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

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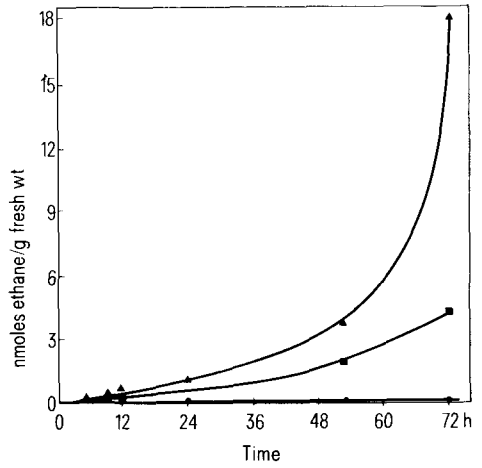
Summary. A chelate with superoxide dismutase activity, D-penicillamine copper complex, was shown to inhibit paraquat toxicity in flax cotyledons (*Linum usitatissimum* var. Reina). Paraquat-stimulated chlorophyll loss and ethane production were markedly reduced by this complex. The role of superoxide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

chloroplasts, have shown that this D-penicillamine copper chelate (PA-Cu) has an action similar to the Cu-Zn and Mn superoxide dismutase enzymes in a range of reactions involving superoxide[10].

Flax cotyledon leaves were floated on solutions of paraquat and PA-Cu and the herbicidal effect was initially followed by assessing the breakdown of chlorophyll, the most obvious phytotoxic symptom. The table shows that after 72 h illumination, the breakdown of chlorophyll in paraquat treated leaves was significantly retarded by the additional presence of PA-Cu.

Previous work has shown that disruption of the tonoplast and plasmalemma were the earliest structural changes observed in leaf cells following paraquat treatment[11]. This is brought about by various reactions generally known as lipid peroxidation which are initiated by free radicals. It is unlikely that superoxide possesses the necessary reactivity to abstract protons from unsaturated fatty acids in the membrane to instigate this deteriorative chain reaction, it is more probable that initiation is by a more reactive species derived from superoxide, such as singlet oxygen[12] or .OH radicals[13]. The process of lipid peroxidation can be monitored by the release of ethane from the damaged tissue[14–16]. The figure shows that the release of this simple hydrocarbon from flax cotyledon leaves was considerably promoted by paraquat treatment, however this was minimised when PA-Cu was also present. Although PA-Cu has a high superoxide dismutating capability ($K = 10^9\ M^{-1}\ S^{-1}$)[17], it failed to completely prevent paraquat induced damage.

Chlorophyll levels in treated flax cotyledons

Treatment	Chlorophyll content (µg/g fresh weight)
Control	540
Paraquat (10^{-6} M)	300
Paraquat + PA-Cu	430

20 cotyledon leaves from 7-day-old flax seedlings were used for each treatment and were incubated in 70-ml screw top flasks fitted with serum rubber material to allow sampling of the flask atmosphere. The addition of paraquat was delayed for 24 h to allow PA-Cu to penetrate the leaf tissue. A concentration of 50 nmoles of PA-Cu in 3 ml was equivalent to 50 superoxide dismutase units, as determined by the ability to inhibit nitrite formation from hydroxylamine[10]. Chlorophyll was determined upon conclusion of the experiment (72 h illumination with 5500 lux), according to the method of Arnon[19].

This can be explained by the fact that only a very small proportion of the superoxide produced need escape dismutation for the damage process to assume a more complex nature which is then more difficult to control.

These experiments have provided good evidence for the actual generation of superoxide in vivo in paraquat treated leaves. They also demonstrate that PA-Cu, which is already in pharmaceutical use in the nonchelated form[18], might be efficacious in the reduction of paraquat poisoning in humans.

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Genetic changes of pupa weight in *Tribolium castaneum* under domestication[1]

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Summary. The evolution of the genetic and phenotypic parameters of pupa weight in 6 wild populations of *Tribolium castaneum* under domestication was studied. A gradual increase of the phenotypic and additive genetic variances of the populations was detected accompanied by small or non-significant changes of the mean. These results are interpreted as short-term consequences of the relaxation of centripetal (stabilizing) selection forces under laboratory conditions.

Natural selection is considered to be the main process responsible for the adaptation of populations to their particular environments. The action of natural selection is directly exerted on fitness and indirectly on any other trait genetically correlated with it. A consequence of this indirect selective action is the temporal change of the genetic and phenotypic distributions of quantitative traits[2]. It is of interest to study these changes in populations subject to a given environmental stimulus over a number of generations. These changes can be more easily observed when the