HOST PLANT INDUCTION OF MICROSOMAL MONOOXYGENASES IN RELATION TO ORGANOPHOSPHATE ACTIVATION IN FALL ARMYWORM LARVAE

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ABSTRACT

Various host plants were found to induce microsomal monooxygenase activity that activates organophosphorus insecticides in larvae of the fall armyworm, *Spodoptera frugiperda* (J. E. Smith). Of these plants, cotton and parsley were best inducers of microsomal organophosphate desulfuration and sulfoxidation, respectively. The induction was associated with increased toxicity of numerous phosphorothionate and thioether-containing insecticides. Analyses of internal insecticide revealed that, at various intervals, induced larvae retained less phorate and its oxidative metabolites than did the controls, indicating that induction caused an overall increase in the rate of insecticide metabolism in this insect.

RESUMEN

Se encontraron varias plantas hospederas que inducen la actividad monooxigenase microsomal que activa los insecticidas de organofosfatos en las larvas del gusano cogollero, *Spodoptera frugiperda* (J. E. Smith). De estas plantas, el algodón y el perejil fueron las mejores inducidoras de la desulfatación de organofosfatos microsomales y de sulfaoxidación. La inducción estaba asociada con aumentos de toxicidad de numerosos insecticidas conteniendo fosforothionate y thioether. Análisis de insecticidas internos reveló que a varios intervalos, las larvas inducidas retuvieron menos foratos y sus metabolitos oxidativos que los testigos, indicando que la inducción en este insecto causó un aumento general en el grado de metabolismo de los insecticidas.

It is well established that host plants can induce detoxication enzymes in herbivorous insects resulting in decreasing insecticide toxicity (Yu 1985a). For example, peppermint (*Mentha piperita* L.) leaves were found to stimulate microsomal epoxidase activity in variegated cutworms (*Peridroma saucia* Hübner) causing the insects to become less susceptible to various commonly used insecticides such as carbaryl, methomyl, acephate and malathion (Yu et al. 1979, Berry et al. 1980). Feeding peppermint leaves to larvae of cabbage looper (*Trichoplusia ni* (Hübner)) and alfalfa looper (*Autographa californica* (Speyer)) also increased the epoxidase activity which provided increased tolerance for carbaryl and methomyl. In addition, increased microsomal epoxidase activity by corn (*Zea may* L.) leaves in fall armyworm (*Spodoptera frugiperda* (J. E. Smith)) larvae (FAW) was associated with increasing tolerance for a variety of insecticides including methomyl, acephate, trichlofon, diazinon, methamidophos, monocrotophos, permethrin and cypermethrin (Yu 1982). The induction was apparently due to allelochemicals in the host plants (Brattsten et al. 1977, Yu 1985a).

Although microsomal monooxygenases are capable of detoxifying insecticides, in some cases, these enzymes can activate proinsecticides to active insecticides (Dahm and Naktsugawa 1968, Nakatsugawa and Morelli 1976, Yu 1985b). Microsomal desulfuration

and sulfoxidation of phosphorothionate and thioether-containing insecticides, respectively, are typical examples of such oxidative activation. The question then arises as to whether host plants can stimulate these organophosphate (OP) activation enzymes in insects; such induction would increase an insect's susceptibility to insecticides that are activated by the microsomal oxidase systems. If so, control might be achieved by application of a low rate of appropriate insecticides to crops which possess inducing capability. I now report experiments with FAW larvae which support these possibilities.

MATERIALS AND METHODS

Insects: Larvae of the FAW were reared on an artificial diet and maintained in environmental chambers at 25°C with a 16:8, L:D photoperiod as described previously (Yu 1982).

Chemicals: The chemicals used in this study and their sources were indole 3-carbinol, indole 3-acetonitrile, flavone, and β-naphthoflavone, Sigma Chemical Company, St. Louis, MO; oxydemetonmethyl, azinphosmethyl, isofenphos, demeton, disulfoton, and fenthion, Mobay Chemical Corporation, Kansas City, MO; malathion, and phorate and its sulfoxide and sulfone, American Cyanamid Company, Princeton, NJ; diazinon, Ciba-Geigy Corporation, Greensboro, NC; parathion, Monsanto Chemical Company, St. Louis, MO; and carbophenothion, Stauffer Chemical Company, Westport, CT. All other Chemicals were of analytical quality and purchased from commercial suppliers.

