Formosan Entomologist

Journal Homepage: entsocjournal.yabee.com.tw

【Research report】

羧基酯[酉每]在家蠅體上之誘發研究【研究報告】

馬堪津

*通訊作者E-mail :

Received: Accepted: 1987/02/18 Available online: 1987/03/01

Abstract

摘要

本文旨在證實羧基酯[酉每]每可以在 Rutgers 家蠅品系體上被誘發。誘發劑為一種類似昆蟲青春激素的合成品，Hydroprene。將 Hydroprene 以表皮處理法施於家蠅成蟲體表，由奈乙酸酯[酉每]被水解之多寡，可知羧基酯[酉每]確實可以被誘發。並發現在羽化後六日之雌成蟲體上可得到最高之羧基酯[酉每]活性，故此誘發作用與試驗成蟲之蟲齡有關。此外，本實驗也以一種可以 抑制核 酸轉錄機制的抗生素，Actinomycin D 來反證家蠅成蟲經處理後新增之羧基酯[酉每]活性確實是由新合成之蛋白所致。以生化及電泳法來測定酯[酉每]之異構在膠柱中之分佈，發現絕大部分 Hydroprene 酯[酉每]皆分佈於陰極處，可惜回收實驗的活性太小，不能就此證實此項誘發實驗所得之酯[酉每]有其特殊之專一性。

Key words:

關鍵詞:

Full Text: PDF(0.58 MB)

下載其它卷期全文 Browse all articles in archive: http://entsocjournal.yabee.com.tw

中華昆蟲 7: 49-58 (1987)
Chinese J. Entomol. 7: 49-58 (1987)

HYDROPRENE INDUCTION OF CARBOXYLESTERASE ON HOUSE FLY (*MUSCA DOMESTICA*)

Maa, Can-Jen William

Institute of Zoology, Academia Sinica
11529, Taipei, Taiwan, Rep. of China

(Accepted February 18, 1987)

Abstract

Profile study on carboxylesterase induction in Rugters house fly, *M. domestica*, was investigated. Hydroprene, a juvenile hormone analog, was topically treated on the insect. The enzymatic activity, monitored with naphthylacetate, revealed that the induction was successful in the 6-day-old femle adult flies. Induction was evidently an age-dependent mechanism. Actinomycin D was used to justify the increasing enzyme activity was caused by new protein synthesis through transcriptional level. The induced isozymes were further characterized electrophoretically and biochemically. The hydrolytic activity to hydroprene was mostly distributed in area at cathode end of the gel. However, the induced specific esterase was unable to be certified because of the low recovery of enzyme activity from the acrylamide gel. Significance of the induction was discussed.

Introduction

Metabolism of insect growth regulators' ester by esterase has been intensively studied in many insect species including: *Musca domestica, Phormia regina, Sarcophaga bulluta, Culex pipiens pipiens, Aedes aegypti, Munduca sexta, Hyalophora ceroia, P. brunmea, Schistocerca vage, Samia cynthis, Tenebrio moltor, Blaberus giganteus,* and *Solennopsis invicta* (Yu and Terriere, 1973, 1975, 1977a, 1977b; Maa and Terriere, 1983a, 1983b; Terriere and Yu, 1973; Quistad *et al.*, 1975; Weirich and Wren, 1973; Georghiou *et al.*, 1978; Mumby *et al.*, 1979; Bigley and Vinson, 1979).

Hydroprene, an ethyl ester, was metabolized by both soluble and microsomal esterase of dipteran species in the order of house fly, flesh fly, blow fly, with the susceptible housefly being most active (Yu and Terriere, 1977a). The level of juvenile hormone (JH) hydrolytic activity in the hemolymph or in the microsomal fraction of the dipteran insects is totally age- or stage-dependent (Yu and Terriere, 1975). In *Drosophila hydei* hemolymph, this enzyme was found only in the prepupal stage (Klages and Emmerich, 1979). In lepidopterous insects the highest hemolymph JH esterase (JHEase) activity was restricted to the last instar larvae (Slade and Wilkinson, 1974; Sanburg *et al.*, 1975). In Colorado beetle, JHEase occurred during all the stages tested but showed characteristics change in activity during development (Kramer and De Kort, 1976). Quistad *et al.* (1975) found that in *Aedes aegypti* and *Culex pipiens quinque-fasiatus* larvae the methoprene ester is cleaved to give hydroxy acid. Hooper (1976), however, found very low esterase activity to JH analogues in *Culex pipiens* larvae. The estimated esterase activity to naphthylacetate

(NA) was high in the mitochoriion fraction, but was low in the soluble and microsomal fraction.

