2 h. The reaction mixture was poured into crushed ice and acidified with hydrochloric acid. The residue was isolated and crystallized from ethanol to yield 2′-hydroxy-4′,6′,3,4-tetramethoxychalcone (2.75 g, 80%).

The 2′-hydroxy chalcones prepared now have been identified by mixed m.pt with samples available from our sample collection, co-TLC and also by ^{1}H n.m.r.

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CARBARYL AND ENDOSULPHAN INDUCED ALTERATIONS IN THE INTESTINAL α-AMYLASE ACTIVITY OF *PHERETIMA POSTHUMA*

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Our environment is getting polluted day by day due to human activities, urbanization and rapid industrialization. Almost all the agrochemicals which are used to kill the pests ultimately reach the soil and disturb the population of non-target organisms like earthworms[1,2] which are beneficial to our farmers as well as the future source of pollution control. The soil surface becomes highly contaminated due to the fall out of insecticides and worms are exposed to high concentrations of chemicals when they come out on the surface at night in search of their food. The importance of earthworms in the ecosystem is very well-reviewed[3]. The soil fertility is closely associated with the population of earthworms in the soil besides other factors and the population of earthworms is directly related with the microclimatic conditions of the soil.

It is well-known that earthworms are omnivorous[4]. They digest not only the dead organic matter of the soil but also decomposed animals, living protozoa, rotifers and other minute organisms[5]. The digestive enzymes of only a few oligochaetes have been studied[6,7]. The presence of amylase in the intestinal caecae of the segments 22–26 of *Pheretima*[6] and in every part of the gut was detected in *Lumbricus terrestris* and *Eisenia foetida*[7]. The effect of phosphamidon on α-amylase of *Pheretima posthuma* has been worked out[8]. We have attempted here to determine the effect of carbaryl (1-naphthyl methylcarbamate, Sevin) and endosulphan (1,2,3,4,10,10-hexachloro-1,5,5a–6,9,9a-hexahydro-6,9-methano 2,4,3-benzodioxathiepin-3-oxide, Thiodan) on the α-amylase (1,4-α-D-Glucan glucanohydrolase, E.C. 3.2.1.1.) activity of *P. posthuma*.

The mature worms *P. posthuma* were treated with 2 mg/kg, 4 mg/kg, and 8 mg/kg of carbaryl and endosulphan by moist soil treatment. The worms were sampled at 0.5 h, 1 h, 2 h, 4 h, and 24 h intervals alongwith the normal worms, washed first in tapwater and finally with distilled water. Each worm was incisioned, intestine was taken out and washed properly in chilled double distilled water. A 10% homogenate of whole intestine was prepared in

Table 1 *α-amylase activity in the intestine of Pheretima posthuma*

Normal value	Treatment	Exposure time (h) 0.5	1	2	4	24
0.24 ± 0.007	Carbaryl					
	2 mg/kg	0.220 ± 0.001 > 0.05	0.206 ± 0.012 > 0.05	0.187 ± 0.008 > 0.01	0.160 ± 0.008 > 0.001	0.108 ± 0.011 > 0.001
	4 mg/kg	0.201 ± 0.022 NS	0.183 ± 0.009 > 0.01	0.180 ± 0.013 > 0.01	0.133 ± 0.010 > 0.001	0.084 ± 0.006 > 0.001
	8 mg/kg	0.163 ± 0.013 > 0.01	0.132 ± 0.004 > 0.01	0.112 ± 0.002 > 0.001	0.096 ± 0.003 > 0.001	0.036 ± 0.006 > 0.001
0.26 ± 0.030	Endosulphan					
	2 mg/kg	0.251 ± 0.007 NS	0.250 ± 0.032 NS	0.234 ± 0.011 NS	0.213 ± 0.018 NS	0.178 ± 0.016 > 0.05
	4 mg/kg	0.248 ± 0.010 NS	0.223 ± 0.018 NS	0.198 ± 0.009 NS	0.171 ± 0.012 > 0.05	0.159 ± 0.006 > 0.02
	8 mg/kg	0.223 ± 0.006 NS	0.198 ± 0.012 NS	0.169 ± 0.008 NS	0.154 ± 0.007 > 0.02	0.127 ± 0.003 > 0.01

NS = Not significant. Mean value calculated from 4 determinations ± SE. Probability evaluated by Student's *t* test. Unit of enzyme μmol of maltose liberated/min/g fresh tissue equivalent.

chilled double distilled water for enzyme assay and it was centrifuged at 12,000 g for 20 min at −4°C. Amylase activity was assayed by the method of Ishaaya *et al*[9]. The réaction mixture contained 1 ml substrate solution (1% soluble starch, BDH in 0.05 M phosphate buffer) and 1 ml of enzyme extract. The reaction mixture was incubated at 25°C for 1 h. The reaction was stopped by adding 2 ml of 3,5 dinitrosalicylic acid reagent and the optical density was measured at 540 nm by Spectronic 21 Spectrophotometer.

The changes in the α-amylase activity of the intestine of *P. posthuma* exposed to different concentrations of carbaryl and endosulphan are presented in table 1. Worms exposed to higher concentration of carbaryl (4 and 8 mg/kg) started wriggling out of the soil after 4 h but again kept under the soil to avoid dehydration. Carbaryl caused immobility and rigidity, and midsegmental swellings after 24 h beyond the clitellum. This type of behavioural and morphological change was not seen in endosulphan treatment.