Treatment of Insects: Newly-molted sixth-instar larvae that had been maintained on artificial diet were placed in individual plastic cups and fed artificial diets containing allelochemicals for 2 days. Controls were fed the artificial diet only. After 48 h, larvae were removed from each diet and used for enzyme assays. No mortality was observed due to the treatments.

When host plants were used as inducers, newly-molted sixth-instar larvae were placed in plastic containers in groups of 25 larvae each and fed for 2 days on freshly-excised leaves of the plants listed in Table 1. All plants were grown under field conditions with no insecticide application.

Enzyme Assays: Groups of 225 midguts were dissected from the larvae and their gut contents removed. They were then washed in 1.15% KCl and homogenized in 20 ml of ice-cold 0.1 M sodium phosphate buffer, pH 7.5, in a motor-driven tissue grinder for 30 sec. The crude homogenate was filtere through cheesecloth and then centrifuged at 10,000 g max for 15 min in a Beckman L5-50E ultracentrifuge. The pellet was discarded and the supernatant, which had been filtered through glass wool, was recentrifuged at 105,000 g max for 60 min. The microsomal pellet was suspended in ice-cold 0.1 M sodium phosphate buffer, pH 7.2 to make a final protein concentration of 1 mg/ml and was used immediately as the enzyme source. The procedure was conducted at 0° to 4° C.

Microsomal desulfurase activity was determined with parathion as substrate following the optimum assay conditions described below. The 5-ml incubation mixture contained 1 ml of microsomal suspension; 0.1 M sodium phosphate buffer, pH 7.2; an NADPH-generating system consisting of 1.8 μmol NADP; 18 μmol glucose 6-phosphate, 1 unit of glucose 6-phosphate dehydrogenase; and 100 μg of parathion in 0.1 ml of methyl Cellosolve. The mixture was incubated by shaking the tubes in a water bath at 30C in an atmosphere of air for 15 min. The reaction was stopped and extracted with 10 ml of hexane, and the hexane fraction was analyzed for the desulfuration product, paraoxon. Analyses were performed on a Varian Model 3740 gas chromatograph equipped with a thermionic specific detector. The column used was 6 ft x 2 mm i.d. glass packed with

1.5% OV-17/1.95% QF-1 on Chromosorb WHP. No detectable paraoxon hydrolysis was observed by this system.

Bioassays: Newly-molted sixth-instar larvae maintained on an artificial diet were divided into two groups, one reared on freshly-excised leaves of cotton (*Gossypium aroreum* L.) or parsley (*Petroselinum crispum* (Mill) Nym.) and the other on the artificial diet as control. After 48 h, larvae were placed individually in a scintillation vial and starved for 3 h. They were then fed individually a leaf disk of 'crisphead' lettuce (*Lactuca sativa* L.), 0.7 cm in diameter, which had been topically treated with 1 μl of acetone containing various insecticides. All tests were replicated twice with 10 larvae per replicate. Mortality counts were made after 24 h. Probit analysis was performed using the SAS computer program (Ray 1982).

For the *in vivo* phorate sulfoxidation experiments, larvae were topically treated with phorate in 1 μl acetone and kept individually in a scintillation vial. At various time intervals after treatment, duplicate groups of three larvae each were collected, and internal phorate and its oxidative metabolites were analyzed as described by Yu (1985b).

RESULTS

Induction of Organophosphate Activation Enzymes: The effect of host plants and allelochemicals on microsomal parathion desulfurase activity in FAW larvae is shown in Table 1. Of these plants, cotton was the best inducer resulting in a 2.4-fold increase in the desulfurase activity. Soybeans (*Glycine max* (L.) Merril) and peanuts (*Arachis hypogeaea* L.) significantly reduced the enzyme activity. Various allelochemicals which were inducers of microsomal monooxygenases (Yu 1983, Yu and Ing 1984) also induced parathion desulfurase activity. Among those tested, indole 3-acetonitrile was the best inducer causing a 3.4-fold increase in the desulfurase activity. Gossypol, which is found in cotton leaves, had no effect on the enzyme.

TABLE 1. PARATHION DESULFURASE ACTIVITY OF FALL ARMYWORM LARVAE FED HOST PLANTS AND ALLELOCHEMICALS.

