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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References and Notes

1. W. M. Mitchell and W. F. Harrington, *J. Biol. Chem.* **243**, 4683 (1968).
2. Kindly provided by Dr. B. Merrifield.
3. D. Gross, *Nature* **184**, 1298 (1959).
4. F. Irreverre, *Biochim. Biophys. Acta* **111**, 551 (1965).
5. Supported by PHS grant AM-10833 and a special PHS fellowship 1 F3 AM-34, 798.

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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

cultures was established by inoculating tenfold dilutions into ten mice per group and found to be 1×10^3 LD_{50} per milliliter (lethal dose effective for 50 percent of the animals) of medium for both cultures.

Proteolytic biochemical activity of the cultures, first noted in cooked meat medium, was further tested on iron milk, gelatin, and coagulated egg albumin media. All four media were digested by the organisms, thus indicating the presence of proteolytic enzymes.

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References and Notes

1. V. Møller and I. Scheibel, *Acta Path. Microbiol. Scand.* **48**, 80 (1960).
2. B. B. Jensen and F. Hahnemann, *Ugeskrift Laeger* **121**, 1363 (1959).
3. C. E. Dolman and L. Murakami, *J. Infec. Dis.* **109**, 107 (1961).
4. M. W. Eklund and F. Poysky, *Science* **149**, 306 (1965).
5. J. M. Craig and K. S. Pilcher, *ibid.* **153**, 311 (1966).
6. M. W. Eklund, F. T. Poysky, D. I. Wieler, *Appl. Microbiol.* **15**, 1316 (1967).
7. M. W. Wentz, R. A. Scott, J. W. Vennes, *Science* **155**, 89 (1967).
8. B. Q. Ward, B. J. Carroll, E. S. Garrett, G. B. Reese, *Appl. Microbiol.* **15**, 629 (1967).
9. J. T. Graikoski, personal communication.
10. Supported by Bureau of Commercial Fisheries contract 14-17-0004-273. Antitoxins were obtained from the National Communicable Disease Center (HEW), Atlanta, Georgia. I thank D. E. DeFoor and E. Gasper for technical assistance.

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Color: A Motion-Contingent Aftereffect

Abstract. *After human observers alternately view green stripes moving up and red stripes moving down for periods of ½ to 4 hours, they see a pink aftereffect when white stripes move up and a green aftereffect when white stripes move down. Longer exposures produce aftereffects which are visible 20 hours after stimulation. Thus, experience which pairs simple attributes (color and motion) of visual stimulation can result in a lasting modification of perception.*

McCullough (*1*) described negative color aftereffects, dependent on the orientation of lines in the test field, which were obtained by presenting a horizontal grating of one color alternately with a vertical grating of another color. These color aftereffects are orientation specific and persist for 1 hour after brief adaptation exposure periods.

The experiments reported here pair stimulus attributes of color and motion to produce color aftereffects which are motion contingent. An observer alternately views green stripes moving up and red stripes moving down on a black ground. When the stripes are later illuminated by tungsten (white) light, he perceives a color aftereffect which is direction specific; white stripes appear pink when they move up and green when they move down. These color aftereffects may persist for 20 hours (*2*).

Seven male and nine female university students who had no experience with visual aftereffects were paid observers. All had uncorrected normal vision without anomalies of color perception when tested with OSA pseudoisochromatic plates. While fixating the center of a moving grid with his head positioned in a chin rest, each observer viewed red stripes moving in one direction and green stripes moving in the opposite direction. A grid of 3-mm stripes was seen moving up or down at 2 degrees visual angle per second. A black mask restricted the grid area to 6.3 by 7.6 degrees visual angle at viewing distance of 83 cm. Magenta and yellow-green filters (*3*) provided the colors paired with ascending and descending motion. When these filters were placed in a projector behind the grid, the observer saw transilluminated colored stripes moving on a black field in the dark test room. Integrated visible transmission values were 35.6 percent for the magenta filter, 49.4 percent for the yellow-green filter, and the luminance of the grid in white light was 232 mlam. A timing circuit alternated color filters and reversed the direction of the motor powering the grid every 5 seconds. Each exposure period of alternating stimulation continued for 24 to 33 minutes.

Table 1. Color paired with motion: minutes of exposure required to produce color aftereffects which were direction-specific and visible immediately and 20 hours after stimulation. R, red; G, green; arrows indicate direction of motion.

Exposure condition		Time (min)	
		Immediate test	Delayed test*
R↑	G↓	198	192
R↑	G↓	132	501
R↑	G↓	231	384
R↑	G↓	120	48
G↑	R↓	33	288
G↑	R↓	33	312
G↑	R↓	216	216
G↑	R↓	120	216
		mean=135	mean=270

*$N = 8$ in both immediate and delayed groups.

Eight of the 16 observers were tested immediately after each exposure period. They judged the appearance of the moving stripes seen in white light from the projector bulb (3200°K). After repeated periods of exposure on successive days (one period per day), all observers reported that they clearly saw a negative color aftereffect specific to each direction of motion. They reported no colors when the grid remained in a stationary position. Data in Table 1 show that an immediate test produced reliable reports of color aftereffects that were direction specific when observers had experienced a mean of 135 minutes (2.3 hours) of paired stimulation.

To determine whether a persistent change had been produced, eight of the observers were not tested until 20 to 27 hours after each exposure period. (These observers did not judge the appearance of moving stripes immediately after exposure since a decision made at that time might have influenced their delayed reports.) At this delayed test interval [after a mean of 270 minutes (4.5 hours) of exposure to paired stimulation (Table 1)], observers saw color aftereffects which were direction specific. They did not see lasting colors after shorter exposure periods; perception of persisting color aftereffects required about twice as much experience as perception of similar aftereffects seen immediately after stimulation.

Test instructions simply requested a description of the moving stripes and did not mention the possibility of a color aftereffect. However, observers may have expected to see a color difference. A naive observer might easily think that he should report the color that was originally paired with a particular direction. Instead, all observers reported negative color aftereffects, and it seems unlikely that they could have known which color should be associated with a particular direction of motion. Another explanation is that paired exposure in these experiments associated perception of different colors with distinctive patterns of eye movements elicited by ascending and descending motion. Color adaptation, and the resulting complementary aftereffect,