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SOME METABOLIC ALTERATIONS INDUCED BY AN ORGANOPHOSPHATE INSECTICIDE, MALATHION, ON CHICK MUSCLE*

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Abstract: An organophosphate insecticide malathion (57 EC) was administered orally to seven week old broilers as three different doses *i.e.*, 600, 400 and 250 mg (a.i) /kg body weight/day for the total durations of 40 hours, 12 days and 4 weeks, and were designated as short term (ST), long term-I (LT-I) and LT-II experiments, respectively. After stipulated periods, a group of 3-5 birds were sacrificed along with a group of control animals, their muscle samples were quickly excised and processed differently for various biochemical analyses.

The muscle acid phosphatase (AcP) activity, although showed a gradual rise in ST experiment but it was significant, with 32% rise, only at 40 hours. In LT-I treatment AcP activity shot up by 21 and 79% at 3 and 6 days malathion feeding. The alkaline phosphatase (AP) activity in ST experiment showed a significant increase of 40, 45 and 38% at 10, 20 and 40 hours, respectively. The glutamate pyruvate transaminase activity remained uneffected in ST and LT-I experiment until day 6 after which 100% rise at 12 day and 33, 29 and 33% rise at 2nd, 3rd and 4th week insecticide feeding was observed, respectively. Significant increase in free amino acids (FAA; 338% and 145%) and glycogen (63% and 38%) contents was also noticed at 5 and 10 hour treatments, respectively in ST experiment. The rise in FAA contents was 54% (12 days) and 62% (2 weeks) in ST-I and ST-II experiments respectively. Glycogen content also increased by 55 and 54% at 6 and 12 days and 31 and 20% at 3rd and 4th week of insecticide feeding. Soluble proteins remained unchanged in ST experiment but showed variable results in LT-I and LT-II experiments. It is concluded that malathion is relatively less toxic to muscle tissue under the present experimental conditions as for as the above analysed parameters are concerned. This was probably due to the fact that muscle is not a primary target of foreign toxic compounds in the animal systems.

Key words: Malathion, chick muscle, biochemical effects, enzymes

INTRODUCTION

Organophosphate pesticides are important agrochemicals which have wide range of activity against insect pests of crops, vegetables, fruits, and also active against the pests of medical and veterinary importance (Jayarathnam *et al.*, 1991; Kao and Tzeng, 1992; Stevens, 1992; Toma *et al.*, 1992; Aboul Ela *et al.*, 1993; Chakraborti *et al.*, 1993; Mourya *et al.*, 1993). These pesticides have replaced the earlier organochlorine compounds which are considered as highly persistent, due to their lipophillic nature, with consequent accumulation and induction of resistance in animals, plants and other systems of the environment (Mugambi *et al.*,

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1989; Sorokin and Zharov, 1992; Rao, 1992; Winter and Streit, 1992; Fischer and Pecher, 1993; Hellou *et al.*, 1993; Lin *et al.*, 1993; Urdaneta *et al.*,1995). Their degradation by animals and other systems of the environment was very slow, the factor which plays very significant role in the development of long term toxic effects in non-target systems, including man (Shakoori and Haq, 1987; Ali *et al.*, 1988 a,b; Ali and Shakoori, 1990, 1993; Shahida and Solangi, 1990; Meera *et al.*, 1993; Rani *et al.*, 1993).

Organophosphate, being phosphate esters, have comparatively low cumulative potential. These are relatively easy to biodegrade by various systems of the organism and environment into non toxic or less toxic metabolites which indicated that these pesticides are less hazardous to the environment (Ali and Shakoori, 1981; Tripathi and Shukla, 1992). There are many reports regarding the development of toxic effects by these phosphate esters in non-target systems. These pesticides are well known for their ability to inhibit the acetylcholine esterase activity at the synapse with the result of loss of nervous co-ordination (Kumar *et al.*, 1992; Sawyer *et al.*, 1992; Satyadevan *et al.*, 1993). In addition, metabolic alterations induced by organophosphate pesticides have also been reported in different tissues of non-target organisms (Qadri *et al.*, 1987; Ali and Shakoori, 1988; Blasiak, 1993; Dutta *et al.*, 1994; Begum and Vijayavaghavan, 1995; Tsuda *et al.*, 1995).

