control value (Fig. 1A). Since this finding suggested that the decrease was at the level of uridylate kinase, we repeated the experiment with ^{3}H-5-UMP as substrate. The decrease of ^{3}H-UTP formation amounts to about 40 percent of the control value (Fig. 1B), a figure comparable to the inhibition of ^{3}H-uridine incorporation into RNA.

We have demonstrated that uridylate kinase activity is decreased in mouse salivary glands after injection of isoproterenol. The magnitude of this decrease is sufficient to account for the decreased incorporation of ^{3}H-uridine into RNA. In conjunction with earlier findings that there is a decreased specific activity of the phosphorylated uridine pool (*2*), it becomes clear that there is no change in the rate of RNA synthesis detectable with the methods employed at the times studied. The significance of a decreased uridylate kinase activity is not clear at the present time. Since the changes occur only under conditions which stimulate DNA synthesis, they may be involved in metabolic alterations which precede cell proliferation. The situation in isoproterenol-stimulated salivary glands clearly differs from that found in another model of stimulated cell proliferation, phytohemagglutinin-stimulated lymphocytes. In this latter model there is a stimulation of ^{14}C-uridine incorporation into RNA between 10 and 50 hours after stimulation (*8*). This stimulation is accompanied by an increase in uridine kinase activity, with no change in uridylate kinase (*8*).

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Hydrolysis at Arginylproline in Polypeptides by Clostridiopeptidase B

Abstract. *Clostridiopeptidase B, a sulfhydryl protease from* Clostridium histolyticum, *hydrolyzes the arginylproline bond in the synthetic polypeptide methionyllysyl-bradykinin. Hydrolysis also occurs at a reduced rate at the lysylarginine bond, further delineating the environment necessary for the minor proteolysis seen with this enzyme at lysine residues. The specificity of clostridiopeptidase B differs from trypsin, which hydrolyzes this synthetic polypeptide only at the lysylarginine bond.*

Clostridiopeptidase B (E.C. 3.4.4.20), a sulfhydryl protease from *Clostridium histolyticum*, has been obtained recently from a commercial collagenase preparation in a state of apparent homogeneity (*1*). The enzyme has a narrow specificity range against synthetic esters and amides as well as against polypeptide substrates. Hydrolysis of polypeptides of known sequence has always been less than that obtained with trypsin. The major specificity is directed at the carboxyl side of arginine. Lysyl bonds are also attacked, but at rates generally much slower than hydrolysis of arginyl bonds. The unique specificity of clostridiopeptidase B and the judicious use of controlled enzymatic hydrolysis should facilitate sequence analysis by allowing the isolation of larger peptide fragments without chemical modification of the substrate. This communication extends the specificity data for this bacterial protease.

Synthetic methionyllysyl-bradykinin NH_2-Met-Lys-Arg-Pro-Pro-Gly-Phe-Pro-Phe-Arg-COOH (*2*) was hydrolyzed by clostridiopeptidase B and trypsin (~ 1:50, enzyme : substrate) for 1 hour at 30°C in sodium phosphate buffer (*p*H 7.7, ionic strength, 0.025) containing 1 m*M* dithiothreitol. The products were analyzed by paper electrophoresis in formate buffer (*3*) at *p*H 2.0 at 2000 volts on Whatman 3 MM paper (46 by 57 cm) for 75 minutes. Peptides were identified by ninhydrin tests, and arginine-containing peptides were identified with the Sakaguchi stain (*4*). A lysine standard was used for reference positions. Peptides adjacent to the ninhydrin and Sakaguchi markers were eluted with water (overnight), hydrolyzed for 18 hours in 6*N* HCl, and analyzed with a Technicon amino acid analyzer; the peptide fragments were identified by the molar ratios of component amino acids.

The difference in specificity of trypsin and clostridiopeptidase B for the synthetic peptide methionyllysyl-bradykinin is shown in Fig 1. The parent peptide, represented in column V, showed a single spot that was both Sakaguchi- and ninhydrin-positive ($\varepsilon f = 0.72$).