As to the reaction of these enzymes with inhibitors, Quistad *et al.* (1975) indicated that triorthocresyl phosphate decreased the propertion of methoprene esterases present in the larvae. Yu and Terriere (1977a) found that diisopropylfluorophasphate inhibited microsomal esterase activity in flesh fly and blow fly, but not in the house fly. Paraoxon of 10^{-5} M had low inhibitory effect on house fly microsomal esterase, but inhibited these esterases in blow fly and flesh fly. Maa and Terriere (1983a, 1983b) demonstrated that the enzyme system metabolizing the JH analogue was possibly composed of several esterase, each with a somewhat different substrate preference. It was also indicated that the soluble JHEase, or the general NA esterase activity was age-dependent in house fly and other fly species as well, In this report the author provided some experimental evidences to illustrate how the hydroprene esterase in a homeostatic condition would respond to the hydroprene treatment as a mechanism of enzyme induction in housefly.

Materials and Methods

Insect

The house fly used was Rugters, an insecticide resistant strain. It is resistant to diazinon, an organophosphate compound and propoxur, a carbamate compound. The resistance is due to a considerable extent to the increased metabolism of these insecticides by the microsomal oxidase system (Terriere and Yu, 1976).

The flies were reared under constant temperature 24±1°C and a light: dark condition (16:8). House fly larvae were reared on an alfalfa meal-wheat barn mixture (1:3) and adults were fed a mixture of powdered milk, sugar, and powdered egg yolk (12:12:1). The species reproduced normally under these rearing conditions. Stocks of newly emerged adults of each generation were synchronized with twelve hours for experiments. Adults were used at various ages depending on the necessity.

Chemicals

All chemicals and reagents were of analytical grade or the best grade available. Cold and hot hydroprène (ethyl (2E, 4E)-3, 7, 11-trimethyl-2, 4-dodecadienoate 0, 5-(^{14}C), specific activity 5-8 mCi/mM) containing 98.4 percent trans, trans and essentially no cis, trans form, was from Zoecon Corporation, USA. Chemicals for Bray's counting solution were purchased from New England Nuclear Corporation, USA. Silica gel G-1, 2-naphthol, 2-naphthalene acetate, laryl sulfate (sodium laryl sulfate, dodecyl sodium sulfate, approximately 95%), diazoblue (*O*-dianisidine, tetrazotized $ZnCl_2$ complex, practical grade 30% pure), phenylthiourea, eserine (physotigmine) para-benzyate mercuribenzate (PHMB, crystalline sodium salt), Actinomycin D, coomassie brilliant blue G-250, were all purchased from Sigma Corporation, USA. Acrylamide, bis acrylamide, *N, N, N, N*-tetramethylenediamine, riboflavin, ammonium persulfate, bromophenol blue, all electrophoresis purity grade were purchased from Bio-Rad Laboratories, USA.

Enzyme source for assay

The hemolymph samples were withdrawn from female adult flies of various ages according to Maa and Terriere (1983a). At least 100 μl hemolymph was pooled for each sample was then centrifuged into 1,000 g for 15 min. The suppernatant was withdrawn and the upper lipid layer and the bottom precipitate were discarded. In this study the hemolymph sample was used immediately as soon as enough samples were collected. The

soluble fraction of decapted flies were obtained according to Yu and Terriere (1971). The suppernatant above the 100,000 g pellet, after centrifugation, contained an unknown quantity of hemolymph, was obtained from homogenates of insects from which a hemolymph sample had been taken. There was no attempt to remove remaining hemolymph prior to the homogenization of the insect. Preliminary assay on these two tissue fractions showed that about equal titer of esterase activity to hydroprene were present in both fractions.

