ought to be measured in biological fluids rather than the major urinary metabolite alone before conclusions can be drawn whether a drug inhibits only prostaglandin biosynthesis or catabolism or both. We are presently exploring this more complete approach towards the effect of drugs on the PG system with several other compounds which we have found to inhibit prostaglandin catabolism[20,21].

[20] We thank Drs. J. E. PIKE and U. AXEN, The Upjohn Co., Kalamazoo, U.S.A. for their generous supplies of prostaglandins.
[21] Supported by a grant No. MA-4181 to C.P.A. from the Medical Research Council of Canada.

Zusammenfassung. Es zeigt sich, dass jedes der 3 Enzyme, die den Abbau von Prostaglandin in den Nieren erwachsener Ratten verursachen, durch schwache Konzentrationen von Indomethacin in steigendem Ausmass inhibiert werden können: 9-PGDH > 13-PGR > 15-PGDH.

C. PACE-ASCIAK and S. COLE

Research Institute, The Hospital for Sick Children, 555 University Avenue, Toronto M5G 1X8 (Canada), 9 September 1974.

Identification of Precoccinellin in the Ladybird Beetle, *Coleomegilla maculata*[1-3]

Coccinellid beetles have long been known to possess defensive compounds associated with 'reflex bleeding'[4], and the investigation of several European lady bugs has yielded the structures of the specific alkaloids[5-7]. Likewise, during an investigation of the volatiles of the lady beetle, *Coleomegilla maculata*, we have isolated and identified a defensive alkaloid, precoccinellin.

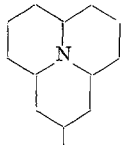
Isolation procedures. The beetles were steam-distilled for 2 h in an all-glass system, and the distillate was extracted with methylene chloride. The extract was concentrated in vacuo and chromatographed by GLC on a 20 ft, 1/8 inch O.D. stainless steel column packed with 10% SE-30. Column temperature was 160 °C, and carrier gas pressure at the inlet was 60 ψ. Retention time of the alkaloid was 10 min. I_k = 1475. The compound was collected from a stream splitter attachment on the gas chromatograph. *IR-spectrum.* The IR-spectrum in CCl_4 included ν_{max} 1020, 1040, 1120, 1130, 1325, 1385, 1450, 2775, 2860, 2925, 2950 CM^{-1}.

PMR-spectrum. The PMR-spectrum in CCl_4 showed ppm (δ) 0.89, 1.24, 1.38, 1.55, 1.64.

Mass spectrum m/e. 41 (100), 192 (66), 150 (51), 151 (47), 55 (47), 42 (44), 137 (34), 136 (32), 164 (28), 67 (26), 122 (25), 93 (22), 82 (19), 178 (18), 108 (15), 96 (13); M^+ = 193. Essentially the same as the precoccinellin isolated by TURSCH et al.[5].

Reactions. Hydrogenation (Pd on charcoal; hydrogen under pressure) – no effect. Lithium aluminum hydride reduction – no effect. NaOH treatment – no effect. HCl treatment – hydrochloride formed.

Based on these experiments and on data presented by TURSCH et al.[6], we propose that the compound isolated from *C. maculata* is precoccinellin:



(Dodecahydro-2-methylpyrido[2,1,6-*de*]quinolizine)

Extraction of whole beetles with methanol revealed no additional alkaloids. The compound is bitter to the taste, and it is assumed that it performs in a defensive mode as do other similar coccinellid alkaloids.

Zusammenfassung. Isolierung und Strukturzuteilung für ein Alkaloid aus *Coleomegilla maculata* (Coleoptera: Curculionidae).

R. D. HENSON[8], A. C. THOMPSON[8], P.A. HEDIN[8], P. R. NICHOLS[9] and W. W. NEEL[9]

United States Department of Agriculture, Agricultural Research Service, Boll Weevil Research Laboratory, P. O. Box 5367, Mississippi State (Mississippi 39762, USA), 1 August 1974.