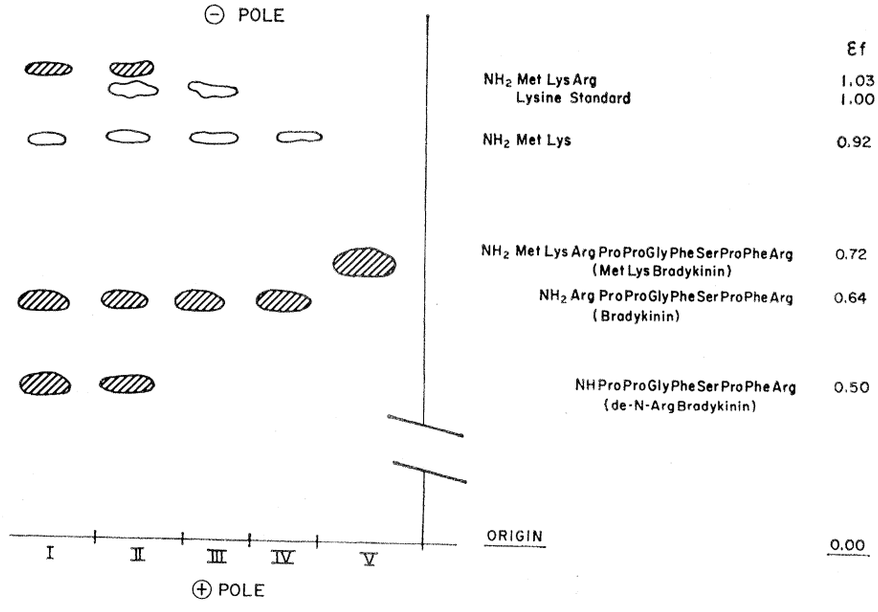

Fig. 1. Paper electrophoresis of metlysyl-bradykinin and degradation products. All spots are ninhydrin-positive. Shaded spots indicate Sakaguchi-positive peptides. Column I, hydrolysis by clostridiopeptidase B; II, I plus lysine standard; IV, hydrolysis by trypsin; III is IV plus lysine standard; V is metlysyl-bradykinin, the parent substrate. Content of the fragments is indicated on the right as judged by mobility, staining reactions, and apparent amino acid content after elution from paper. Mobility relative to lysine (εf) is given at the far right. Arginine has an εf of 0.92.

Trypsin digestion (columns III and IV) results in the expected hydrolysis at the Lys-Arg bond, the adjacent Arg-Pro resisting proteolysis, as demonstrated by the degradation to two ninhydrin-positive spots, the slower ($\varepsilon f = 0.64$) being Sakaguchi-positive and the faster ($\varepsilon f = 0.92$) being Sakaguchi-negative. The composition of eluted peptides corresponds to hydrolysis at Lys-Arg. Digestion of methionyllysyl-bradykinin with clostridiopeptidase B (columns I and II) reveals a marked specificity difference as compared to trypsin. Although ninhydrin-positive spots are seen corresponding to tryptic hydrolysis at Lys-Arg, two new peptides appear. Both are Sakaguchi-positive with the slower peptide ($\varepsilon f = 0.50$) yielding a yellow ninhydrin color, an indication of an imino terminal. The composition of the new peptides corresponds to hydrolysis at the Arg-Pro bond. No free arginine was noted, indicating that the enzyme competes for each site and that the Met-Lys fragment is not the result of further degradation of an original Met-Lys-Arg peptide. The yields of the individual peptides, as estimated from their content of amino acids, indicates that hydrolysis at the Arg-Pro bond by clostridiopeptidase B proceeds at approximately one and one-half to two times the rate at the Lys-Arg bond.

