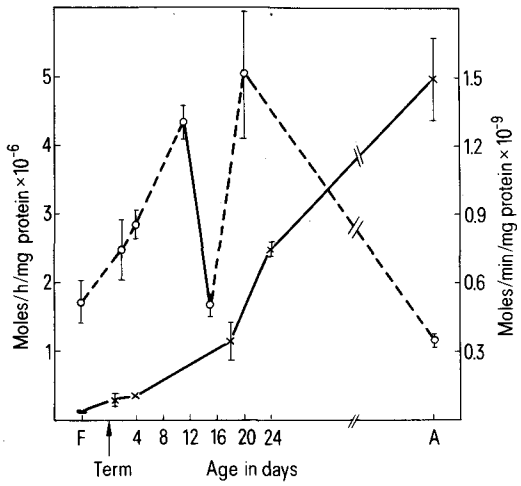
postnatal day. Afterwhich it falls gradually to the adult value.

Discussion. Although the γ-glutamyl cycle has been demonstrated to occur in adult kidney[3], results obtained in this study as well as those of Tate and Meister's[4] suggests that it is unlikely to be operational in foetal tissue. The enzymes of other metabolic processes maintaining the homeostasis, like that of gluconeogenesis[7] and of the urea cycle[8], also appear only after birth.

In the present study, the estimation of γ-glutamylcysteine synthetase activity was performed in ammonium sulfate fractionated extract as interference of the assay occurs in crude extract. It might be argued that the absence of enzyme activity in foetal extract could be a result of loss of enzyme activity through fractionation with ammonium sulfate. Work from Orlowski and Meister[6] suggests this is unlikely, as more than 97% of enzyme activity might be recovered after the fractionation procedure. As for the assay of the enzyme, the method of Orlowski and Meister[6] was used as it is simpler than that of Minnich et al.[9]. The latter assay was only used to confirm the absence of enzyme activity in foetal samples, as it is more sensitive. Since both enzymes show marked increases in activities during weaning, it is likely that dietary factor is involved in the normal regulation of the 2 enzymes. In this respect, it is of interest to note that γ-glutamyl transpeptidase, another enzyme of the γ-glutamyl cycle, is reported to be affected by the nutritional state of the animal[10]. Thus if the γ-glutamyl cycle does participate in the manner proposed by Meister's group[2] it is logical to expect such a response to dietary intake of amino acids.



Development of oxoprolinase and γ-glutamylcysteine synthetase in rat kidney. Each point represents the mean of determination on 4–6 animals drawn from different litters. The vertical bars represent 1 SD of the mean. A, Adult rats, 3 months old. Activities of oxoproline (---○---○---) and γ-glutamylcysteine synthetase (——×——×——) are determined by procedures described in the text.

1 Acknowledgment. The work was supported by a research grant awarded by the Committee on Research and Conference Grants, University of Hong Kong.

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Xanthumin and 8-epi-xanthatin as insect development inhibitors from *Xanthium canadense* Mill.[1]

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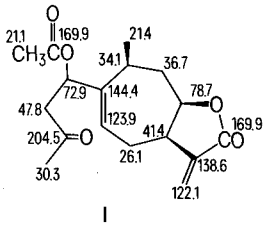
Summary. Two insect development inhibitors against *Drosophila melanogaster* have been isolated from the leaves of *X. canadense* and identified as xanthumin and 8-epi-xanthatin.

In recent years much interest has centered on the use of natural products for controlling insect pests[3–7]. For the purpose of exploring such natural products, suitable bioassay method, of which the criterion is development retardation or interruption as well as death, would be required. We devised a novel bioassay method using *Drosophila melanogaster*. The features of this method is to administer a plant extract orally, dermally and respiratorily to the insects throughout their development from egg to adult, and to observe precisely its effect on every stage of their development[8].

The bioassay was carried out as follows: 10 newly laid eggs of *D. melanogaster* were seeded on 2 g of the diet containing the test material in a glass tube and reared for 2 weeks with a photoperiod 16 h light/8 h dark at 25±2 °C and 93% relative humidity. The development state was observed, and the number of survivors at every stage of development was recorded every other day and compared with that of a control.