[1] Coleoptera: Curculionidae.
[2] In cooperation with the Mississippi Agricultural and Forestry Experiment Station, Mississippi State, Mississippi 39762. Received for publication.
[3] Mention of a proprietary product does not necessarily imply endorsement of this product by the USDA.
[4] R. M. HAPP and T. EISNER, Science *134*, 329 (1961).
[5] B. TURŠCH, D. DALOZE, M. DUPONT, J. M. PASTEELS and MARIE-CLAIRE TRICOT, Experientia *27*, 1380 (1971).
[6] B. TURSCH, D. DALOZE, M. DUPONT, C. HOUTELE, M. KAISIN, J. M. PASTEELS, and D. ZIMMERMANN, Chimia *25*, 307 (1971).
[7] R. KARLSSON and D. LOSMAN, J. chem. Soc., Chem. Commun. *11*, 626 (1972).
[8] Boll Weevil Research Laboratory, USDA, ARS, Mississippi State, Mississippi 39762, USA.
[9] Department of Entomology, Mississippi State University, Mississippi State, Mississippi 39762, USA.

Microheterogeneity of Staphylococcal Enterotoxin C_2

Staphylococcal enterotoxin C_2 belongs to a closely related group of simple proteins that are causative agents of staphylococcal food poisoning. The toxin has been purified to homogeneity, and several of its physical-chemical properties have been determined[1]. We also isolated enterotoxin C_2 in a homogeneous form as judged by gel filtration, immunodiffusion, ultracentrifugation, and N-terminal amino acid analysis (to be published elsewhere). However, disc-gel electrophoresis showed 2 bands that formed an immunoprecipitate with specific

antiserum when overlayered with antiserum-agar. In this investigation we attempted to characterize further the heterogeneity of purified enterotoxin C_2.

Materials and methods. Enterotoxin C_2 was purified by a new method employing QAE-Sephadex (to be published) and subjected to disc-gel electrophoresis at pH 4.5. The gels were prepared according to REISFIELD et al.[2] except that the *Bis*/acrylamide monomer weight ratio was increased to 1:30 (3). Small pore gel concentration was varied from 3.7 to 10%, and the *Bis*/acrylamide ratio was kept constant by the method of HEDRICK and SMITH[3]. Enterotoxin C_2 was trace-labeled with ^{125}I using lactoperoxidase (CalBiochem) and H_2O_2 according to THORELL and JOHANSSON[4]. To remove remaining unreacted iodine and lactoperoxidase, the labelled toxin (10 µg) was filtered through a column of Sephadex G-100. Isoelectric focusing was performed in a LKB 8102/440 ml column[5]. When isoelectric points were determined in the presence of urea, water was replaced by 6 *M* urea-0.01 *M* dithiothreitol, which was passed through a mixed-bed ion-exchange resin before filling the column. A correction of 0.42 pH unit was applied to the apparent isoelectric points for the effect of 6 *M* urea on measured pH[6]. Immunological reactivity was determined by solid-phase radioimmunoassay[7,8].

Results and discussion. When electrophoresis was performed according to the method of HEDRICK and SMITH[3], a plot of the logarithm of the relative mobility

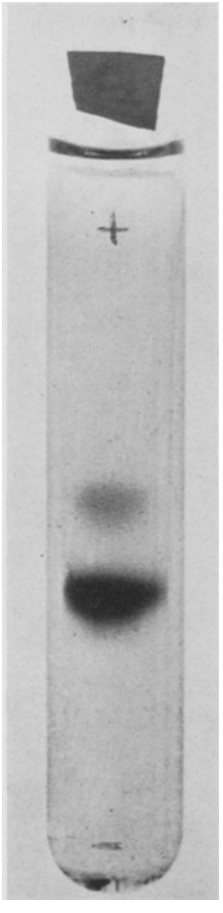


Fig. 1. Polyacrylamide gel electrophoresis of enterotoxin C_2. About 40 µg of toxin applied on 7.5% acrylamide in β-alanine-acetic acid buffer, pH 4.5.

