crystals were found in the fibroblasts of the valves. Definite virus-like particles and valvular cell damage have been previously demonstrated by electron microscopy in mice infected with Coxsackie B_4 virus[10]. These studies show viruses to be pathogenic for the valves of the mouse and indicate further the possible role of viruses in the production of valvular heart disease in man.

Résumé. Un cristal du virus EMC fut trouvé dans le fibroblaste valvulaire d'une souris atteinte de la maladie provoquée par ce virus. La description de ce cristal et des changements pathologiques corrélatifs survenus dans le tissu valvulaire ont établis que ce virus est au moins capable d'envahir des valvules du cœur et de produire la maladie valvulaire.

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Enzymatic and Biological Studies of Cholera (*Vibrio cholerae*) Toxin

It has been well recognized that death due to cholera (*Vibrio cholerae* or *V. comma*) infection is due to the loss of enormous amounts of fluid and electrolytes. A recent study indicates, that pathogenesis is caused by the toxin produced by the bacterium and not by the organism itself[1–5]. Choleragen was isolated from cholera toxin and found to be responsible for choleraic diarrhea in infant rabbits[1], and in humans[6]. Cholera toxin is a mixture of different proteins of which identity and function have not been well investigated. It is, therefore, important to know not only the choleragen present in the toxin but also other components which may contribute to the pathogenic effect in cholera infection.

In this study the investigation of cholera toxin other than choleragen was made. This report will describe the presence or absence of certain enzymes, hemolytic activity, and capillary tube permeability activity.

Cholera toxin was prepared by the method of FINKELSTEIN et al.[7] at the Merck, Sharp and Dohme Company. The lyophilized toxin was generously supplied by Dr. JOHN R. SEAL, of the National Institute of Allergy and Infectious Diseases, National Institutes of Health. Before the investigation was made, the absence of intact bacterium was confirmed by inoculating the cholera toxin on agar plates.

Hyaluronidase activity was followed by measuring the decrease in turbidity of a hyaluronic acid-protein complex[8]. Acetylocholine esterase activity was assayed by following the hydrolysis of indophenyl acetate as described by KRAMER and GRAMSON[9]. Phosphodiesterase activity was spectrophotometrically measured by following the hydrolysis of the substrate Ca-*bis*-(*p*-nitrophenyl)-phosphate[10]. Proteolytic activity using casein as the substrate was carried out following the method of KUNITZ[11].

For the coagulation test, 90% clottable fibrinogen was dissolved in 10 ml of 0.001 *M* phosphate, 0.8% NaCl at pH 6.2, 0.5 ml of clear supernatant solution after centrifugation was incubated with 0.5 ml toxin solution (10 mg/ml). Hydrolyses of N-benzoyl-L-arginine ethyl ester, *p*-toluene-sulfonyl-L-arginine methyl ester, and acetyl-L-tyrosine ethyl ester were carried out as described previously[12]. Leucine aminopeptidase activity was determined by a modified method of GOLDBARG and RUTTENBURG[13]. Acid and alkaline phosphatase activities were determined spectrophotometrically on the substrate *p*-nitrophenyl phosphate and *o*-carboxyphenyl phosphate[14]. Phospholipase A was measured by titrating released fatty acids from lecithin[15].

For the hemolytic assay, rabbit erythrocytes were washed first with physiological saline solution then followed by 2, 4, 6-trimethyl pyridine containing 0.005 *M*

Enzyme or biological activities	Substrate	Presence or absence of activities
Hyaluronidase	Hyaluronic acid	+
DNase	DNA	+
Acetylcholinesterase	Indophenyl acetate	+
Phosphodiesterase	Ca-*bis*-(*p*-nitrophenyl)-phosphate	+
Proteolytic enzyme	Casein	+
Coagulation	Fibrinogen	+
Amino acid esterase	N-benzoyl-L-arginine ethyl ester	—
	p-toluenesulfonyl-L-arginine methyl ester	—
	Acetyl-L-tyrosine ethyl ester	—
Leucine amino peptidase	L-luecyl-β-naphthylamide	—
Acid phosphatase	*o*-carboxyphenyl phosphate	—
Alkaline phosphatase	*p*-nitrophenyl phosphate *o*-carboxyphenyl phosphate	—
RNase	RNA	—
Phospholipase A	Lecithin	—
Hemolysis		—
Capillary permeability		+

+, presence of enzyme or biological activity; —, absence of activities.

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$CaCl_2$ and 0.012 *M* NaCl at pH 6.5. The erythrocyte suspension in 0.5 ml of the barbiturate buffer at pH 7 was mixed with 0.5 ml cholera toxin (10 mg/ml) for 45 min at 37 °C. The incubation mixture was centrifuged and the supernatant liquid was read at 410 nm for possible release of hemoglobin.

For the capillary permeability experiment, 0.25 ml Evans Blue was injected into the tail vein of a mouse. 0.05 ml toxin (30 mg/ml) in saline solution was injected s.c., immediately after the injection of Evans Blue. The skin was removed 6 h later and the stained area under the skin was examined. The control animal without the injection of toxin was also made for comparison.

