

Studies on L-Ascorbic Acid Metabolism in Rats Under Chronic Toxicity Due to Organophosphorus Insecticides: Effects of Supplementation of L-Ascorbic Acid in High Doses¹

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ABSTRACT The effects of chronic administration of two organophosphorus insecticides, parathion and malathion on the growth rate, ascorbic acid metabolism and some other nutritional and physiological parameters in rats were studied. Both parathion and malathion toxicity retarded the growth rate of rats. Inhibition of brain acetylcholinesterase was taken as an index of organophosphorus insecticide toxicity. Haemoglobin concentration of blood and organ weights were not affected under the toxic conditions. Parathion and malathion administration stimulated the activity of L-gulonolactone oxidase along with a simultaneous increase in the tissue storage and urinary excretion of vitamin C. The activities of other enzymes of ascorbic acid metabolism, dehydroascorbate, uronolactonase, and L-gulonate dehydrogenase and decarboxylase were altered under the experimental conditions. Only minor histological changes of the liver and kidney tissues were noted under parathion and malathion toxicities. Excess intake of vitamin C under the toxic conditions was found to be very effective in counteracting the growth retardation and also the alterations produced by parathion and malathion both at the enzymatic and histological levels. *J. Nutr.* 108: 973-980, 1978.

INDEXING KEY WORDS parathion · malathion · toxicity · ascorbic acid

Recent decline in the use of chlorinated hydrocarbon insecticides, especially DDT and lindane has resulted in a substantial increase in the use of organophosphorus insecticides, which are far less resistant to chemical break-down than the persistent insecticides. Organophosphorus insecticides owe their lethal properties to the marked anticholinesterase activity (1). Our earlier studies using various environmental agents have shown that many of these compounds can significantly alter vitamin C metabolism in rats, either at the level of tissue storage of this vitamin or at the level of activities of the enzymes metabolizing vitamin C and

excess intake of vitamin C has been found, in certain cases, to be beneficial in partially reversing some of the toxic effects of these environmental factors (2-6). In this communication, studies have been carried out on the effects of chronic administration of two organophosphorus insecticides, parathion and malathion, on L-ascorbic acid metabolism in rats and also on several physi-

Received for publication October 26, 1977.

¹This work has been financed by the University Grants Commission, India.
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ological parameters like growth rate, organ weights, haemoglobin concentration, histological pattern of liver and kidney tissues etc. in rats as experimental animals. Investigations have also been carried out to find out whether excess intake of vitamin C can reverse the toxic signs produced by these organophosphorus compounds. Inhibition of brain acetylcholinesterase activity has been taken as an index of organophosphorus insecticide toxicity in rats.

ANIMAL EXPERIMENTS

Male albino rats³ weighing 30 to 35 g which had been fed basal diet (7) were divided into six groups. The first group of rats which served as control were given basal diet only. Rats of the second and third groups received along with the basal diet an oral administration of parathion⁴ in olive oil at a dose of 1 mg/kg body weight/day. The rats in the control group were given an equal amount of olive oil. In addition to parathion administration rats of group three were given an oral supplementation of L-ascorbic acid at a dose of 20 mg/100 g body weight/day. Experiments were designed similarly using malathion⁵ at a dose of 200 mg/kg body weight/day with the remaining three groups of rats (groups 4, 5, and 6). The rats of groups 1 and 3 were pair fed with respect to the rats of group 2 and those of groups 4 and 6 were pair fed with respect to the rats of group 5. Each of the rats was given an oral supplementation of B vitamins: (in μg) thiamin, 100; riboflavin, 100; niacin, 200; calcium pantothenate, 400; pyridoxin·HCl, 50; biotin, 2; folic acid, 4; B₁₂, 0.4 and (in mg) inositol, 2 and choline chloride, 30 daily; 0.1 ml of a concentrate⁶ of vitamin A and cholecalciferol once in a week. The rats were weighed biweekly, and killed by decapitation after 15 days of experimental period, and the tissues were used for biochemical investigations.