Of the methanol extracts of several hundred species of terrestrial and marine plants tested, the extract of the leaves of *Xanthium canadense* showed remarkable inhibitory ac-



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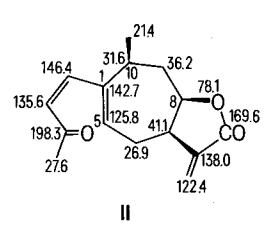

II

Number of survivors observed every other day after seeding 10 newly laid eggs of *D.melanogaster* on rearing medium containing xanthumin and 8-epi-xanthatin

Day*	Xanthumin						8-epi-Xanthatin					
	2	4	6	8	10	12	2	4	6	8	10	12
Dose**												
5.0	$1L_s$	$1L_s$	0	0	0	0	$2L_s$	$2L_s$	$2L_s$	$2L_s$	$1L_s$	0
2.5	$6L_s$	$6L_s$	$6L_s$	$5L_s$	$2L_s$	0	$6L_s$	$2L_s$	$2L_s$	$1L_s$	0	0
1.3	$10L_s$	$10L_s$	$10L_s$	$7L_s3P_s$	$1L_s7P_s$	$1L_s7P_s$	$8L_s$	$8L_s$	$8L_s$	$8L_s$	$5L_s3P_s$	$1L_s7P_s$
0.6	10L	10L	10L	10P	10P	10A	10L	10L	10L	10P	10P	10A
Control	10L	10L	10L	10P	10P	10A	10L	10L	10L	10P	10P	10A

* After seeding; ** mg in 2 g medium. L: larva, P: pupa, A: adult; s: smaller than control.

tivity against the larval growth. By conventional solvent partition and subsequent chromatography, 2 compounds: *I* colorless crystals, m.p. 100–100.5 °C, $[\alpha]_D$ −49° (c = 1.0, dioxane), and *II* a viscous oil, $[\alpha]_D$ +70° (c = 1.0, dioxane) have been isolated as the active principles. Larval growth inhibitory activities of *I* and *II* are shown in the table.

These compounds have been identified as xanthumin *(I)* and 8-epi-xanthatin *(II)* by comparison of their m.p., $[\alpha]_D$, UV, IR and ^{1}H-NMR spectral data with those reported in the literature[9]. All the ^{13}C-NMR signals have been assigned. Their chemical shifts ($\delta^{CDCl_3}_{TMS}$) are shown with the structures.

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2 This investigation was supported in part by grants (No.056141 and No.376065) from the Ministry of Education of Japan. The authors express their sincere thanks to Prof. Junkichi Iwasa of this department for his encouragement and to Dr Kenji Uneyama, Department of Industrial Chemistry of this University, for ^{13}C-NMR measurements.

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Inhibition of paraquat phytotoxicity by a novel copper chelate with superoxide dismutating activity[1]

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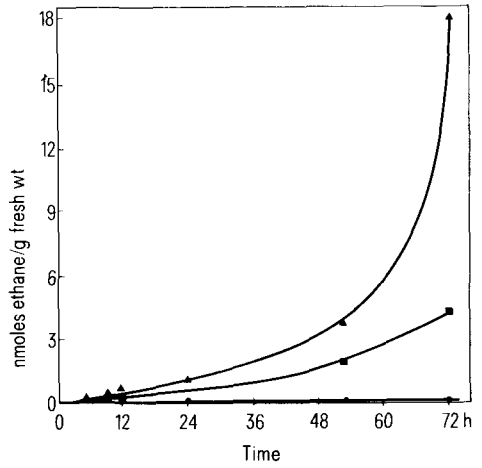
Summary. A chelate with superoxide dismutase activity, D-penicillamine copper complex, was shown to inhibit paraquat toxicity in flax cotyledons (*Linum usitatissimum* var. Reina). Paraquat-stimulated chlorophyll loss and ethane production were markedly reduced by this complex. The role of superoxide in the action of paraquat is briefly discussed.

It is well-known that the action of the herbicide paraquat is dependent upon light and oxygen[2]. In treated plants as in the normal photosynthetic reaction, light induces chloroplast electron transport which leads to a reduction of paraquat by a one electron transfer process. The immediate reoxidation of this free radical by oxygen was shown to generate hydrogen peroxide[3], which was thought to be the primary toxic agent. However, more recent work has suggested that one electron transfer to oxygen initially gives rise to the superoxide free radical[4]. Superoxide production mediated by paraquat (= methyl viologen) has been demonstrated in experiments with isolated chloroplasts[5]. Although superoxide dismutase enzymes are present within the chloroplast[6], it is assumed that the level of superoxide formed in vivo following treatment of the leaves with paraquat is in excess of the capabilities for enzymic dismutation and this leads to cellular damage[7]. In the present study, we have provided evidence for the generation of superoxide in vivo by the use of a superoxide dismutating copper chelate of D-penicillamine.

The reaction of Cu(II) with D-penicillamine results in the formation of a mixed valence Cu(I) Cu(II) cluster with a mol.wt of about 2200[8,9]. Experiments, also with isolated



Ethane generation by flax cotyledons. The conditions were as detailed for the table. Ethane was determined as described previously[20] in a Varian Aerograph model 1400 gas chromatograph. ●——● Control; ▲——▲ paraquat treated; ■——■ paraquat plus PA-Cu.