Protein content determinaton and esterase assay

Both the determinations were performed as described by Maa and Terriere (1983a) according to Bradford (1976) and Van Asperen (1962). The inhibitor used for characterization of esterase were: eserine, to inhibit the choline esterases, and PHMB, to inhibit the arylesterases. The concentration of inhibition assay was determined to be 10^{-4} M. When the inhibitors were used they were incubated with the enzyme sample (hemolymph, soluble fraction, or enzyme source recovered from gels) for 30 min. prior to the addition of naphthylacetate.

The concentration of the labeled hydroprene-^{14}C compound was with 100,000 cpm per ml. The unlabeled chemical was used for the determination of proper concentration for the esterase assay. Incubation time was 3 hrs for hydroprene, 30 min. for naphthylacetate. At the completion of hydroprene incubation, 2.0 ml of distilled water and 1$\frac{1}{2}$ ml mixture of ether-ethanol were added to the mixture. After shaking and centrifuging, the solvent phase was removed. The extraction was repeated twice ether-ethanol mixture. This procedure recovered all of the added radioactivity. The extracts were combined and dried[5] over sodium sulfate. Aliquots were then evaprated to approximately 0.25 ml and applied on silica gel G thin layer chromatoplates ($0.25\,mm \times 5 \times 20\,cm^2$). The chromatoplates were developed unidimensionally with hexane-ethyl acetate (100:15), then with benzene-ethyl acetate-acetic acid (100:30:3) (Quistad *et al.*, 1975). The radioactive metabolites and unreacted substrates were detected by passing the plates under a radioactive chromatogram scanner. The zones of radioactivity were scraped from the plates, placed in scintillation vials with 10.0 ml of scintillation mixture and counted. Recovery of added radioactivity carried through the complete procedure was more than 75%.

Enzyme induction

Groups of 50 house flies of 4 to 10 day-old were anethetized with CO_2 and topically treated with hydroprene in 1.0 μl acetone. The microdrops were applied to the tip of of the abdomen. Controls were treated with 1.0 μl acetone. The flies were then released into the cages with food and water provided. Doses of hydroprene were 10.0, 5.0, or 2.5 μg per fly. The hemolymph and the soluble fractions were collected from the treated and control flies every twelve hours.

When actinomycin D was used to characterize the induction, the flies were anesthetized with CO_2 and injected into the thorax through the scutellar suture with 1 μl of an aqueous solution containing 0.1 μg of the compound. Controls were injected with 1 μl distilled water. Neither treatment caused mortality in flies.

Electrophoretic procedures and esterase activity recovery

The disc electrophoretic procedure of Davis (1964) was used. The electrophoresis was carried on for 4 hrs at 4°C. All the procedures were at low temperature except the staining. This was modified from Ahmad (1974). The zymogram of the esterase was examined two days after the two changes of 7% acetic aqueous solution. When it is necessary for quantitation, the gel was scanned in a photovolt densitometer.

Recovery of the enzyme from the gels was also done in the cold. The procedure was modified from Whitmore *et al.* (1972). The gel was stained with naphthylacetate for 5 min. to locate the peak area. The appropriate gel sections were removed, chopped into fine pieces for diffusion at 0°C with 2.0 ml of distilled water. After 40 min. for protein diffusion, the chopped pieces with the aliquate was centrifuged to 1,000 g for 15 min. The supernatants were used for protein determination and enzymatic assay. In most cases, four major sections of the gels were taken for esterase characterization with hydroprene as substrate. The soluble fraction as well as the hemolymph, with one fly equivalent for Rugters strain, was used for each electrophoretic gel. The method for esterolytic assay was from Yu and Terriere (1975).