ANALYTICAL PROCEDURE

Details of the methods for the determination of total ascorbic acid of the tissues and preparation of rat liver and kidney homogenates, of the test systems for studying the dehydroascorbate activity and the synthesis of L-xylulose were the same as

described by Mukherjee et al. (8). The incubation media used for the biosynthesis of L-ascorbic acid from L-gulonolactone was the same as described by Chatterjee et al. (7). The urinary excretion of L-ascorbic acid was determined titrimetrically against 2,6-dichloroindophenol-phenol (9) and of D-glucuronic acid was measured by the carbazole reaction according to the method of Dische (10). The activity of uronolactonase was determined by the method described by Eisenberg and Field (11). Protein was determined by the Biuret method (12). Haemoglobin concentration of blood was measured against International standard by cyanmethemoglobin method as described by Dacie and Lewis (13). Acetylcholinesterase activity was measured according to the method of Ellman (14) in the 1,500 × g supernatant fraction of whole brain homogenate.

In order to eliminate the effect of L-ascorbic acid supplementation a separate control group was maintained which was treated with L-ascorbic acid only at a dose of 20 mg/100 g body weight/day. It has been noted⁷ that the L-ascorbic acid supplemented control group of rats do not show any appreciable difference from the normal control group with respect to any of the parameters studied. Hence results of only control group have been presented in this communication.

The evaluation of *P* values was made from the Fisher and Yates statistical table (15).

RESULTS

No gross degenerative changes in the normal histological pattern of liver (figs. 1 and 2) or kidney (figs. 3 and 4) tissues were noted under either parathion or malathion toxicity. Appearances of intercellular spaces and increase in the number of cytoplasmic vacuoles and eosinophilic granules were noted in the liver cells of parathion and malathion treated rats. The nuclei

³ Wistar strain, obtained from CIBA Pharmaceuticals, Bombay, India.

⁴ Parathion (99% pure) was manufactured by Central Insecticides and Fertilizers, Bombay, India.

⁵ Malathion (99% pure) was manufactured by Sandoz India Ltd., Bombay, India.

⁶ Adexoln, Glaxo Laboratories, Bombay, India (12,000 IU of vitamin A and 2,000 IU of cholecalciferol per ml).

⁷ Chakraborty, D. in Ph.D. thesis, Studies on L-ascorbic acid metabolism: 1977, Calcutta University.

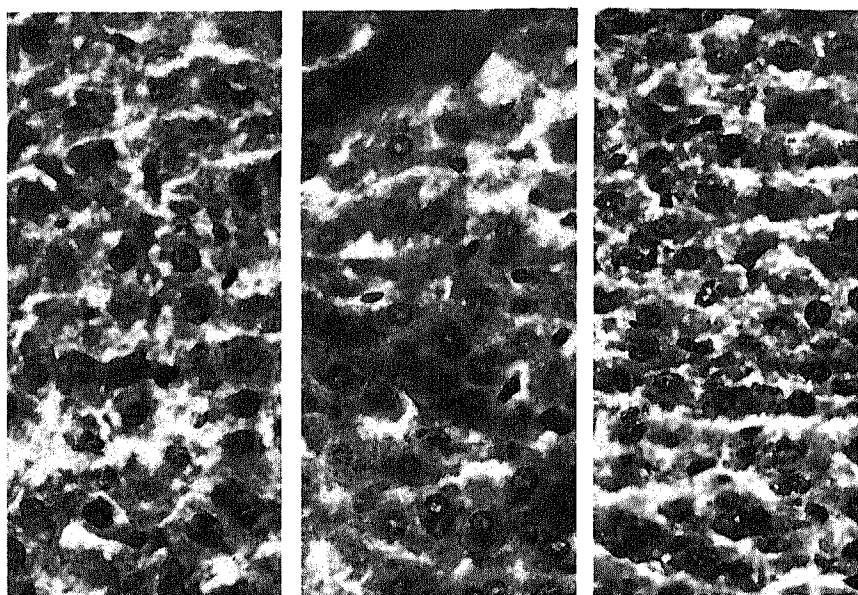


Fig. 1 Histological examination of liver tissues of rats under parathion administration (eosin-hematoxylin). (a) Normal liver cells ($\times 575$). (b) Liver cells of parathion treated rats ($\times 575$). (c) Liver cells of Vitamin C supplemented parathion treated rats ($\times 575$).