The results of the investigation are summarized in the Table. The toxin contains hyaluronidase, DNase, acetylocholinesterase, phosphodiesterase, proteolytic enzyme, and coagulation promoting enzyme. No amino acid esterase activity was detected using 3 different substrates. Since the above compounds are common substrates for the assay of trypsin and chymotrypsin, the proteolytic enzymes present in the cholera toxin are different from trypsin and chymotrypsin. The fibrinogen coagulated in 10 min as compared to 1 min for 0.5 ml thrombin (1 mg/ml). The toxin converted fibrinogen into a fibrin like clot. However, the enzyme responsible for coagulation is not identical to thrombin as the *p*-toluenesulfonyl-L-arginine methyl ester was not hydrolyzed by the toxin.

The toxin does not contain leucine aminopeptidase, acid phosphatase, alkaline phosphatase, RNase, or phospholipase A. The toxin lacks the ability to hemolyze erythrocytes. However, the toxin contains potent capillary permeability promoting factors[16].

Zusammenfassung. Untersuchung der Enterotoxine von *Vibrio cholerae* auf eine Reihe von Enzymaktivitäten und im Hinblick auf die Aufklärung des pathogenetischen Mechanismus.

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[16] Acknowledgment. This work was supported by a NIH Career Development Award No. 1 K04 GM 41768 to A. T. Tu.

[17] We wish to thank Dr. J. Ogg, Department of Microbiology, for the advice we received and Mr. Ronald O'Quinn for technical assistance.

Effect of Insect Hormones on Nematodes in Axenic Culture

Insect juvenile hormones (JH) or JH mimetics have been shown to affect development of nematodes: *Trichinella spiralis* larvae[1] and fourth stage *Phocanema decipiens*[2] were inhibited, and abnormal morphology was seen in *Heterodera schactii*[3]. The effects of insect hormones and analogues on development of several free-living and parasitic nematodes cultured axenically are described in the present paper.

Methods were designed to expose nematodes to hormones in a growth-supporting medium. To grow the parasitic nematodes *Aphelenchus avenae* and *Strongyloides fülleborni*, chemically defined basal medium CbMM[4] supplemented with 25% fresh chick embryo extract plus 10% human serum was used; CbMM containing hemin and supplemented with γ-globulin[5] was used to culture other species. Juvenile hormones and their analogues, 3,4-methylenedioxyphenyl 6,7-epoxygeranyl ether and ethyl 6,7,10,11-tetrahydrofarnesoate, were added from ethereal solution to aseptically lyophilized γ-globulin or chick embryo extract. After evaporating the ether at room temperature, the lyophilized fraction was suspended in water and the other components of the medium added. Pronasterone A was added in ethanol and evaporated aseptically with nitrogen; ecdysone was added in water. Ether, or alcohol-treated γ-globulin, or ether treated chick embryo extract, added to other components of the medium without added hormones served as controls. Nematodes were inoculated and observed throughout their life cycles for possible hormone induced effects. Species examined included the free-living *Caenorhabditis briggsae* and *Panagrellus redivivus*, the insect parasites *Neoaplectana glaseri* and *N. carpocapsae* DD136, the plant parasite *Aphelenchus avenae* and the animal parasite *Strongyloides fülleborni*, stercoral phase.

The characteristics observed to determine the effects of insect hormones on nematode development are listed in Table I. While Pronasterone A and ecdysone had no effect, compounds with JH activity had marked effect. They caused a delay in *C. briggsae* maturation which was proportional to the hormone concentration in the growth medium; at 200 µg/ml, maturation was prevented and inhibitory effects were seen to a dilution of 50 µg/ml. With *N. carpocapsae* DD136, there was marked toxicity to young larvae during a holding period of 24 h in γ-globulin supplemented medium with or without hemin.

The relative activities of some of the JH type compounds tested, using percent survivors of first stage *N. carpocapsae* larvae as the assay, are shown in Table II. For each hormone level, duplicate tubes containing 0.20 ml of CbMM with hemin and supplemented with γ-globulin plus hormone were inoculated with 5 larvae

Table I. The effects of insect hormone type compounds on nematode development

Characteristic	Species Affected	Not affected
Egg laying	–	*C. briggsae* *A. avenae* *N. carpocapsae* DD136
Egg hatch	–	*C. briggsae* *A. avenae*
Viability	*N. carpocapsae* DD136	*C. briggsae* *P. redivivus* *S. fulleborni*
Exsheathment	–	*N. carpocapsae* DD136 *N. glaseri*
Sex development changes	–	*A. avenae*
Maturation time and population	*C. briggsae*	*A. avenae*

Nematodes were inoculated into 0.20 ml of medium in duplicate tubes and incubated at their optimum growth temperature. For *Aphelenchus avenae* and *Strongyloides fulleborni*, the growth medium was CbMM supplemented with 25% fresh chick embryo extract and 10% human serum; for the other nematodes, CbMM was supplemented with γ-globulin and hemin. Hormones were added to aseptically lyophilized γ-globulin or chick embryo extract. Periodic observations of the specific characteristics were made.