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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

Table 1 *Distribution of iron in tissues after iron treatment*

Contents	Controls (μg/mg protein)	Iron-treated (μg/mg protein)
Liver	0.7 ± 0.035	7.14 ± 0.14
Kidney	0.37 ± 0.02	1.07 ± 0.03
Brain	0.23 ± 0.06	0.25 ± 0.05
Heart	0.14 ± 0.02	0.22 ± 0.01
Lung	0.14 ± 0.01	0.23 ± 0.01
Plasma	0.04 ± 0.001	0.64 ± 0.009

Values are the mean ± SD from 15 different rats from each group.

liver, kidney and plasma. In table 2 is given the effect of addition of tissue homogenates and plasma of iron-treated and control rats on the rate of auto-oxidation of ascorbic acid on incubation in air under assay conditions as described earlier. All the tissues of control rats showed a definite antioxidant property on ascorbic acid auto-oxidation. However the tissues from iron-treated rats excepting brain were less effective than the corresponding tissues of the control rats in preventing auto-oxidation. The above results indicate that in the iron-overloaded rats the antioxygenic potential of the tissues towards ascorbate auto-oxidation is definitely suppressed as compared to the control tissues. The maximum effect was observed in the kidney followed with liver and heart, lung and plasma. The differences were found to be statistically significant by the application of Student's t test with the $P < 0.001$ in each case. However, in the case of brain homogenates, the difference between test and control was marginal and was not statistically significant. Gavino *et al*[7] reported that in rats intra-peritoneal injections of 100 mg iron-dextran resulted in an increased level of iron in all tissues excepting the brain. Our results (table 1) confirm the above findings although it may be noted that in our experiments the rats were administered nearly 140 mg of iron-dextran during the course of 14 days. It was also reported[7] that in iron overloading of vitamin E-deficient rat lipid peroxidation of the tissues was markedly increased with the kidney showing maximum susceptibility. In the present study, maximum inhibition of ascorbatic auto-oxidation was seen in the kidney.

The ascorbate is shown to function as a potential antioxidant in tissues[9,10] while iron is a catalyst of lipid peroxidation[16]. Gavino *et al*[7] reported that the increased concentration of iron in the tissues enhances lipid peroxidation. The present results show that the inhibitory factor that prevents L-ascorbate auto-oxidation normally present in the tissues, is depressed in the iron-overloaded rat tissues. It is possible therefore to hypothesize that the enhancement of lipid peroxidation in rat tissues of iron overload as reported earlier[6,7] may at least be partly correlated with the decreased capacity of tissues in preventing the auto-oxidation of ascorbic acid, a natural antioxidant thus reducing the overall antioxygenic potential of the tissues against lipid peroxidation.

Table 2 *Auto-oxidation rate of ascorbic acid on incubation in air at 37°C (pH 7.4) as measured by absorbancy at 265 nm in the presence of iron-treated and control rat tissues*

Contents	Controls		Iron-treated		(T − C)*
Additions	Fe-content (μg/140 μg protein)	μmol ascorbate-oxidized	Fe-content (μg/140 μg protein)	μmol ascorbate-oxidized	Difference (%)
Liver	0.098	0.025 ± 0.002	0.99	0.046 ± 0.003	84.00
Kidney	0.051	0.023 ± 0.002	0.15	0.065 ± 0.002	182.00
Brain	0.032	0.04 ± 0.003	0.035	0.043 ± 0.003	7.50
Heart	0 019	0.045 ± 0.003	0.03	0.06 ± 0.002	33.30
Lung	0.019	0.045 ± 0.002	0.03	0.06 ± 0.002	33.30
RBC	(-)	0.06 ± 0.002	(-)	0.08 ± 0.002	33.30
Plasma	0.005	0.05 ± 0.002	0.09	0.065 ± 0.002	30 00

The incubation mixture contains 1.135 μmol ascorbate. The amount of ascorbate oxidized in μmol in buffer alone is 0.17 ± 0.01 per h on incubation in air; Tissue homogenates and plasma were used at a concentration of 140 μg protein in the incubation mixture. Results are a mean ± SD from 15 different rats. The differences between the control and test excepting brain is statistically significant ($P < 0.001$) Brain not statistically significant; * The values indicate the percentage increase of ascorbate oxidation in iron-treated over that of controls; (-) The iron content in RBC was not estimated

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DISTRIBUTION AND ABUNDANCE OF BENTHIC DIPTERAN INSECTS IN HOOGHLY ESTUARY, SAGAR ISLAND, INDIA

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ONLY a fraction of the total insect population is marine and estuarine. Dipterans by their abundance and diversity constitute a major group of marine and estuarine insects; they spend part of their life in the littoral belt as benthic fauna[1]. The present note outlines the characteristics, composition distribution and variation of estuarine benthic dipterans in the littoral zone of the Hooghly estuary.