showed diffused chromatin bodies under the experimental conditions. After supplementation of L-ascorbic acid to the toxic groups of rats the intercellular spaces disappeared and the cytoplasmic vacuoles and eosinophilic granules became less abundant, but the nuclei still showed diffused chromatin bodies. In the case of kidney tissues, under parathion and malathion toxic conditions there was an increase in the space between glomerulus and the capsule in the

nephrons. Occasional hemorrhages in the kidney tissues were also noted under these conditions. There was an increase in the eosinophilic granules. Supplementation of L-ascorbic acid to the toxic groups of rats could reverse these defects significantly.

Chronic administration of parathion and malathion to rats brought about a considerable lowering in the growth rate (table 1). Subsequent supplementation of L-ascorbic acid to these rats could definitely counter-

TABLE 1

Effect of chronic administration of parathion and malathion on the changes in growth rate, haemoglobin concentration and organ weights of rats: Influence of L-ascorbic acid supplementation

Groups	Changes in growth rate from initial weight	Haemoglobin concentration	Organ weight/body weight (%)	
			Liver	Kidney
	<i>g</i>	<i>g%</i>		
1. Basal diet fed	+41.6 \pm 2.8 ^{1,2}	9.6 \pm 0.8	3.3 \pm 0.1	0.70 \pm 0.06
2. + Parathion	+28.2 \pm 1.8 ^a	9.2 \pm 0.7	3.4 \pm 0.3	0.71 \pm 0.05
3. + Parathion + L-ascorbic acid	+36.7 \pm 1.6 ^b	9.6 \pm 0.6	3.6 \pm 0.3	0.69 \pm 0.07
4. Basal diet fed	+40.2 \pm 2.4	9.4 \pm 0.8	3.1 \pm 0.1	0.70 \pm 0.06
5. + Malathion	+30.1 \pm 1.8 ^a	9.2 \pm 0.6	3.1 \pm 0.2	0.72 \pm 0.07
6. + Malathion + L-ascorbic acid	+36.2 \pm 2.8 ^c	9.4 \pm 0.7	3.3 \pm 0.3	0.73 \pm 0.06

¹ Each result is expressed as mean value \pm SD of 5 rats per group. ² Groups 2 and 3 have been compared to group 1 and groups 5 and 6 have been compared to group 4; mean value significantly different from basal diet fed group at ^a $P < 0.001$, ^b $P < 0.01$, or ^c $P < 0.05$.

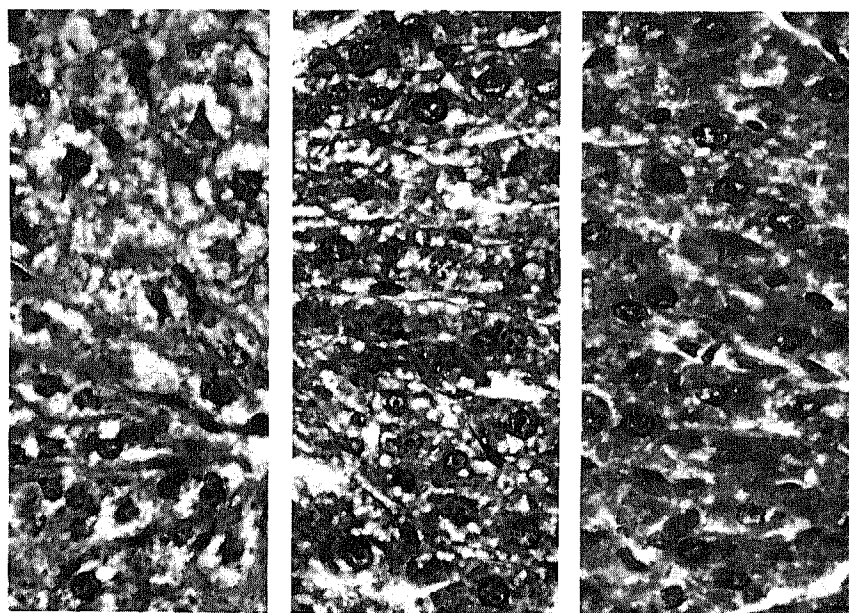


Fig. 2 Histological examination of liver tissues of rats under malathion administration (eosin-hematoxylin). (a) Normal liver cells ($\times 575$). (b) Liver cells of malathion treated rats ($\times 575$). (c) Liver cells of Vitamin C supplemented malathion treated rats ($\times 575$).