Results

Age-profile of hydroprene induction of esterase

Naphthylacetate was used as substrate for monitoring the activity of carboxylesterase induced by hydroprene treatment on adult females of various age. This experiment was carried on in order to narrow down the proper gate of adult age for induction. Fig. 1 shows that a biphasic status of both induction and depression was observed. Somehow, the depression was a minor phase and was found only at 4-day- and 7-day-old flies. It was estimated that the average of depression of esterase caused by hydroprene treatment on fly was approximately 7%. It is comparatively in significant to the induction of the esterase.

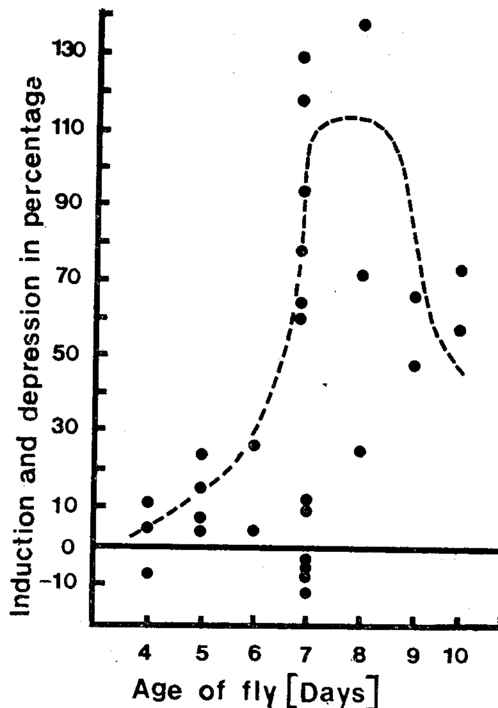

Fig. 1. Age-profile of hydroprene induction of soluble carboxylesterase of female Rugters house fly. Naphthyl acetate was used as the substrate for carboxylesterase assay in vitro; each dot represented sample size of 50 flies; the zero reading was a calibration of control.

Enzyme induction was initiated with a lower level at 4-day-old female. It climbed up gradually from 110% of the control during days 5 and 6 to its maximum of 230% at days 7 and 8, and declined down to about 140% at days 9 and 10 afterward. It seems that the 6-day-old flies expressed with high enzyme regulation mechanism to hydroprene induction. It is interesting to know that in normal condition, the Rugters fly has a maximum esterase activity at day 8 after adult eclosion (Maa and Terriere, 1983b).

Apparently, hydroprene induction of esterase in this fly strain is age-dependent. An enzyme assay with hydroprene as substrate revealed that the total esterolytic activity was at its maximum of 238% as the control counterpart 36 hrs after treatment. The induction potential dropped down to 150 to 180% the second day after the treatment on the 6-day-old flies (data not shown).

Induction of esterase through a transcription level

In this experiment hydroprene was used as substrate and the hydroprene esterase was induced as a regulated enzymes. Actinomycin D was used to block the protein synthesis. The inhibition effect of actinomycin D was checked at 36, 48, and 72 hrs after treatment. Table 1 shows that flies treated with the inhibitor and hydroprene were with less esterase activity than those treated with the inhibitor and acetone. This revealed that hydroprene esterases were induced to 27% of net increase of activity by 36 hrs after treatment, 72% by 48 hrs, and 65% by 72 hrs. The low esterase induction at 36 hr (data not shown) was possibly associated with the injury by injection. Conclusively, the induced esterase was at least specifically, although only partially, responded to hydroprene.

Electrophoresis and enzyme recovery

Enzyme source taken from hydroprene-treated and control flies were applied to the gels for isozyme separation. After the run, the gels were stained for isozyme and were scanned with a densitometer. The isozymes were visiously sorted into 8 peak areas

Table 1. Actinomycin D inhibition of hydroprene esterase of Rugters house flies[a]

Treatments[2]	Duration of treatment (hr)	*p* mols hydroprene acid/mg protein/ 3 hr	Enzyme activity % of controls	
			Induction	inhibition
H_2O 1.0 μl				
Acetone 1.0 μl	48	295.1±13.94	172.0	
	72	272.9±61.61	164.8	
H_2O 1.0 μl				
Hydroprene 5 μg	48	507.5±65.31		
	72	448.1±29.53		
Act. D. 0.1 μg				
Acetone 1.0 μl	48	346.4±72.78		100
	72	442.1±74.72		100
Act. D. 0.1 μg				
Hydroprene 5 μg	48	260.8±85.28		
	72	392.7±13.35		

[a] 1. Average of duplicates, with 6.0-day-old female flies.
2. Aqueous Actinomycin D given by injection with water alone as control; hydroprene given by topical treatment with acetone alone as control.

