

standard rat TSH (rat TSH PR-1, specific activity 0.22 USP units/mg), pooled amniotic fluid was measured at 4 dilutions (50 μ l, 100 μ l, 200 μ l and 400 μ l) in a total incubation volume of 0.7 ml.

Results and discussion. Dilutions of pooled amniotic fluid exhibited parallelism with the standard curve of rat TSH (fig.), demonstrating the immunological identity of amniotic fluid and standard rat TSH. Moreover we found TSH immunoactivity in all 12 samples obtained from 2 different experiments with a mean concentration of 305.4 ± 24.1 (SE) ng/ml, which was about 25% of the level found in adult rat serum. We have demonstrated unequivocally the presence of TSH im-

munoactivity in rat amniotic fluid, although El-Znheri and his coworkers failed to detect TSH in the amniotic fluid of rat fetuses at term⁸. The discrepancy between the results may be due to a difference in the assay methods and/or sample volumes used, as Chopra and his co-workers⁹ also failed to detect TSH in human amniotic fluid in earlier studies. The amniotic TSH levels found in this study were similar to those found in rat fetal blood^{7,8,10}. In view of this, and the previous finding that hypothyroidism resulted in elevated TSH in the amniotic fluid of lamb fetuses⁵, we infer that this amniotic TSH is probably of fetal origin. This aspect of the problem is currently under investigation in our laboratory.

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- 3 Tyson, J.E., Hwang, P., Guyda, H., and Friesen, H.G., Am. J. Obstet. Gynec. 113 (1972) 14.
- 4 Tuimala, R., Kauppila, A., and Haapalahti, J., Br. J. Obstet. Gynaec. 83 (1976) 853.
- 5 Hollingsworth, D.R., Davis, S.L., Chopra, I.J., Belin, R.P., and Reid, M.C., Proc. Soc. exp. Biol. Med. 157 (1978) 106.

- 6 Kourides, I.A., Heath, C.V., and Ginsberg-Fellner, F., J. clin. Endocr. Metab. 54 (1982) 635.
- 7 Tonooka, N., and Green, M.A., Endocrinology 102 (1978) 852.
- 8 El-Zaheri, M.M., Vagenakis, A.G., Hinerfold, L., Emerson, C.H., and Braverman, L.E., J. clin. Invest. 67 (1981) 1126.
- 9 Chopra, L.J., and Crandall, B.F., New Engl. J. Med. 293 (1975) 740.
- 10 Kuhn, E.R., Bollen, M., and Dorras, V., J. Endocr. 93, (1982) 55.

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Cadmium-induced inhibition of brain monoamine oxidase in the freshwater catfish¹

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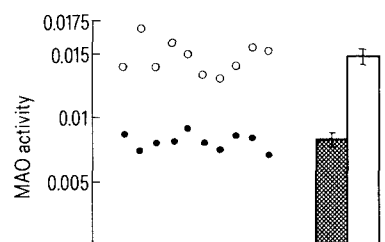
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Summary. The freshwater catfish *Clarias batrachus* (L.), exposed to 50 ppm of cadmium (Cd) chloride for a period of 135 days exhibited marked inhibition of brain monoamine oxidase (MAO) ($p < 0.001$). The retarded gonadal growth observed in the present study suggests that Cd may be capable of creating imbalance in the aminergic activity of the hypothalamus, which modulates the secretion and release of gonadotropins.

Cadmium is one of the apparently nonessential metals which can enter the bodies of animals, including man, through food, water and air. It activates or inhibits several enzymes, and causes developmental abnormalities, anemia, bone mineral loss, hypertension, cardiac enlargement, enteropathy, gonadal atrophy and kidney damage²⁻⁵. Although it has been suggested that the toxic effects of Cd are due to induced deficiencies of essential nutrients which are accompanied by increased Cd absorption, little is known about the biochemical basis of its chronic toxicity^{4,6}. In mature rats, Cd exerts its neurotoxic effects on the sensory ganglia^{7,8} whereas in immature ones it causes damage to the central nervous system accompanied by hemorrhagic lesions and dysfunction of neurons.