act this growth retardative effect of parathion and malathion. Liver and kidney weights were, however, not altered under these toxic conditions. Both parathion and malathion administration could increase L-ascorbic acid levels of liver and kidney tissues significantly, but no change was observed in the adrenal content of L-ascorbic acid (table 2). Haemoglobin concentration

in the blood of the rats was not changed under the experimental conditions (table 1). Urinary excretion of L-ascorbic acid and of D-glucuronic acid was raised considerably after chronic administration of both parathion and malathion (table 2).

Oral administration of both parathion and malathion stimulated the activity of L-gulonolactone oxidase but depressed the

TABLE 2
Effect of chronic administration of parathion and malathion on the urinary excretion of L-ascorbic acid and D-glucuronic acid and tissue concentration of L-ascorbic acid: Influence of L-ascorbic acid supplementation

Groups	Urinary excretion of		Tissue concentration of L-ascorbic acid		
	L-ascorbic acid	D-glucuronic acid	Liver	Kidney	Adrenal
	mg/24 hr	mg/24 hr	mg/100 g		
1. Basal diet fed	1.6 \pm 0.1 ^{1,2}	2.1 \pm 0.1	28.5 \pm 1.6	20.5 \pm 1.2	681 \pm 24
2. + Parathion	2.9 \pm 0.1 ^a	3.0 \pm 0.2 ^a	38.0 \pm 2.0 ^a	24.4 \pm 1.4 ^a	724 \pm 47
3. + Parathion + L-ascorbic acid	4.7 \pm 0.1 ^a	3.1 \pm 0.2 ^a	43.1 \pm 2.2 ^a	28.7 \pm 2.4 ^a	832 \pm 42 ^a
4. Basal diet fed	1.7 \pm 0.1	2.0 \pm 0.1	26.7 \pm 1.4	18.6 \pm 1.2	637 \pm 34
5. + Malathion	3.1 \pm 0.2 ^a	2.8 \pm 0.2 ^a	38.3 \pm 1.1 ^a	23.4 \pm 1.7 ^a	678 \pm 54
6. + Malathion + L-ascorbic acid	4.4 \pm 0.2 ^a	3.6 \pm 0.2 ^a	44.6 \pm 2.1 ^a	26.4 \pm 2.0 ^a	853 \pm 48 ^a

¹ Each result is expressed as mean value \pm SD of five rats per group. ² Groups 2 and 3 have been compared to group 1 and groups 5 and 6 have been compared to group 4 with mean value significantly different from basal diet fed group at $P < 0.001$.