The activity of α amylase in all the three concentrations in the carbaryl-exposed earthworms decreased significantly from 1 h to 24 h treatment except in 30 min exposure where the inhibition was very little. The inhibition of activity was dose- and exposure time-dependent. Phosphamidon also caused significant inhibition in α-amylase activity of *P. posthuma* in our previous studies[8]. But in the case of endosulphan the degree of inhibition was found less as compared to carbaryl. It is evident that phosphamidon (organophosphate) and carbaryl (carbamate) behave in the same way on α-amylase but endosulphan (organochlorine) is not the potent α-amylase inhibitor.

Amylase is secreted in the pharynx by the pharyngeal gland itself and pharyngeal nephridia[10]. Thus it is clear that the digestion of starch begins in the pharynx. High concentration of amylase is also secreted in the intestinal caecae attached to intestine as well as intestinal tissue. After passing through the pharynx the semi-digested food gets amylase in the anterior intestine and the rest of the starch is digested here. Thus the starch appears to be the most important carbohydrate to get energy for very active life of the worm. Present investigations revealed that due to carbaryl, the amylase activity was inhibited to an appreciable extent. Thus it is clear that the worm cannot utilize this most important and energy giving food material in the carbaryl-treated plots and the vital activity of the worm like continuous burrowing would be affected.

The implication of abuse of several potentially toxic pesticides at much larger quantities and contamination of our soil and water and the consequences on organisms like earthworms which are beneficial to soil fertility deserve extensive investigations. Further work in this direction is in progress.

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INCREASED AUTO-OXIDATION OF L-ASCORBATE BY IRON-TREATED RAT TISSUES

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In a previous communication[1] we have demonstrated that human blood plasma and erythrocytes significantly inhibit L-ascorbate oxidation *in vitro*. Similar antioxidant reactivities towards L-ascorbate oxidation are present in rat serum and brain[2], and in rat kidney and liver[3]. Iron overload increases the *in vivo* lipid peroxidation in rat liver organelles[4,5]. Recently Bacon *et al*[6] reported significant increase in diene formation indicating lipid peroxidation in liver mitochondria and microsomes of iron-treated rats. Iron-overload increases total ethane and pentane release, volatile products of fatty acid hydroperoxide decomposition, by all tissues in the rat except the brain[7]. Ascorbic acid is a known antioxidant and oxygen radical scavenger[8]. Furthermore, ascorbic acid functions as a specific antioxidant towards lipid peroxidation in mammalian and rat tissues[9–12]. The presence of an antioxidant substance towards ascorbate oxidation, in the tissues, is therefore a beneficial factor, to maintain the vitamin in its reduced form. The effect of tissue homogenates from iron overloaded rats on ascorbate auto-oxidation on incubation in air is reported here.

Transitional metals like Fe^{2+} or Cu^{2+} are known promoters of ascorbate oxidation. To achieve minimum interference from metal ion contamination all glassware were washed first with 30% HNO_3 followed by washing with deionized double-distilled water. Similarly all reagents were prepared in the same deionized double-distilled water. Male albino rats (150–200 g) of this College strain maintained on a commercial pellet diet were used in this study. One group of 15 rats was given intra-peritoneal injections of iron-dextran 100 mg/kg body weight on alternate days for seven days to produce iron-overload. Another group of the same number of rats received intra-peritoneal injections of saline in place of iron-dextran and served as controls. The animals were continued on stock pellet diet and water *ad lib* during the experiment. The dosage of iron used did not lead to any over-toxic symptoms or loss of body weight. The animals were sacrificed one day after the last injection after overnight fast by withdrawing as much blood as possible from the heart into heparinized syringes while under light ether anaesthesia. The red blood cells (RBC) and plasma were separated immediately and used for the study on the same day. Brain, kidney, liver, heart and lung were extirpated, washed free of blood with ice-cold normal saline and homogenized (10% w/v) in saline phosphate buffer (5 mM sodium phosphate buffer containing 0.15 M NaCl, pH 7.4) prepared in double distilled water. The crude homogenates were centrifuged at 500 *g* for 10 min to remove the cell debris and unbroken cells. The unoxidized ascorbic acid was assayed as described earlier[1] and the mixture (10 ml) containing 0.2 mg of L-ascorbate (113.7 μM) in the saline phosphate buffer was incubated at 37°C with shaking for the required time intervals. The ascorbate concentration employed was within the physiological limits as present in human plasma. Two ml aliquots was withdrawn and the absorbance at 265 nm measured for determining the amount of oxidized ascorbic acid[13]. The inhibitory activity was measured by incorporating plasma, RBC, and tissue homogenates equivalent to 140 μg protein in the buffer. Aliquots of tissue homogenates and plasma were analysed for the total iron content following the method of Ramsay[14] and proteins by the method of Reinhold[15].

The mean ± SD values of iron content from the tissues and plasma of control and iron-treated rats are summarized in table 1. All the tissues excepting brain were overloaded with iron when compared to controls, the maximum increase being registered in