Fifteen *C. batrachus* were exposed to 50 mg/l of Cd chloride for 135 days, changing the medium every alternate day after feeding. An equal number was kept as control. The experiment was started in February 1982 when the gonads were in the resting phase and terminated in June 1982, when the control exhibited spawning phase stage IV gonads. All the fish were sacrificed by decapitation and the whole brain MAO was estimated adopting the enzyme isotopic technique of Parvez and Parvez⁹. 50 μ l of 0.44 nM ¹⁴C-tryptamine bisuccinate (specific activity 49.6 mCi/mmol; obtained from New England Nuclear, USA) was used as the enzyme substrate. MAO is a mitochondrial enzyme responsible for oxidative deamination of monoamines. Hence quantitative variations in MAO are an indirect indication of changes in monoamine activity. In Cd-exposed rats, Magour et al.¹⁰ reported inhibition of MAO in the mitochondrial extract of brain and liver. In the rat, intraocular injection of lead was

accompanied by hyperinnervation but Cd did not show any change in nerve fiber density whereas mercury caused degeneration of adrenergic fibers¹¹. *C. batrachus* exhibited a significant ($p < 0.001$) (44%) depletion of whole brain MAO in response to Cd chloride treatment (fig.). It is well established that the monoaminergic system of brain is not only involved in neurotransmission but also modulates the various tropic hormones secreted by the pituitary. In Cd chloride exposed *C. batrachus* the gonads remained in the resting phase whereas in the controls they were fully matured. Apart from its probable direct effect on the gonads, Cd may impair the aminergic system responsible for the modulation of gonadotropic function.



Brain MAO activity (nM/mg tissue/h) in cadmium-exposed and control groups (n = 10); statistical analysis was ratified through Student's t-test ($p < 0.001$). □, control; ○, control individual value; ▨, Cd-treated; ●, Cd-treated individual value.

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- 2 Vallee, B. L., and Ulmer, D. D., A. Rev. Biochem. 41 (1972) 91.
- 3 Friberg, L., Piscator, M., Nordberg, G., and Kjellstrom, T., Eds, Cadmium in the environment, Cleveland CRC Press, Cleveland 1974.
- 4 Fox, M. R. S., in: Clinical, biochemical, and nutritional aspects of trace elements, p. 537. Ed. A. Prasad. Alan R. Liss Inc., New York 1982.
- 5 Kopp, S. J., Glonek, T., Perry, H. M., Jr, Erlanger, M., and Perry, E. F., Science 217 (1982) 837.
- 6 Sandstead, H. H., in: Toxicology of trace elements, p. 241. Eds R. A. Goyer and M. A. Mehlman. Halsted press, New York 1977.
- 7 Gabbiani, G., Experientia 22 (1966) 261.
- 8 Arvidson, B., Acta Neuropath. 49 (1980) 213.
- 9 Parvez, H., and Parvez, S., Clinica chim. Acta 40 (1973) 85.
- 10 Magour, S., Cumpelick, O., and Paulus, M., J. clin. Chem. clin. Biochem. 17 (1979) 777.
- 11 Bjorklund, H., Hoffer, B., Olson, L., and Seiger, A., Envir. Res. 26 (1981) 69.

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Photosensitization and feeding deterrence of *Euxoa messoria* (Lepidoptera:Noctuidae) by α -terthienyl, a naturally occurring thiophene from the Asteraceae¹

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Summary. Alpha-terthienyl, a phototoxic thiophene derivative from species in the Asteraceae reduced feeding and growth of the phytophagous lepidopteran, *Euxoa messoria*, when incorporated into artificial diets at a concentration of 100 ppm. The effects of this substance were substantially enhanced by including photosensitizing near-UV radiation in the trials. The results suggest that the phototoxic properties of this secondary substance provide significant protection to the plants containing it.

Our recent investigations^{3,4} have documented some of the effects of naturally occurring polyacetylenes and thiophenes on insects. A remarkable property of these secondary metabolites of the plant family Asteraceae is their greatly enhanced activity in the presence of sunlight or near-UV. 9 of 14 substances tested had photosensitizing lethal activity on mosquito and blackfly larvae at 0.5 ppm. One of these substances, α -terthienyl (α -T) (fig. 1) which forms the basis of the current investigation, was found to be more toxic ($LC_{50} = 19$ ppb) to *Aedes aegypti* larvae in the presence of near-UV, than DDT³.