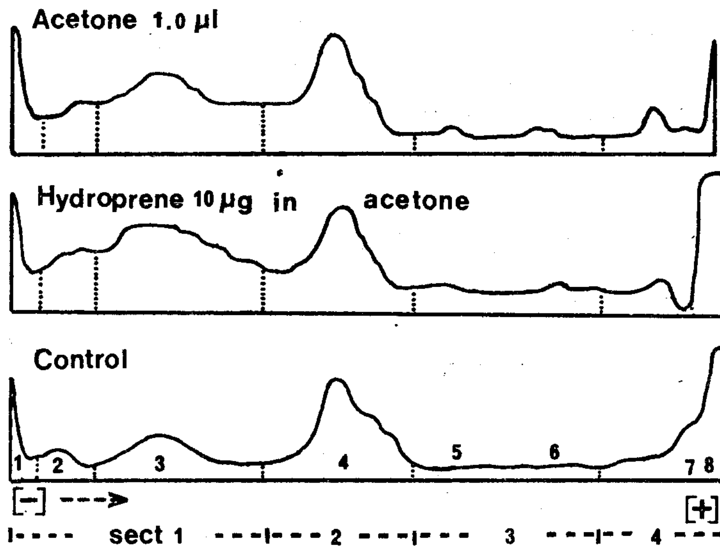

Fig. 2. Densitometric scans of electropherograms showing changes of carboxylesterase zymogram with treatment or no treatment on Rugters flies. Eserine and PHMB were used for preincubation; 8.0 μl haemolymph for sample of the acetone treated larvae, 7.0 μl for that of hydroprene treated ones, and 4.0 μl for that of control; arrow indicating the movibility of the isozyme from anode to cathode end of the gel.

according to their mobility along the gel. These peak areas were furtherly grouped into four major sections for recovery study (Fig. 2). The electrophoregram paper of the stained gel with corresponding peak area, or section were cut off. Each peak area or section of the graph paper was weighed. The weights of the electrophoregram paper was used for monitoring the hydrolytic activity of naphthylacetate of the corresponding isozymes.

The densitometric electrophoregrams were shown in Fig. 2. Each of them represents zymograms of esterases from normal adults, adults treated with acetone and adults treated with acetone and adults treated with hydroprene, respectively. The induction ratio based on paper weight was calculated.

Table 2 shows that peak area 2, 7 and 8 were with increased weight in hydroprene-treated

Table 2. Estimation of hydroprene induction of carboxylesterase isozymes by densitometric electrophoregram[a]

Peak area for isozymes	Enzyme activity (weight of graph paper) mg/10.0 gel		Enzyme induction in percentage
	Hydroprene treated	Acetone trested	
1	1.65±NC	2.33±NC[2]	70(−30)
2	5.12±NC	3.13±NC	163(+63)
3	16.37±1.76	13.43±1.38	122(+22)
4	11.30±0.07	11.75±0.09	96(−4)
5+6	7.37±NC	4.67±NC	158(+58)
7+8	4.75±NC	1.94±NC	245(+145)

[a] 1. Average of duplicates; Naphthylacetate and DBLS for staining, with 10^{-4}M of eserine and PHMB for preincubation; Isozyme distribution, see Fig. 2.

2. NC; wieght of the paper variation was too light to be counted.

larvae. Peak area 1 was with decreasing weight to 70% of its original. There was not much change being found in peak area of others. It shows that the second peak area and the combination peak areas of 5 and 6 were with a net increase by 60%. The induction was most obvious with peak area at cathode end of the gel. The enhancement was approximately two and half folds as the control counterpart. These results hinted that the net increase of esterolytic activity to hydroprene found in previous assay was likely to be specific.

