds known to be active against tumors, using very small quantities of test compound (3). A study was also made to determine the existence of additive or synergistic properties when two inhibitory compounds were used simultaneously.

The present paper reports the results of these studies.

EXPERIMENTAL

The free amino acids, except for the isomers of β-hydroxymethoxinine and β-hydroxyhomomethionine which were prepared in this laboratory (4), were obtained commercially. The sources and purity data for the acyl derivatives are shown in Tables I–III. The commercially obtained free amino acids and acyl compounds were recrystallized, and all compounds were checked for purity by elemental analysis, melting-point determination, optical rotation determination, and Van Slyke nitrous acid determination of primary amino nitrogen (5) before use (Tables I–III). In addition, the free amino acids were checked by paper chromatography in at least four different solvent systems (*cf.*, 2).

The chloroacetyl and propionyl derivatives were prepared in this laboratory by acylation of the precursors by the conventional Schotten–Baumann procedure (*cf.*, 6). The chloroacetyl chloride[1] and propionyl chloride[1] used for the acylation and the propionic acid[1] and monochloroacetic acid[2] were freshly redistilled before use. The optically pure amino acids were prepared by asymmetric enzymatic hydrolysis of the racemic chloroacetyl derivatives and subsequent acid hydrolysis of the unsusceptible chloroacetyl-D-amino acid. The optically pure chloroacetyl-L-amino acids were prepared by acylation of the pure L-isomer, and the chloroacetyl-D-isomers were isolated from the resolution mixture as described (11). Hog renal acylase[3] was used for the hydrolysis of the acyl aliphatic amino acids, and pancreatic carboxypeptidase[4] was used for the hydrolysis of the acyl aromatic amino acid (11).

For the microbiological assay, the test compounds were dissolved in a small volume of water and the pH was adjusted to 6 by the addition of an appropriate volume of 0.1 *N* NaOH. L-Tyrosine required equimolar amounts of the base for solution, resulting in a basic solution. In some cases, gentle warming was required for complete solution. The test solutions were sterilized by filtration using an all-glass bacterial filter (ultrafine porosity).

The concentration of the test solution as prepared was 2 mg/ml and was equivalent to a concentration of 1 mg/ml in the assay system. For the experiments comparing the relative activities of the "active" compounds, the concentration of the test solution was 89.4 μmoles/10 ml as prepared and was equivalent to a concentration of 4.47 μmoles of the test compound/ml in the assay system. This concentration is equivalent to 1 mg of *N*-chloroacetyl-β-hydroxy-D-norleucine B/ml, the compound found previously to be the most active isomer of chloroacetyl-β-hydroxynorleucine (2).

All glassware used in the microbiological assay was cleaned in a metal ion-free cleaning agent[5].

[1] Eastman Organic Chemicals, Rochester, N.Y.
[2] Fisher Scientific Co., Silver Spring, Md.
[3] Nutritional Biochemicals, Cleveland, Ohio.
[4] Calbiochem, La Jolla, Calif.
[5] No-chromix, Godax Laboratories, New York, N.Y.