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Clostridium botulinum Type F: Isolation from Crabs

Abstract. *Two proteolytic strains of* Clostridium botulinum *type F have been isolated from crabs* (Callinectes sapidus) *from the York River in Virginia. This is the first time proteolytic strains of this type have been isolated in the United States and the first demonstration of the presence of type F on the eastern coast of the United States.*

In the course of an investigation on the prevalence of *Clostridium botulinum* type E in crabs in the middle Atlantic region of the United States, samples of the gills and viscera of two crabs (*Callinectes sapidus*) collected at the mouth of the York River channel yielded cultures of *Clostridium botulinum* type F. This immunologic type of *Cl. botulinum* was first isolated in 1960 (*1*) after its toxin, present in homemade liver paste, had caused an outbreak of botulism on the Danish island of Langeland (*2*). The proteolytic organism subsequently isolated from the liver paste was designated as the prototype strain of *Cl. botulinum* type F (*3*). The only other isolations of this immunologic type from natural sources have been made from materials collected along the western coast of the United States. It was isolated once from Pacific Ocean sediments (*4*) and once from the gills and viscera of a sockeye salmon (*5*). The strains thus far characterized in the United States have differed from the prototype Langeland strain in being nonproteolytic (*6*). The *Cl. botulinum* type F toxin has recently been identified in mud samples from a stream in eastern North Dakota (*7*) and in a composite mixture of viscera and gills from a fish caught in the Atchafalaya River, Louisiana (*8*), but the causative organism itself was not isolated by these investigators. I now report the first isolation of a proteolytic *Cl. botulinum* type F in the United States and the first reported demonstration of the natural occurrence of this immunologic type on the eastern coast of the United States.

Crabs were collected at the York River in Virginia, placed alive in plastic bags on wet ice in styrofoam coolers, and returned to the laboratory for processing. Two tubes of culture medium were inoculated from each crab, one with gills and one with viscera aseptically dissected from the crabs. Approximately 1 g of each sample was inoculated into 80 ml of freshly prepared beef infusion broth containing one-tenth part (by volume) of meat particles and supplemented with proteose peptone, soluble starch, sodium chloride, dibasic sodium phosphate, and sodium thioglycollate (*9*). After 14 days of incubation at 20°C, portions of the culture supernatants were centrifuged at 3000 rev/min (1500 relative centrifugal force) for 30 minutes at 3°C. The supernatants were tested for toxicity by injecting ICR strain white mice (13 to 18 g, male) with 0.5 ml of supernatant diluted 1:10 with gelatin phosphate buffer (*p*H 6.8). Initially the supernatants were tested for toxin by inoculating two mice each with either unheated supernatant or supernatant heated at 100°C for 10 minutes. They were observed for 4 days for typical symptoms of botulism and death.

Those cultures which contained heat-labile toxin were typed for the specific type of toxin present with monovalent antitoxins against *Cl. botulinum* types A, B, C, D, E, or F. Each antitoxin was reconstituted to contain 10 international units per milliliter. Mice were injected with 0.5 ml of a mixture containing 0.4 ml of centrifuged undiluted supernatant and 0.1 ml of a specific antitoxin. At the same time, as controls, equal groups of mice were injected with boiled and unboiled supernatant. In the crab cultures from which isolation of type F *Cl. botulinum* organisms was later attempted, complete protection was provided by type F antitoxin only.

The *Cl. botulinum* type F organisms were isolated initially by streaking the original mixed cultures from the crab materials onto plates of nutrient agar. These were incubated at 30°C for 18 hours in a Gaspak anaerobic jar. Feathery colonies were picked and inoculated into 30 ml of freshly exhausted, cooked meat medium (Difco) in screw-capped tubes. After these cultures were incubated at 30°C for 3 days, the purity of the cultures was verified by the Gram stain and the malachite green spore stain. Cultures were typed again as described, but ten mice per group were used and tetanus antitoxin was included in the testing. Neutralization data in support of the identification of the cultures as *Cl. botulinum* type F are summarized in Table 1. Toxicity of the

Table 1. Neutralization pattern of cultures identified as *Clostridium botulinum* type F. Results are given as number of mice dead over number tested. Abbreviations: S, centrifuged supernatant; HS, heated supernatant (100°C for 10 minutes).

Source of toxin	Anti-toxin	Results	
		Crab 4-V-I	Crab 8-G-I
S	None	10/10	10/10
S	Tetanus	10/10	10/10
S	A	10/10	10/10
S	B	10/10	10/10
S	C	10/10	10/10
S	D	10/10	10/10
S	E	10/10	10/10
S	F	0/10	0/10
HS	None	0/10	0/10