Larval and pupal populations were sampled from the soil every fortnight during July 1982 to June 1984 from a selected station situated in the south-eastern sector of the Sagar Island. They were recovered from the soil by standardized magnesium sulphate flotation technique[2], counted and expressed as no./25 cm^2. Simultaneously with sampling, various environmental parameters, viz., air temperature, soil temperature, soil moisture, rainfall, salinity, dissolved oxygen, pH of interstitial water, organic carbon, available phosphorus and texture of the soil were also recorded following standard methods[3]. Correlation coefficient analysis was made to understand the relationship with different environmental parameters and species populations.

The ecological parameters during different months are highly dynamic and present a cyclic pattern (figure 1a and b). In total, 13 species of dipterans have been recorded of which 7 species under the family Ceratopogonidae, 1 species each under the families Chironomidae and Psychodidae, 2 species both under the families Ephydridae and

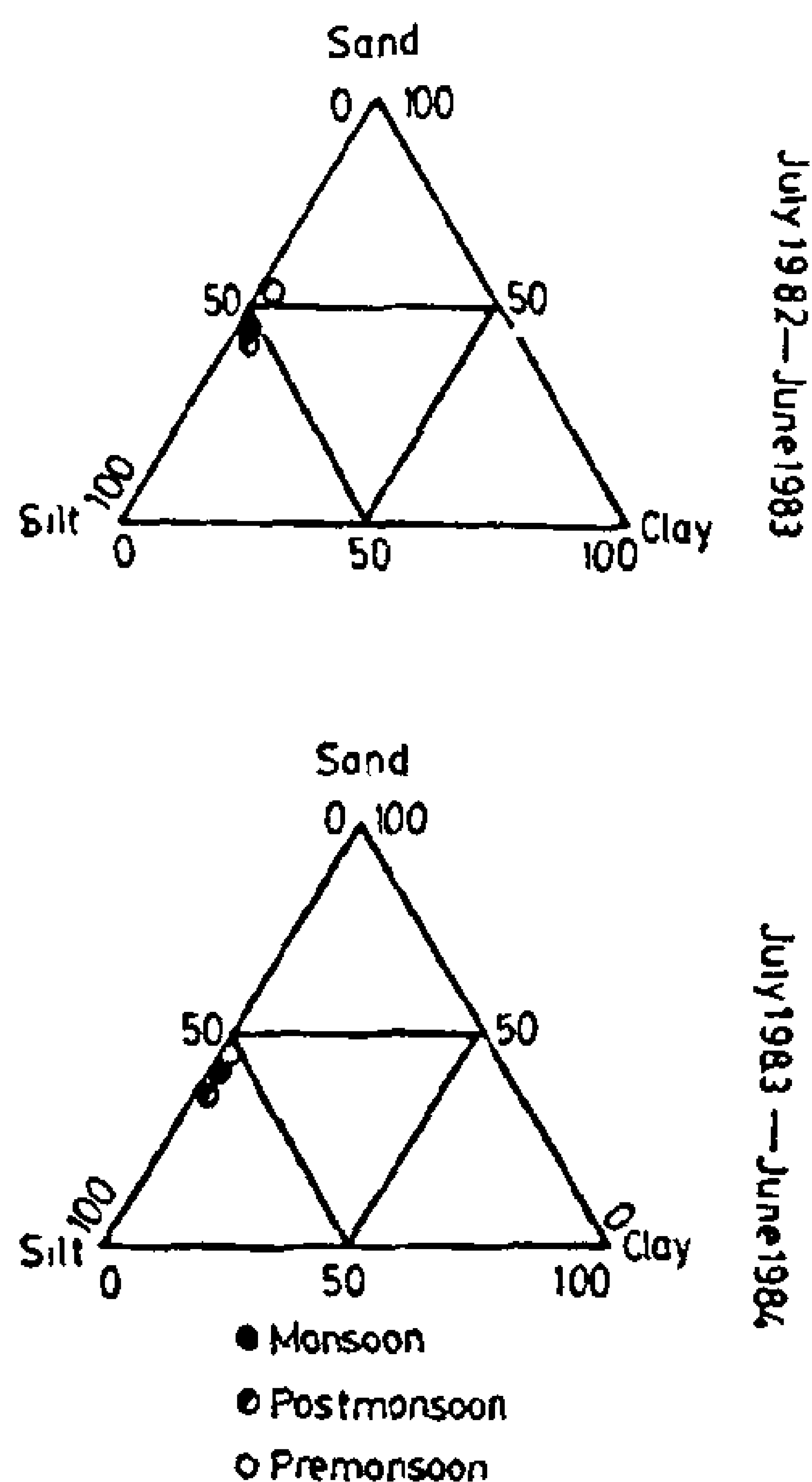

Figure 1b. Seasonal variation of the percentages of sand, silt and clay.