$CaCl_2$ 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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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.

each and incubated at 20 °C overnight. The mortality of the nematodes was observed using an inverted Leitz microscope at 60 × magnification. The MEYER/SCHNEIDERMAN JH[6] was more active than the RÖLLER JH[7]. A synthetic analogue was active while a synthetic compound lacking the important epoxide group was relatively inactive. These JH type compounds had the same relative activities when tested using inhibition of *C. briggsae* as the assay system.

We conclude that the JH type compounds affect the growth and development of nematodes in axenic culture, and can be compared by toxicity to *N. carpocapsae* or by inhibition of development of *C. briggsae*. No effect on specific developmental stages, moulting, or sex development was detected[9,10].

Table II. Toxicity of juvenile hormone (JH) type compounds to first stage larvae of *Neoaplectana carpocapsae* DD136

JH-type compound (μg/ml)	Survivors at 24 h (%)					
	400	200	100	50	20	0
RÖLLER	67	72	75	72	88	100
MEYER/SCHNEIDERMAN	0	0	0	11	64	100
Synthetic[a]	0	0	0	6	93	100
Compound lacking epoxide[b]	89	–	84	100	100	100

[a] 3,4-methylenedioxyphenyl 6,7-epoxygeranyl ether[8]. [b] Ethyl 6,7, 10,11-tetrahydrofarnesoate. For each hormone level, 5 larvae were inoculated into duplicate tubes containing 0.20 ml of CbMM with hemin and supplemented with hormone treated γ-globulin. Control medium (0 μg/ml JH) was supplemented with ether treated γ-globulin. After 24 h incubation at 20 °C nematodes were examined for viability.

Zusammenfassung. Insektenjuvenilhormone und -analoge wurden an verschiedenen freilebenden und parasitischen Nematoden getestet. Bei *Neoaplectana carpocapsae* wurden toxische Effekte und bei *Caenorhabditis briggsiae* Reifungshemmung festgestellt.

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AD STIMULANDUM

Phenylacetic Acid as a Potential Therapeutic Agent for the Treatment of Human Cancer

In the summary of a previous work, ROBERTS and SIMONSEN[1] remarked that apparently 'tumor cells are operating at a marginal level of glutamine availability by comparison with other tissue'. They believed that glutamine antagonists ought to have anti-tumour activity but found that competitive inhibitors such as γ-L-glutamylhydrazide were not particularly successful as tumour inhibitors. They concluded that 'in order to be able to employ glutamine antagonists in a maximally effective manner it will be necessary to direct attention to methods of decreasing formation and liberation of glutamine into the circulation by normal tissues as well as to disturbing its uptake and utilization in the tumor cells themselves'.

It is now suggested that a simple method for depleting the glutamine reserves of cancer patients might be based on the ability of humans to detoxify phenylacetic acid by conjugation with glutamine. This detoxication reaction which results in the excretion of phenylacetylglutamine in the urine was first detected by THIERFELDER and SHERWIN[2] and later studied in more detail by SHERWIN et al.[3]. POWER[4] found that the same conjugation reaction occurred in the chimpanzee but other mammalian species excreted the acid as a glycine conjugate and birds detoxified phenylacetic acid by conjugation with ornithine[5].

Since phenylacetic acid is not markedly toxic for humans it is considered that this material merits investigation as an anti-tumour agent by itself or in conjunction with glutamine antimetabolites or cancer-chemotherapeutic drugs of other types. Because of the unique mode of detoxication of phenylacetic acid in the human it is expected that there will be no parallel between the results of chemotherapeutic experiments with this acid in tumour-bearing rodents and the outcome of clinical trials in the human cancer patient.

Although the mode of detoxication of phenylacetic acid in the rat has apparently not been investigated in vivo, MOLDAVE and MEISTER[6] found that whereas human liver slices can synthesise radioactive phenylacetylglutamine from [^{14}C]-L-glutamine rat liver slices fail to do so. These experiments suggest that the rat will be unable to conjugate phenylacetic acid with glutamine in vivo. Thus if glutamine deprivation is important for tumour inhibition, phenylacetic acid therapy is likely to have little or no effect on the growth of transplantable rat tumours.

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