Induction causing the maximum weight gained at the isozyme of section 4; peak area of 7 and 8 was to be justified by an additional experiment in order to certify whether the isozyme is hydroprene-specific.

The recovery study was unsuccessful because of the low recovery of hydroprene esterase activity to hydroprene. The protein content recovered from the diffusion of the slided gel was somehow satisfactory; approximately 73% of the total. Nevertheless, the recoved activity was found being mostly distributed in the section 4 of the gel. The recovered titer of the esterase was either low or undetectable in the other sections. This result would support the assumption on a hydroprene-specific induction on the fly if the practical problem of low recovery was neglected.

Discussion

As Adams and Edie (1972) indicated, hydroprene has a gonotrophic effect on house fly ovaries inducing vitellogenesis and a switch-over in the sensitivity of the prothoracic gland to externally applied JH and its analogues (Cymborowski and Stolary, 1979). Therefore, it is reasonable to expect that hydroprene may act as JH for JH-ase induction if JH regulation is involved in a feedback mechanism (Sparks and Hammock, 1979; Whitmore *et al.*, 1974; Yu and Terriere, and Terriere, 1971, 1973). However, it was also noted that not all the stages of an insect had the same response to the same inducer. Whitmore *et al.* (1972) could only induce JH-ase in *Hyalophora* pupae and Kramer (1978) were able to induce the hormone hydrolysis enzyme only in *Leptinotarsa* diapausing adult. Similarly, Sparks and Hammock (1979) could induce the hormone esterase activity in *Trichoplusia* larvae and Reddy *et al.* (1979) found that JH reduced esterase and NA esterase activity in larvae, but induced the enzyme in one day-old pupae.

On the other hand, Mumby *et al.* (1979) found that carbamates had a low JH-like activity on dipteran insect, and had an inhibitory effect on house fly JH-ases. This group of *N*-alkyl carbamates of 1-naphthol did not show similar effects on other insect species. Hydroprene has been found to function as a synergis to compete with natural JH in insect (Slade and Wilkinson, 1974) to inhibit other enzymes (Yu and Terriere, 1973), or to induce either the general CE-ase or JH-ase activity or both.

The profile study on carboxylesterase induction of Rugters house fly clearly indicated that several mechanisms were involved with the enzyme regulation. First, it was a age-dependent phenomenon. The induced enzyme activity was somehow closely parallel to the esterase activity of the normal physiological stage of the adult fly. The result showed that the induced esterase activity was low when the fly was newly emerged, and was high when the fly reached the age for the first reproductive cycle at day 8 after eclosion (Maa and Terriere, 1983b). Hydroprene application on the fly might provide an accelerating reaction to the physiological process, for öocyte maturation. Therefore, the biphasic response of esterase induction to the JH analogue, hydroprene, at variously physiological ages of the fly during the interval of examination should be understandable. Some other mechanisms, however, may also be involved (Yu and Terriere, 1971; and Terriere and Yu, 1971).

Secondly, this study revealed that the specific isozyme was enhanced by the treatment

with 10 μg hydroprene. A low dose of hydroprene was found with very minimum enhancement to hydroprene-specific esterase (data not shown). This JH analogue was, on one hand, being able to induce the synthesis of carboxylesterase and hydroprene esterase (peak area 2, 5, 6, and 8), and on the other hand, being with depressing effect to the normal expression of the other isozymes (peak area 1 area 1 and 4). It might be with no effect to another group of isozymes, depending upon the dose of the inducer applied, age of the adult fly for assay, and even tissue fraction used. The dose-dependent variation on hydroprene induction of esterase activity occurred in this fly might involved in a feedback mechanism. Detail of this mechanism was not investigated. Different esterase isozymes responded differentially to same inducer was expectable and has, in fact, been found in other insect species. Most of the enhanced esterase recovered from the acrylamide gel was, somehow, comparatively high in esterolytic activity to hydroprene. The withdrawal of this experiment is that the recoved hydroprene-esterase activity was, however, comparatively low. As the result revealed that the total activity recovered was 52.85 p mole hydroprene acid formed per mg protein for recovered enzyme source (data not shown) and was 270–290 p mole for the initial enzyme source (Table 1). The low recovery of the enzyme activity might be due to the loss in the procedures during extraction, diffusion and deactivation of the esterase. An alternative method with better recovery of the enzyme activity is necessary for further investigation on induction and confirmation of the substrate-specific esterase.