The main objective of the present study was to investigate the effects of malathion, on some metabolically important biochemical parameters of chick muscle which is important as for as human nutrition is concerned.

MATERIALS AND METHODS

Experimental animals and their maintenance

Seventy two one day old broiler chick *(Gallus domesticus)* were obtained from the local poultry breeders and kept in cages (25 cubic ft. area) in the animal house of the Department of Zoology, at controlled temperature (25.00±1.5 °C). Animals were provided with commercial poultry feed and water *ad libitum*.

Administration of insecticide

Sub-lethal doses of an organophosphate insecticide, malathion [*o,o*-dimethyl-S-(1,2-dicarboxyethyl) phosphoro- dithioate], were administered orally to chicks as a single strong dose (600 mg /kg body weight) and two weak doses (400 mg/kg and 250 mg/kg body weight/day) for the total durations of 40 hours (short term experiment), 12 days and 4 weeks (long term experiments), respectively, after consulting the LD_{50} value of malathion for chicks.

Experimental procedure

For short term experiment, malathion was administered as a single strong dose to a group of 16 chicks and the sampling was performed at 5, 10, 20 and 40 hours intervals. In long term treatments, two groups, with 15 and 13 chicks in each, were administered

with weak doses (400 mg and 250 mg, respectively) of malathion. Sampling in former case was done at 3, 6 and 12 days while in later case it was performed at 2, 3 and 4 week after the beginning of insecticide feeding. A group of 4 chicks was used as a control alongwith each treated group in both short and long term experiments.

At the completion of stipulated periods in each case, a group of 3 to 5 malathion treated and a group of 4 control animals were anesthetizes. Their muscle samples were collected quickly from the legs and stored at -10 °C until processed further for various analyses

Muscle processing for biochemical analysis

Weighed amounts of muscle was processed in 0.89% saline, 0.5N sodium hydroxide and boiling ethyl alcohol, separately for the extraction of saline-soluble components, total proteins and nucleic acid (DNA and RNA) contents, respectively.

Saline homogenate was centrifuged at $4x10^3$ rpm for 15 minutes at 5 °C to obtain clear supernatent, which was used for the estimation of some enzyme activities *viz.*, acid phosphatase (AcP, *O*-phosphoric monoester phospho-hydrolase, E.C. 3.1.3.2.) according to Andersch and Szcyphinski (1947); alkaline phosphatase (AP, *O*-phosphoric monoester phosphohydrolase, E.C. 3.1.3.1.) according to the Bessey *et al.* (1946); glutamate oxalo-acetate transaminase (GOT, L-aspartate 2-oxoglutarate aminotransferase, E.C. 2.6.1.1.) and glutamate pyruvate transaminase (GPT, L-alanine 2-oxoglutarate aminotrasferase, E.C. 2.6.1.2.) both according to the method of Reitman and Frankel (1957)

In addition to enzymes some other muscle metabolites like total free amino acids (FAA) according to Moore and Stein (1954) and soluble proteins, according to Lowry *et al.* (1951) were also estimated from the saline muscle extract. Total muscle proteins were extracted in 3 ml of 0.5 N sodium hydroxide in water bath at 55 °C and estimated as soluble proteins. Glycogen was extracted and estimated according to the method mentioned in Hassid and Abraham (1957). Nucleic acids (DNA and RNA) were extracted as mentioned in Shakoori and Ahmad (1973) and estimated according to Schneider (1957).

RESULTS

Some biochemical alterations induced by malathion at various dose (600mg, 400mg and 250mg) and duration (40 hours, 12 days and 4 weeks) levels are being mentioned in Tables I-III as percent variation from control values. Figures 1-3, on the other hand indicate the actual mean values of various parameters in case of control as well as three malathion feeding experiments.