Host Plant or allelochemical[1]	Specific activity[2] (pmol paraoxon min^{-1} mg protein^{-1})
Artificial diet	137.2 ± 11.3
Soybeans (*Glycine max* (L.) Merril) ('Bragg')	47.5 ± 7.9**[3]
Peanuts (*Arachis hypogaea* L.) ('Florunner')	89.4 ± 5.4**
Corn (*Zea may* L.) ('Pioneer')	165.0 ± 17.5*
Potato (*Solanum tuberosum* L.) ('Kenneback')	192.1 ± 19.0*
Cowpeas (*Vigna unguiculata* (L.) Walp) ('Blackeye No. 5')	202.6 ± 17.8*
Cotton (*Gossypium aroreum* L.) ('McNair 220')	339.6 ± 15.9**
Indole 3-carbinol	337.1 ± 30.1*
Flavone	369.1 ± 24.8**
β-naphthoflavone	394.2 ± 21.3**
Indole 3-acetonitrile	463.3 ± 27.4***
Gossypol (0.05%)	121.3 ± 8.4

[1]Newly-molted sixth-instar larvae were fed leaves of host plants or artificial diets containing allelochemicals (0.2% unless otherwise stated) for 2 days prior to enzyme assays.

[2]Mean ± SE of two experiments, each assayed in duplicate.

[3]Values significantly different from the control (artificial diet) based on Student's t test. *, P < 0.05; **, P < 0.01; ***, P < 0.001.

In a previous report (Yu 1985b), I found that another organophosphate activation enzyme activity, microsomal phorate sulfoxidation, was induced by various host plants including corn, cotton, parsnip (*Pastinaca sativa* L.) and parsley. Parsley was the best inducer causing a 3.6-fold increase in the sulfoxidase activity (Table 2). Various allelochemicals including indoles, monoterpenoids, and flavonoids were inducers of the sulfoxidase. Xanthotoxin which is found in parsley and parsnip leaves decreased the sulfoxidase activity by 44% when incorporated in the artificial diet at 0.01% as compared with the control.

Effect of Enzyme Induction on Toxicity of Organophosphorus Insecticides: The toxicity of various OP insecticides to FAW larvae as influenced by host plant induction is shown in Fig. 1-2. In this study, parsley was used as inducer for microsomal sulfoxidase, whereas cotton was used as inducer for microsomal desulfurase. It can be seen that feeding cotton leaves to the larvae enhanced the toxicity of parathion, malathion, and isofenphos as much as 4-fold (Fig. 1). Unexpectedly, the same treatment caused a decrease in the toxicity of diazinon and azinphosmethyl. On the other hand, feeding parsley leaves to the larvae enhanced the toxicity of phorate, disulfoton, carbophenothion, demeton, oxydemeton-methyl and fenthion up to 3-fold (Fig. 2). Probit analysis on a body weight basis revealed that LD_{50} values for the parsley-fed and artificial diet-fed larvae were 32.5 μg phorate/g larva and 69.6 μg phorate/g larva, respectively. The end points of toxicity for these insecticides were about 8 h post-treatment with the exception of carbophenothion and parathion which required 24 h. In most cases, mortality occurred

TABLE 2. PHORATE SULFOXIDASE ACTIVITY OF FALL ARMYWORM LARVAE FED HOST PLANTS AND ALLELOCHEMICALS.[1]

Host plant or allelochemical[2]	Specific activity[3] (nmol phorate sulfoxide min^{-1} mg protein^{-1})
Artificial diet	2.50 ± 0.13
Soybeans	1.81 ± 0.10
Broccoli	1.87 ± 0.10
Potato	2.43 ± 0.13
Peanuts	2.50 ± 0.04
Cowpeas	2.83 ± 0.28
Corn	3.46 ± 0.22**[4]
Cotton	5.73 ± 0.28**
Parsnip	6.65 ± 0.96**
Parsley	9.04 ± 0.57***
Artificial diet	2.41 ± 0.05
Indole 3-acetonitrile	11.83 ± 0.96**
Indole 3-carbinol	12.19 ± 1.24**
Flavone	14.73 ± 1.54**
β-naphthoflavone	12.23 ± 0.65***
(+) α-pinene	3.83 ± 0.11**
Peppermint oil	7.70 ± 0.33***
Xanthotoxin (0.01%)	1.07 ± 0.04***