This substance was also toxic, without near-UV activation, at 100 times this concentration. Other workers have also demonstrated the non-photosensitizing effects of these substances. For example, cis-dehydromatricaria ester and tridec-1-ene-3,5,7,9,11-pentayne were found to be ovicidal to the fruit fly *Drosophila melanogaster* and the housefly *Musca domestica*⁵. Although these studies have suggested that the presence of polyacetylenes and thiophenes may protect the host plant from insect attacks, little work has been undertaken with truly phytophagous species. In the only quantitative study to date, we reported that the polyacetylene phenylheptatriyne was a feeding deterrent to larvae of the cutworm *Euxoa messoria*, a highly polyphagous lepidopteran whose diet exposes it to a wide range of secondary substances⁶. These trials were also conducted without irradiation.

In the present study, we have investigated the feeding deterrence of the thiophene (α -terthienyl, α -T) to *Euxoa messoria* with and without near-UV exposure. In a subsequent experiment, the effects of chronic exposure of larvae to α -T and near-UV were examined.

Materials and methods. a) *Growth studies.* All larvae were reared according to the procedure described by Devitt et al.⁷ in individual 2.5 × 4 cm polystyrene vials capped with UV-transparent Stretch'n Seal perforated to prevent condensation. 4 groups of 15 larvae were used: 1 group exposed to 100 µg/g α -T prepared as described previously⁸ and near-UV light, 1 group exposed to 100 µg/g α -T without UV, and 2 other groups exposed to untreated diet with and without UV. Larvae were weighed, and diets were changed, every 2 days until all of the α -T+UV-group had died. Illumination was provided by a bank of 12 Vita-Lights (Duro-Test No. 48T12) producing 10 W/m² including 1 W/m² near-UV. Control groups without UV

were shielded by a Kodak Wratten 2-B filter (400 nm cut-off), and so were exposed to visible light and the same photoperiod (16 L:8 D) as insects in the +UV groups.

b) *Feeding studies.* Groups of 10 sixth instar cutworm larvae (200–400 mg), reared under visible + near-UV light and not previously exposed to α -T, were individually weighed and offered a weighed appropriately treated diet cube. Remaining diet, frass, and the larvae were weighed after 24, 48, and 72 h. The 4 combinations of α -T and UV as described for the growth experiments were examined.

Results and discussion. In a feeding deterrence trial, groups of sixth-instar larvae that had not previously been exposed to α -T were presented with diets containing 100 ppm α -T and divided into groups treated with and without near-UV (table). In the absence of near-UV, 100 ppm α -T reduced feeding and weight gain of larvae. These effects were also observed in trials with a feeding specialist, *Manduca sexta* (Champagne et al., unpublished), and are similar to results obtained previously with the polyacetylene phenylheptatriyne⁶. Thus, despite their very different structures, thiophenes and polyacetylenes have comparable feeding deterrent activities towards insects.

The presence of near-UV alone did not significantly change feeding rates compared to the non-UV treated group; however, exposure to both α -T and near-UV decreased feeding activity to a level well below that resulting from exposure to α -T alone. The interaction of α -T and UV has been observed in every replicate of this experiment. Hence, it appears that photosensitization can affect feeding behavior. Weight gain was also decreased by exposure to both α -T and UV, to a larger extent than was expected if α -T and UV did not interact.

The efficiency of diet conversion to insect biomass, expressed as the ratio of larval weight gain to diet consumption, was markedly decreased by exposure to α -T. The presence of near-UV did not enhance this expression of toxicity. These results suggest that non-specialized insects feeding on plants containing α -T not only consume less, but are less well able to utilize what is consumed.

In a 2nd experiment, larvae were exposed to α -T (100 ppm) during their entire development period (fig. 2). Larvae exposed to α -T without near-UV initially showed poor growth relative to controls, but growth performance improved after 2 weeks. A similar pattern was observed in insects exposed only to near-