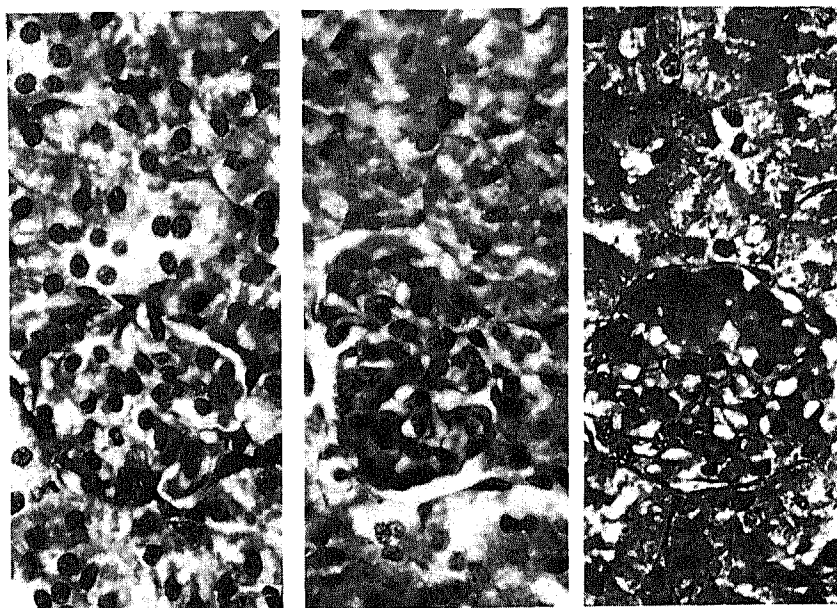


Fig. 3 Histological examination of kidney tissues of rats under parathion administration (eosin-hematoxylin). (a) Normal kidney cells ($\times 575$). (b) Kidney cells of parathion treated rats ($\times 575$). (c) Kidney cells of Vitamin C supplemented parathion treated rats ($\times 575$).

activity of dehydroascorbate (table 3). The synthesis of L-xylulose was also inhibited in the kidney tissues of the toxic rats. Subsequent supplementation of L-ascorbic acid to the toxic groups of rats could partially counteract these enzymatic altera-

tions produced by organophosphorus insecticides.

The activity of brain acetylcholinesterase was inhibited under either parathion or malathion (table 4). Vitamin C supplemented groups of rats exhibited a signifi-

TABLE 3

Effect of chronic administration of parathion and malathion on the biosynthesis and catabolism of L-ascorbic acid, uronolactonase activity and L-xylulose biosynthesis in rats: Influence of L-ascorbic acid supplementation

Groups	Activity of L-gulonolactone oxidase (nmoles of ascorbic acid synthesized/mg of protein)	Activity of dehydroascorbate (μ moles of 2:3 dioxogulonic acid formed/mg of protein)	Activity of uronolactonase (μ moles of p-glucuronolactone hydrolysed/mg of protein)	Activities of L-gulonate dehydrogenase and L-gulonate decarboxylase (μ moles of L-xylulose formed/mg of protein)
1. Basal diet fed	33.6 \pm 2.1 ^{1,2}	0.58 \pm 0.02	1.9 \pm 0.08	0.36 \pm 0.03
2. +Parathion	42.7 \pm 2.2 ^a	0.49 \pm 0.04 ^a	2.0 \pm 0.13	0.27 \pm 0.02 ^a
3. +Parathion + L-ascorbic acid	37.8 \pm 2.3 ^b	0.53 \pm 0.04 ^c	2.1 \pm 0.16	0.29 \pm 0.01 ^a
4. Basal diet fed	31.5 \pm 1.7	0.61 \pm 0.04	1.9 \pm 0.07	0.38 \pm 0.02
5. +Malathion	44.3 \pm 1.6 ^a	0.52 \pm 0.03 ^a	1.9 \pm 0.09	0.28 \pm 0.02 ^a
6. +Malathion + L-ascorbic acid	39.6 \pm 2.2 ^a	0.55 \pm 0.04 ^a	1.8 \pm 0.14	0.32 \pm 0.02 ^a

¹ Each result is expressed as mean value \pm SD of five rats per group. ² Groups 2 and 3 have been compared to group 1 and groups 5 and 6 have been compared to group 4 with mean value significantly different from basal diet fed groups at ^a $P < 0.001$, ^b $P < 0.01$, ^c $P < 0.02$, or ^d $P < 0.05$.