[1]Data from Yu (1985b) except xanthotoxin.
[2]Newly-molted sixth-instar larvae were fed leaves of host plants or artificial diets containing allelochemicals (0.2% unless otherwise stated) for 2 days prior to enzyme assays.
[3]Mean ± SE of two experiments, each assayed in duplicate.
[4]See Table 1 (footnotes) for the level of statistical difference from the control (artificial diet).
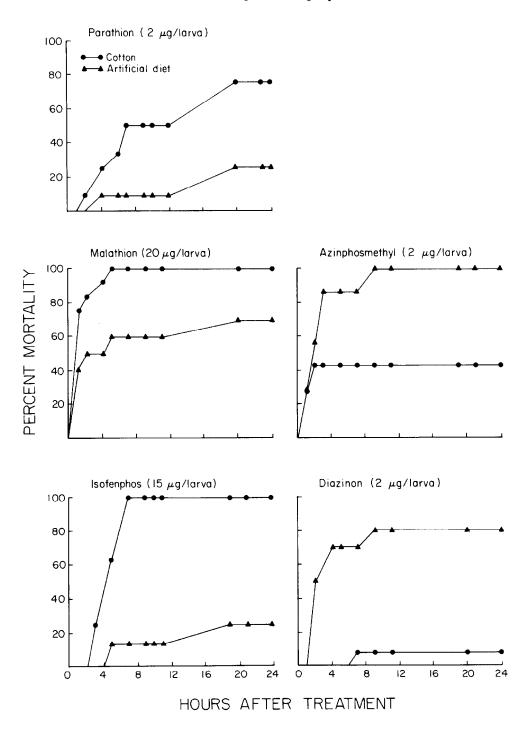
Fig. 1. Effect of cotton on the toxicity of various phosphorothionate insecticides to fall armyworm larvae. Newly molted sixth-instar larvae were fed cotton leaves or artificial diet for 2 days prior to insecticide treatments. Body weights in g per larva (mean ± SE): cotton-fed, 0.28 ± 0.007; artificial diet-fed, 0.30 ± 0.01.
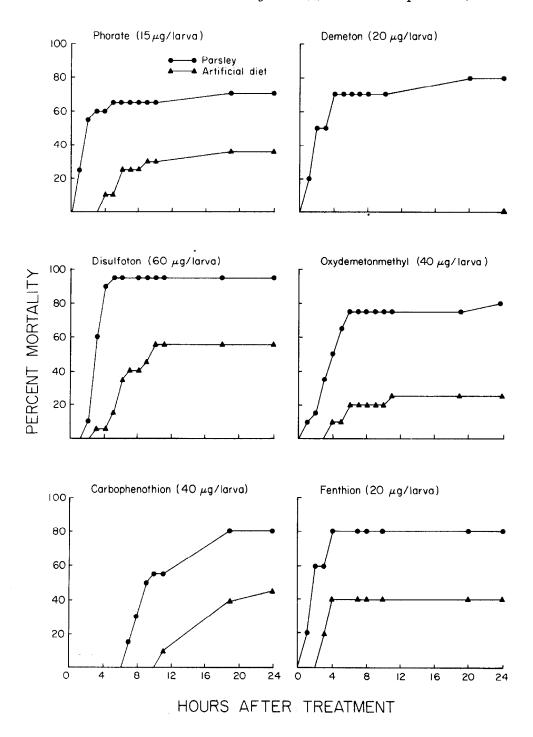
HOURS AFTER TREATMENT

Fig. 2. Effect of parsley on the toxicity of various thioether-containing organophosphorus insecticides to fall armyworm larvae. Newly-molted sixth-instar larvae were fed parsley leaves or artificial diet for 2 days prior to insecticide treatments. Body weights in g per larva (mean ± SE): Parsley-fed, 0.27 ± 0.02; artificial dietfed, 0.29 ± 0.01.

earlier in the induced larvae than in the controls, indicating an increased activation of the insecticides due to host plant feeding.

Analyses of internal phorate and its metabolites showed that phorate was rapidly oxidized to its sulfoxidation products, phorate sulfoxide and phorate sulfone (Table 3). Contrary to the *in vivo* toxicity results, the induced larvae (i.e., parsley-fed) retained less phorate and its oxidative metabolites up to 8 h after topical treatment with the exception of Hr-2 assay in which the induced larvae had more phorate sulfoxide and phorate sulfone than the control.