References

Adams, T. S. and P. E. Eide 1972. A method for the *in vitro* stimulation of house fly egg development with a juvenile hormone analogy. Gen. and Comp. Endo. 18: 12–21.

Abmad, S. 1974. An electrophoretic study of the negative correlation of certain carboxylesterases to insecticide resistance in ***Musca domestica*** L. Experientia 30: 331–333.

Bigley, W. S. and S. B. Vinson 1979. Degradation of (^{14}C) methoprene in the imported fire ant, *Solenopsis invicta*. Pestic. Bioche. Physiol. 10: 1–13.

Bradford, M. M. 1976. A rapid sensitive method for the quantitation of microgram quantities of protein-dye binding. Anal. Biochem. 72: 248–254.

Cymborowski, B. and G. Stolarz 1979. The role of juvenile hormone during larval-pupal transformation of *Spodoptera littoralis*: switchover in the sesitivity of the prothoracic gland to juvenile hormone. J. Insect Physiol. 25: 939–942.

Davis, B. J. 1964. Disc electrophoresis. II. Method and application to human serum proteins. Ann. N. T. Acad. Sci. 121: 404–427.

Georghiou, G. P., S. S. Lee, and A. H. Devries 1978. Developmnt of resistance to juvenoid methoprene in the house fly. J. Econ. Entomo. 71: 544–547.

Hooper, G. S. H. 1976. Esterase medicated hydrolysis of naphthyl ester malathiotn, methoprene, and cecropia juvenile hormone in ***Culex pipiens pipiens***. Insect Biochem. 6: 255–266.

Klages, G. and H. Emmerich 1979. Juvenile hormone metabolism and juvenile hormone esterase titer in hemolymph and peripheral tissues of ***Drosophile hydei***. J. Comp. Physiol. 132: 319–325.

Kramer, S. J. 1978. Regulation of the activity of JH-specific esterases in the Colorada potato beetle, *Leptinotarsa decemineata*. J. Insect Physiol. 24: 743–747.

Kramer, S. J. and C. A. D. de Kort 1976. Age-dependent changes in juvenile hormone esterase and general carboxylesterase activity in the hemolymph of the Colorada potato beetle, *Leptinotarsa decemlineata*. Mol. and Cell. Endo. 4: 43–53.

Maa, C. J. W. and L. C. Terriere 1983a. Age-dependent variation in enzymatic and electrophoretic properties of flesh fly, *Sarcophaga bullata* and blow fly, *Phormia regina* carboxylesterases. Comp. Biochem. Physiol. 74C: 451-460.

Maa, C. J. W. and L. C. Terriere 1983b. Age-dependent variation in enzymatic and electrophoretic properties of house fly, *M. domestica* carboxylesterases. Comp. Biochem. Physiol. 74C(2): 461-467.

Mumby, S. M., B. D. Hammock., T. C. Sparks, and K. Ota 1979. Synthesis and biosynthesis of carbamate inhibitors of juvenile hormone hydrolysis esterases from the house fly, *Musca domestica*. J. Agric. Food Chem. 27: 763-765.

Quistad, G. B., Staiger, L. E., and Schooley, D. A. 1975. Environmental degradation of the insect growth regulator methoprene. Pestic. Biochem. Physiol. 5: 233-241.

Reddy, G., K. H. Hsu, and A. K. Kumaran 1979. Factors influencing juvenile hormone esterase activity in the wax moth, *Galleria mellonella*. J. Insect Physiol. 25: 65-71.