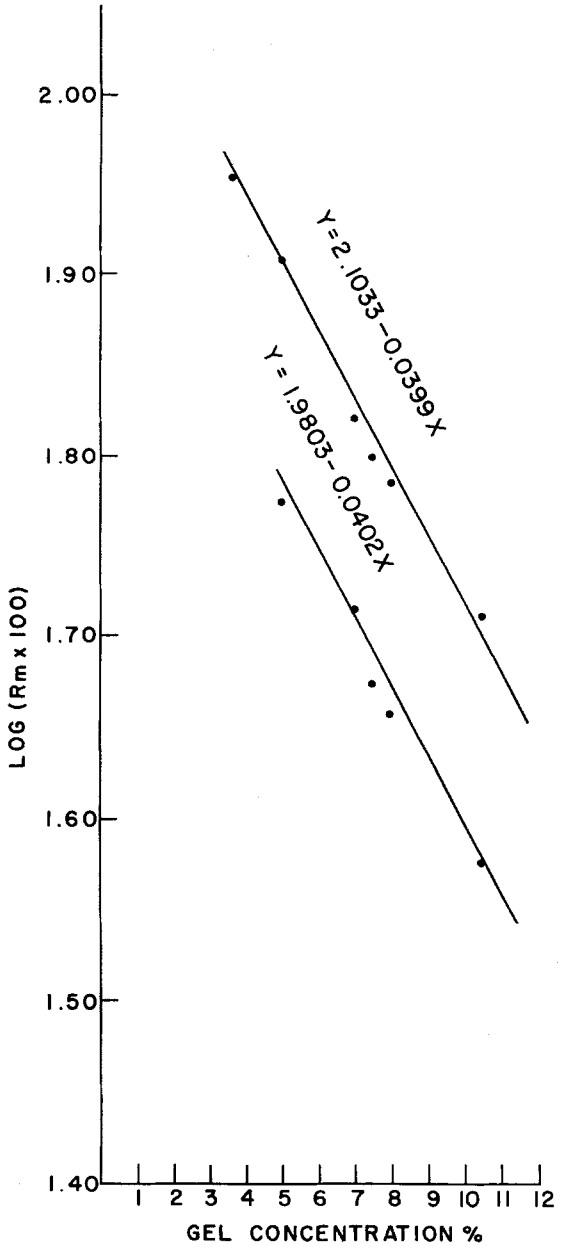


Fig. 2. The effect of different gel concentrations on the mobility of enterotoxin C_2 fractions.

[1] R. M. AVENA and M. S. BERGDOLL, Biochemistry *6*, 1474 (1967).
[2] R. A. REISFIELD, U. J. LEWIS and D. E. WILLIAMS, Nature, Lond. *195*, 281 (1962).
[3] J. L. HEDRICK and A. J. SMITH, Arch. Biochem. Biophys. *126*, 155 (1968).
[4] J. I. THORELL and B. G. JOHANSSON, Biochim. biophys. Acta *251*, 363 (1971).
[5] Instruction manual LKB 8100 Ampholine, LKB Produkter AB, Stockholm-Broma, Sweden.
[6] N. UI, Biochim. biophys. Acta *229*, 567 (1971).
[7] K. CATT and G. N. TREGEAR, Science *158*, 1970 (1967).
[8] N. DICKIE, Y. YANO, C. E. PARK, H. ROBERN and S. STAVRIC, Proceedings Staphylococci in Foods Conference, the Pennsylvania State University, University Park, Pa., March 18–20, 1973, p. 188.

(log R_m) versus gel concentration for the 2 bands of enterotoxin C_2 (Figure 1) gave parallel lines (Figure 2). The regression lines were calculated on a SCM Marchant desk calculator 1016 PR. Parallel lines show that the 2 bands have the same size and therefore exhibit the same retardation coefficient, but differ in charge[3].

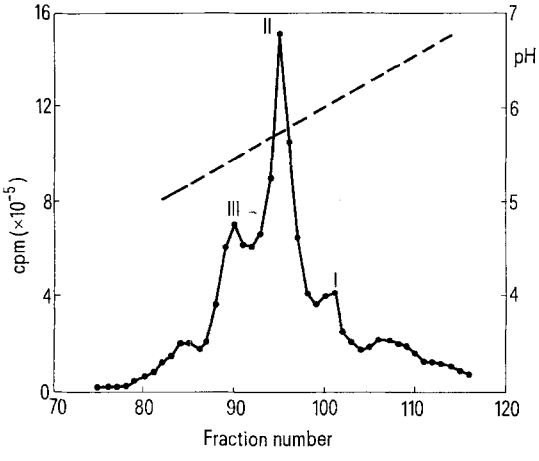


Fig. 3. Isoelectric focusing of ^{125}I-enterotoxin C_2 (46 μCi/μg; 0.6 atom/molecule) using LKB carrier ampholytes with isoelectric point values between pH 3 and 10. 140 3.0-ml fractions were collected and analyzed for radioactivity (●) in a γ-ray spectrometer, and for pH (broken line) at room temperature.