In 40 hours malathion feeding experiment, most of the tested parameters of chick muscle remained unchanged with respect to control group (n=4). However, some prominent changes were observed in phosphatase (AP and AcP) activities, especially AP which was increased by 40% and 45% at 10 (n=3) and 20 (n=4)hours insecticide feeding studies, respectively. On extending the malathion treatment for next 20 hours

the AP showed 35% decrease. The muscle AcP activity exhibited 32% rise at 40 hours while no significant change was observed during the initial 20 hours

Table I: Percent increase (+) or decrease (-) from control values (n=4) in some enzyme activities and metabolite concentrations of chick (*Gallus domesticus*) muscle after malathion administration (600 mg/kg body weight/day) for a period of 40 hours.

Parameters[a]	Malathion Treatment (Hours)			
	5 (n=4)	10 (n=3)	20 (n=4)	40 (n=3)
AcP activity	-1.89	+9.43	+22.64	+32.08*
AP activity	+25.0	+40.0*	+45.00**	-35.00*
GOT activity	+8.11	+5.41	+13.51	-5.41
GPT activity	-2.22	-13.33	-11.11	+6.67
Free amino acid	+337.68*	+144.93*	-33.33	-10.14
Glycogen	+62.5*	+37.50*	-31.25	-37.50
Soluble proteins	-22.38	-3.38	+23.74	+27.60
Total proteins	+0.89	-10.18	+3.59	+22.98
DNA content	+15.15	+44.95	+36.87	+25.76
RNA content	-23.97	+23.86	-22.77	-20.70

Student's `t' test, *P<0.05; **P<0.01.

[a]**Abbreviations used**: AcP, acid phosphatase; AP, alkaline phosphatase; GOT, glutamate oxaloacetate transaminase; GPT, glutamate pyruvate transaminase.

of the experiment. Malathion administration, in this short-term (strong dose) experiment induced significant increase in muscle FAA (338% and 145%) and glycogen contents (63% and 38%) at 5 and 10 hour durations, respectively. The both components showed recovery during next 30 hour malathion treatment. The muscle GOT, GPT activities, soluble and total protein and nucleic acid contents remained unaltered during 40 hours administration of malathion as strong dose (Table I; Fig. 1).