Sanburg, L. L., K. J. Kramer, F. J. Kezdy, and J. H. Law 1975. Juvenile hormone-specific esterases in the hemolymph of the tobacco hornworm, *Manduca sexta*. J. Insect Physiol. 21: 873-878.

Slade, M. and C. F. Wilkinson 1974. Degradation and conjugation of cecropia juvenile hormone by the southern arym worm, *Prodenia eridania*. Comp. Biochem. Physiol. 49B: 99-103.

Sparks, T. C. and B. D. Hammock 1979. Induction and regulation of juvenile hormone esterases during the last instar of cabbage looper, *Trichoplusia ni*. J. Insect Physiol. 25: 551-560.

Terriere, L. C. and S. J. Yu 1973. Insect juvenile hormones: Induction of detoxifying enzymes in the house fly and detoxification by house fly enzymes. Pestic. Biochem. Physiol. 3: 96-107.

Terriere, L. C. and S. J. Yu 1976. Juvenile hormone analoges: *In vitro* metabolism in relation to biological activity in blow flies and flesh flies. Pestic. Biochem. Physiol. 7: 161-168.

Van Asperen, K. 1962. A study of house fly esterases by means of a sensitive colorimetric method. J. Insect Physiol. 8: 401-416.

Weirich, G. and J. Wren 1973. The substrate specificity of juvenile hormone esterase from *Manduca sexta* hemolymph. Life Science 13: 213-226.

Whitmore, D. H., J. E. Whitmore, and L. I. Gilbert 1972. Juvenile hormone induction of esterases: a mechanism for the regulation of juvenile hormone titer. Proc. Nat. Acad. Sci. USA. 69: 1592-1595.

Whitmore, D. H., J. E. Whitmore, and L. I. Gilbert 1974. The origin of hemolymph carboxylesterases induced by the insect juvenile hormone. Mol. Cell. Endo. 1: 37-54.

Yu, S. J. and L. C. Terriere 1971. Hormonal modification of microsomal oxidase activity of the house fly. Life Science 10: 1173-1185.

Yu, S. J. and L. C. Terriere 1973. Phenobarbital induction of detoxifying enzymes in resistant and susceptible house flies. Pestic. Biochem. Physiol. 20: 1901-1912.

Yu, S. J. and L. C. Terriere 1975. Microsomal metabolism of juvenile hormone analogs in the house flies *Musca domestica* L. Pestic. Biochem. Physiol. 5: 418-430.

Yu, S. J. and L. C. Terriere 1977a. Metabolism of (^{14}C) hydroprene (ethyl 3, 7, 11-trimethyl-2, 4-dodecadienoate) by microsomal oxidase and esterase from three species of diptera. J. Agric. Food Chem. 25: 1076-1080.

Yu, S. J. and L. C. Terriere 1977b. Esterase and oxidase activity of housefly microsomes against juvenile hormone analogs containing branched chain ester groups and its induction by phenobarbital. J. Agri. Food Chem. 25: 1333-1336.

羧基酯酶在家蠅體上之誘發研究

馬 堪 津

中央研究院動物所

本文旨在證實羧基酯酶可以在 Rutgers 家蠅品系體上被誘發。誘發劑爲一種類似昆蟲青春激素的合成品，Hydroprene。將 Hydroprene 以表皮處理法施於家蠅成蟲體表，由萘乙酸酯被水解之多寡，可知羧基酯酶確實可以被誘發。並發現在羽化後六日之雌成蟲體上可得到最高之羧基酯酶活性，故此誘發作用與試驗成蟲之蟲齡有關。此外，本實驗也以一種可以抑制核苷酸轉錄機制的抗生素，Actinomycin D 來反證家蠅成蟲經處理後新增之羧基酯酶活性確實是由新合成之蛋白所致。以生化及電泳法來測定酯酶之異構酶在膠柱中之分佈，發現絕大部分 Hydroprene 酯酶皆分佈於陰極處，可惜回收實驗的酶活性太小，不能就此證實此項誘發實驗所得之酯酶有其特殊之專一性。