Binding of various fractions obtained by isoelectric focusing of ^{125}I-enterotoxin C_2 to enterotoxin C_2 antibody

Components	pI	Counts per min[a] added	Counts per min bound (%)
I	6.13	3,342	37.5
		8,719	28.5
II	5.73	15,658	15.7
		38.796	12.3
III	5.45	18,100	17.3
		38,370	13.1
Unfractionated		9,016	18.7
		33,480	10.7

[a] A portion from each of the fractions (I, II, III, of Figure 3) containing peak radioactivity was added to antibody-coated polystyrene tubes.

The heterogeneity of enterotoxin C_2 was corroborated by isoelectric focusing[9]. The toxin can be trace-labelled with radioactive iodine thereby providing for sensitive detection of enterotoxin in the electrofocusing pattern. As shown in Figure 3, a sharp peak focused at pH 5.73, among several other peaks in a pH range between 5.0 and 7.0. Each of the fractions tested showed good binding to solid-phase antibody, as compared with unfractionated enterotoxin C_2 (Table). Also, each isoelectric component of enterotoxin C_2 (peaks I, II, and III shown in Figure 3) was chromatographed in a Sephadex G-75 column, and eluted with the same K_{av} (0.37) as unfractionated enterotoxin C_2, indicating that enterotoxin C_2 had not formed stable aggregates or immunologically active fragments during electrofocusing. In 6 *M* urea-0.01 *M* dithiothreitol an essentially similar isoelectric spectrum was obtained (figure not shown). When a correction[6] was made for the effect of 6 *M* urea on measured pH, no variation in isoelectric point due to the presence of 6 *M* urea was observed with labelled toxin. Therefore, the isoelectric heterogeneity of enterotoxin C_2 does not result from conformation-dependent variations in the dissociation of acidic and basic side chains of the protein molecule. It seems likely that these differences occur due to loss of particular amide groups in the protein as has been demonstrated for enterotoxin B (note cited in[10,11]) and C_1 (L. Spero, private communication cited in[12]). See also Williamson et al.[13].

Résumé. L'hétérogénéité de l'entérotoxine C_2 d'un staphylocoque par électrophorèse en gel polyacrylamide est reliée à la présence d'isomers de charge différente.

S. Stavric, H. Robern and N. Dickie[14]

Food Directorate, Health Protection Branch, Department of Health and Welfare, Tunney's Pasture, Ottawa (Ontario K1A OL2, Canada), 2 September 1974.

[9] O. Vesterberg and H. Svensson, Acta chem. scand. *20*, 829 (1966).
[10] L. Spero, J. R. Warren and J. F. Metzger, J. biol. Chem. *248*, 7289 (1973).
[11] L. Spero, J. R. Warren and J. F. Metzger, Biochim. biophys. Acta *336*, 79 (1974).
[12] M. S. Bergdoll and R. N. Robbins, J. Milk Food Technol. *36*, 610 (1973).
[13] A. R. Williamson, M. R. Salaman and H. W. Kreth, Ann. N.Y. Acad. Sci. *209*, 210 (1973).
[14] Acknowledgments. We thank Debbie Jeffrey, Yukio Yano and Robert Gagné for excellent technical assistance.

Studies on the Adaptive Nature of Cellulolytic Enzyme from *Chaetomium aureum* Chivers

A few microorganisms[1,2] have been reported to produce cellulolytic enzyme adaptively; but information on the nature of cellulase produced by *Chaetomium* spp. is still lacking. *Chaetomium aureum* was therefore tested for the nature of cellulase it produces with a view to exploring the possibility of using the cellulolytic character as a marker in genetic investigations[3]. The culture medium used in the experiment was modified Czapek-Dox[4] adjusted to pH 6.5 before sterilization. Sterilization was done at 10 lbs pressure for 20 min. Glucose, cellulose and combination of cellulose and glucose, in the proportion of 1.5% each, were used as the carbon source. The medium having only the inorganic chemicals was considered as the control medium. Determination of cellulase activity was done qualitatively by depth of clearing[5] and quantitatively by estimation of reducing sugars[6–8]. For qualitative test stab was made with 10 ml of solid medium having 1.5% purified agar[9] in each culture