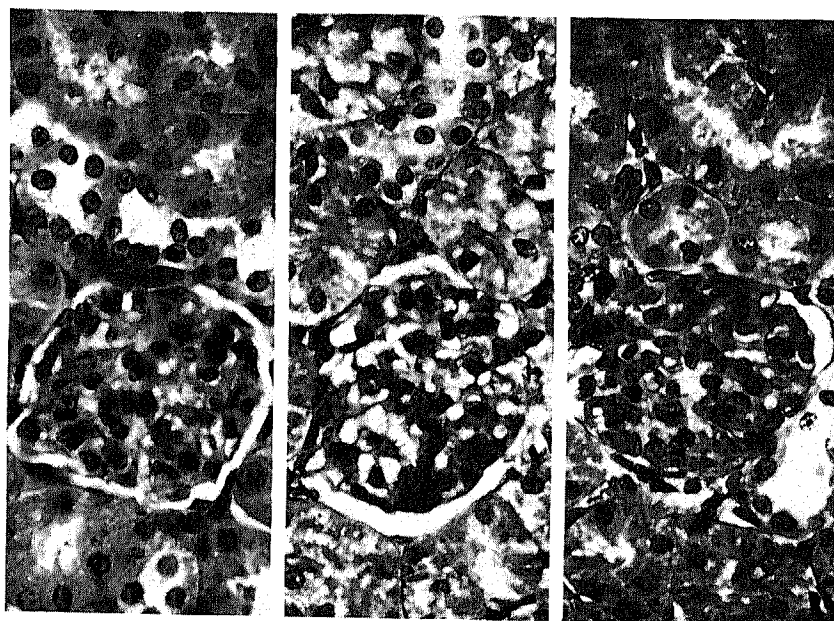


Fig. 4 Histological examination of kidney tissues of rats under malathion administration (eosin-hematoxylin). (a) Normal kidney cells ($\times 575$). (b) Kidney cells of malathion treated rats ($\times 575$). (c) Kidney cells of Vitamin C supplemented malathion treated rats ($\times 575$).

cant decrease in this effect produced by these organophosphorus insecticides.

DISCUSSION

Administration of both parathion and malathion to rats brings about a significant growth retardation, which can be reversed partially by excess intake of vitamin C under the toxic conditions. Stimulation in the activity of L-gulonolactone oxidase

under organophosphorus insecticide toxicity conditions is reflected in the increased tissue concentrations and urinary excretion of vitamin C. The anticholinesterase activity (1) as exhibited by administration of organophosphorus insecticides has been found to be reduced by subsequent administration of vitamin C. The administration of high doses of vitamin C is effective in partially reversing some of the alterations pro-

TABLE 4

Effect of chronic administration of parathion and malathion on the activity of brain acetylcholinesterase: Influence of L-ascorbic acid supplementation

Groups	Activity of acetylcholinesterase (μg of acetylcholine hydrolyzed/min/mg of protein)
1. Basal diet fed	$16.5 \pm 1.0^{1,2}$
2. + Parathion	8.6 ± 0.8^a
3. + Parathion + L-ascorbic acid	11.4 ± 1.1^a
4. Basal diet fed	15.2 ± 0.8
5. + Malathion	10.6 ± 0.6^a
6. + Malathion + L-ascorbic acid	12.1 ± 1.1^a

¹ Each result is expressed as mean value \pm SD of five rats per group. ² Groups 2 and 3 have been compared to group 1 and groups 5 and 6 have been compared to group 4 with mean value significantly different from basal diet fed group at $^a P < 0.001$.

duced by these insecticides either at the enzymatic or histological level.

Many drugs and chemicals including some organochlorine compounds which can stimulate the activity of the hepatic drug oxidation system have been found to decrease the toxicities of several organophosphorus insecticides (16-21). It is also known that the activities of hepatic microsomal drug metabolizing enzymes are dependent on the vitamin C status of animals and depletion with respect to vitamin C can cause lowering in the activities of these enzymes with subsequent increase in the toxicity towards several organochlorine insecticides (22-25). Results in this communication clearly indicate that when rats under toxicity conditions produced by organophosphorus insecticides are supplemented with a high level of vitamin C, some of the toxicity signs can be reversed. The exact mechanism by which vitamin C functions in reversing some of the toxicity signs is not clearly known. Incidentally it may be mentioned that preliminary observations indicate inability of reduced glutathione in replacing vitamin C in this function. It has been shown by Zannoni and Sato (23) that vitamin C has a role in influencing the activity of the hepatic microsomal drug oxidation system in guinea pigs. It is therefore reasonable to assume that excess administration of vitamin C to rats made toxic by organophosphorus compounds e.g. parathion or malathion, causing reversal of some of the toxicity signs, does act by increasing the excretion of these toxic compounds by increased detoxification. This increased detoxification might occur as a result of stimulation in the activity of hepatic microsomal drug metabolizing enzymes under the influence of high vitamin C intake since this vitamin is present in the system of toxic animals was probably not adequate for optimum activity of the hepatic microsomal drug oxidation system.

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