DISCUSSION

One important aspect of research in plant-insect interactions is to learn whether insecticide activation enzymes can be stimulated and to explore consequent effects on the toxicity of insecticides to insects. The results of the present study clearly show that certain host plants can increase the toxicity of OP insecticides to FAW larvae. The enhanced toxicity was apparently due to an increased activity of microsomal desulfuration and sulfoxidation caused by cotton or parsley, respectively. Parathion, malathion, azinphosmethyl, diazinon and isofenphos are phosphorothionate or phosphorothiolothionate compounds containing the P=S moiety. These proinsecticides are known to be activated by microsomal desulfuration to become more potent acetylcholinesterase inhibitors (Nakatsugawa and Dahm 1965, Matsumura 1975, Yang *et al.* 1971, Motoyama and Dauterman 1972). It is not known, however, why enhanced desulfuration caused by cotton did not increase the toxicity of diazinon and azinphosmethyl to the larvae. One possible explanation could be that cotton also induced glutathione S-transferase (Yu 1982) resulting in an increased degradation of diazinon and diazoxon (Yang *et al.* 1971), and azinphosmethyl and its oxygen analog (Motoyama and Dauterman 1972). The possibility of induction by cotton of microsomal monooxygenase activity that oxidatively hydrolyzes these two insecticides and their oxygen analogs (Yang *et al.* 1971, Motoyama and Dauterman 1972) can not be ruled out, however. In any case,

TABLE 3. EFFECT OF MICROSOMAL ENZYME INDUCTION ON THE *IN VIVO* METABOLISM OF PHORATE IN FALL ARMYWORM LARVAE.

Enzyme inducer[1]	Time after treatment (hr)	Internal phorate and metabolites (μg/larva)[2]		
		Phorate	Phorate sulfoxide	Phorate sulfone
Control	2	8.18 ± 0.82[3]	0.83 ± 0.04	0.29 ± 0.02
	4	5.84 ± 1.04	0.76 ± 0.04	0.31 ± 0.01
	6	3.73 ± 0.53	0.77 ± 0.05	0.39 ± 0.03
	8	3.36 ± 0.03	0.71 ± 0.01	0.37 ± 0.01
Parsley	2	2.47 ± 0.28	0.90 ± 0.04	0.42 ± 0.01
	4	0.54 ± 0.15	0.47 ± 0.08	0.22 ± 0.05
	6	0	0	0
	8	0	0	0

[1]Newly-molted sixth-instar larvae were fed parsley leaves or artificial diet (control) for two days prior to enzyme assays.
[2]Larvae (after induction) were topically treated with phorate at 10 μg/larva in 1 μl acetone.
[3]Mean ± SE of two experiments, each assayed in duplicate.

the detoxication rate of diazinon and azinphosmethyl exceeded the activation rate, resulting in the protective effect observed.

On the other hand, phorate, disulfoton, carbophenothion, demeton, fenthion and oxydemetonmethyl are all thioether-containing OP compounds. These proinsecticides are known to be activated by microsomal sulfoxidation to become more potent acetylcholinesterase inhibitors (Yu 1985b, Bull 1965, Menzie 1969, March et al. 1955, Stone 1969). It should be mentioned that with the exceptions of oxydemetonmethyl and the thiol isomer of demeton, these insecticides also contain the P=S group. Therefore, in addition to sulfoxidation, they can be desulfurated by microsomal monooxygenases even though the desulfuration of thioether-containing compounds might be rather insignificant in this insect (Yu 1985b). Furthermore, parsley may not induce microsomal desulfurase activity in insects since it had no marked effect on microsomal epoxidase activity (Yu 1985b).

Increased toxicity of phorate due to parsley was associated with a decrease in the levels of internal phorate and its oxidative metabolites (sulfoxide and sulfone). This suggests that induction of sulfoxidase activity resulted in an overall increase in the rate of phorate metabolism. This observation is in agreement with Yu (1985b) who used the allelochemical indole 3-carbinol as inducer of the enzyme.

These results, in conjunction with those reported elsewhere (Yu 1985a, Yu and Hsu 1985), clearly demonstrated that various host plants can induce a variety of detoxication enzymes including microsomal monooxygenases, glutathione S-transferases, and hydrolases in agricultural insect pests. Since organophosphate activation enzyme activity (desulfuration and sulfoxidation) can be induced in these insects, we may utilize the enzyme activation approach to improve insecticide efficacy in the field and hence lead to a reduction in pesticide usage.

REFERENCES CITED

BERRY, R. E., S. J. YU, AND L. C. TERRIERE. 1980. Influence of host plants on insecticide metabolism and management of variegated cutworm, *Peridroma saucua* (Hubner). J. Econ. Ent. 73: 771-774.

BRATTSTEN, L. B., C. F. WILKINSON, AND T. EISNER. 1977. Herbivore-plant interactions: Mixed-function oxidases and secondary plant substances. Science 196: 1349-1352.

BULL, D. L. 1965. Metabolism of Di-syston by insects, isolated cotton leaves, and rats. J. Econ. Ent. 58: 249-254.

DAHM, P. A., AND T. NAKATSUGAWA. 1968. Bioactivation of insecticides, pp. 89-112. *In* the Enzymatic Oxidation of Toxicants. E. Hodgson (ed.). North Carolina State University, Raleigh, North Carolina.

MARCH, R. B., R. L. METCALF, T. R. FUKUTO, AND M. G. MAXON. 1955. Metabolism of Systox in the white mouse and American cockroach. J. Econ. Ent. 48: 355-363.

MATSUMURA, F. 1975. Toxicology of Insecticides. Plenum Press, New York.

MENZIE, C. M. 1969. Metabolism of Pesticides. U.S. Dept. of the Interior Fish and Wildlife Service. Special Scientific Report-Wildlife No, 127, Washington, D.C.

MOTOYAMA, N., AND W. C. DAUTERMAN. 1972. *In vitro* metabolism of azinphos-methyl in susceptible and resistant house flies. Pestic. Biochem. Physiol. 2: 113-122.

NAKATSUGAWA, T., ANDP. A. DAHM. 1965. Parathion activation enzymes in the fat body microsomes of the American cockroach. J. Econ. Ent. 58: 500-509.

NAKATSUGAWA, T., AND M. A. MORELLI. 1976. Microsomal oxidation and insecticide metabolism, pp. 61-114. *In* Insecticide Biochemistry and Physiology. C. F. Wilkinson (ed.). Plenum Press, New York.

RAY, A. A. 1982. SAS User's Guide: Statistics. SAS Institute, Inc., Cary, North Carolina.

STONE, B. F. 1969. Metabolism of fenthion by the southern house mosquito. J. Econ. Ent. 62: 977-981.

YANG, R. S. H., E. HODGSON, AND W. C. DAUTERMAN. 1971. Metabolism *in vitro* of diazinon and diazoxon in susceptible and resistant house flies. J. Agr. Food Chem. 19: 14-19.

YU, S. J. 1982. Induction of microsomal oxidases by host plants in the fall armyworm, *Spodoptera frugiperda* (J. E. Smith). Pestic. Biochem. Physiol. 17: 59-67.

————. 1983. Induction of detoxifying enzymes by allelochemicals and host plants in the fall armyworm. Pestic. Biochem. Physiol. 19: 330-336.

————. 1985a. Consequences of induction of foreign compound-metabolizing enzymes in insects. *In* Molecular Mechanisms in Insect-plant Associations. L. B. Brattsten and S. Ahmad (eds.). Plenum Press, New York. (In press).

————. 1985b. Microsomal sulfoxidation of phorate in the fall armyworm, *Spodoptera frugiperda* (J. E. Smith). Pestic. Biochem. Physiol. 23: 273-281.

————, R. E. BERRY, AND L. C. TERRIERE. 1979. Host plant stimulation of detoxifying enzymes in a phytophagous insect. Pestic. Biochem. Physiol. 12: 280-284.

————, AND E. L. HSU. 1985. Induction of hydrolases by allelochemicals and host plants in fall armyworm (Lepidoptera, Noctuidae) larvae. Environ. Ent. 14: 512-515.

————, AND R. T. ING. 1984. Microsomal biphenyl hydroxylase of fall armyworm larvae and its induction by allelochemicals and host plants. Comp. Biochem. Physiol. 78C: 145-152.