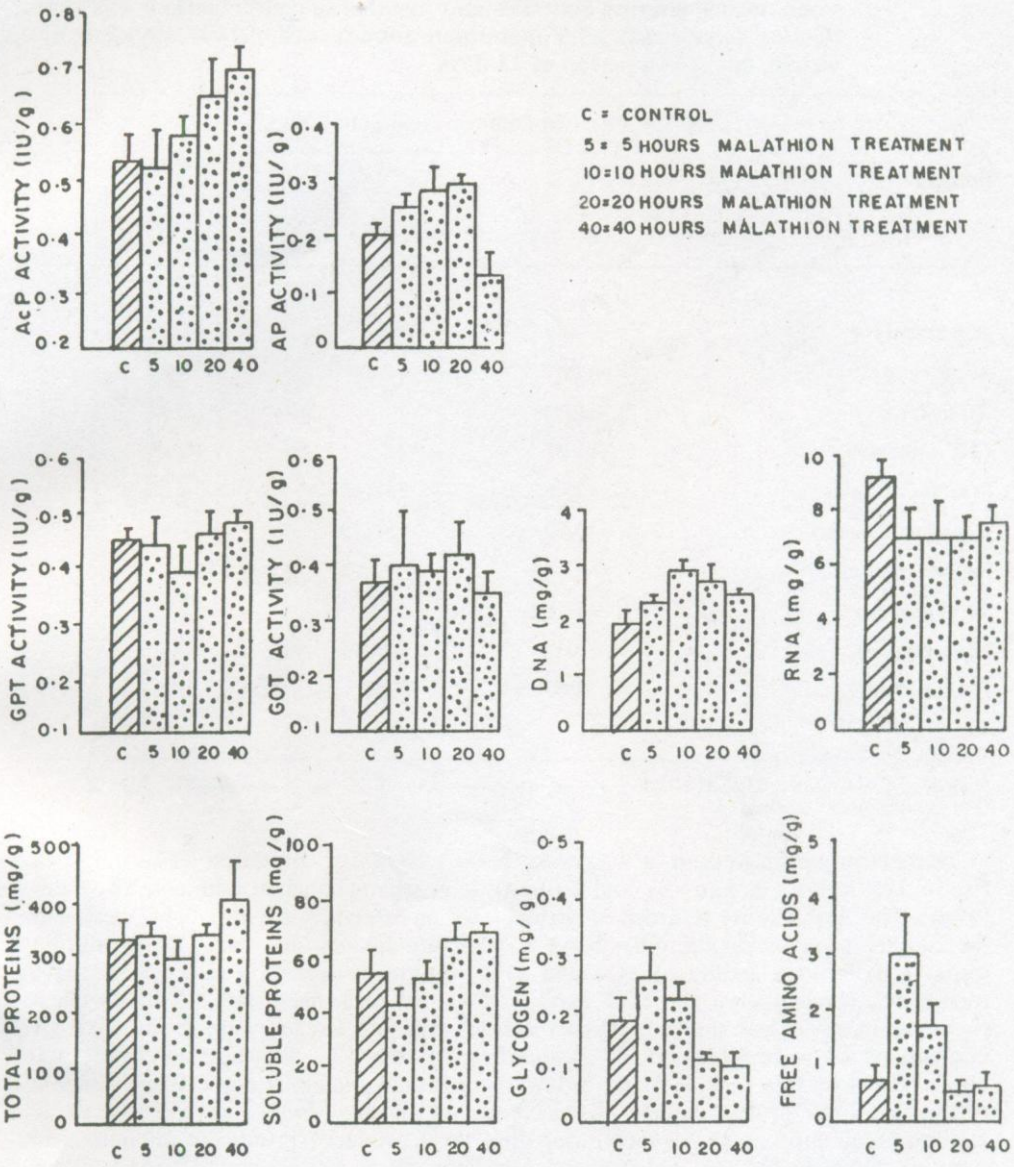

Fig. 1. Effect of malathion (600 mg/kg body weight), administered for a period of 40 hours on some muscle enzyme activities and metabolite concentration in chicks.

Table II: Percent increase (+) or decrease (-) from control values (n=4) in some muscle enzyme activities and metabolite concentrations of chick (*Gallus domesticus*) after malathion administration (400 mg/kg body weight/day) for a period of 12 days.

Parameters[a]	Malathion Treatment (Days)		
	3 (n=4)	6 (n=3)	12 (n=5)
AcP activity	+20.83*	78.95**	+17.39
AP activity	-4.00	-31.03*	-40.74*
GOT activity	+14.29	-40.0	+24.00
GPT activity	-16.67	-31.25	+100.00
Free amino acids	+25.81	-24.24*	+54.17*
Glycogen contents	-40.00	+54.55*	+53.85*
Soluble proteins	+11.63	+30.92	-41.52*
Total proteins	+14.92	-5.59	-16.59
DNA content	-1.02	+28.14	-4.97
RNA content	-24.63	-5.40	-5.76

Student's 't' test, *P<0.05; **P<0.01.
[a]For abbreviations, see Table I.

Malathion administration at 400mg/kg body weight/day, produced 21% and 79% rise in AcP activity at 3 (n=3) and 6 (n=4) days when compared with control values (n=4). The AcP activity returned to normal level on extending the insecticide treatment for another 6 days. On the other hand AP activity did not show any change during initial 3 days while exhibited 31% and 41% significant inhibition at 6 and 12 days treatments, respectively. The GPT activity remained unchanged until 12 hours when two fold increase was noticed. Almost similar type of changes were found in FAA contents which were increased (54%) significantly after 12 days while no change was observed before this *i.e.*, at 3 and 6 days of insecticide administration. The reduction (42%) in soluble protein content of muscle was noted only after 12 days of insecticide feeding. Malathion at above mentioned dose level produced prominent alterations in muscle glycogen content which, were significant at 6 (55%) and 12 (54%) days insecticide feeding. No change was found in GPT activity, total proteins and nucleic acid content (Table II; Fig. 2).

Some prominent changes were also observed after feeding this insecticide @ 250mg/kg body weight/day during four week study period.

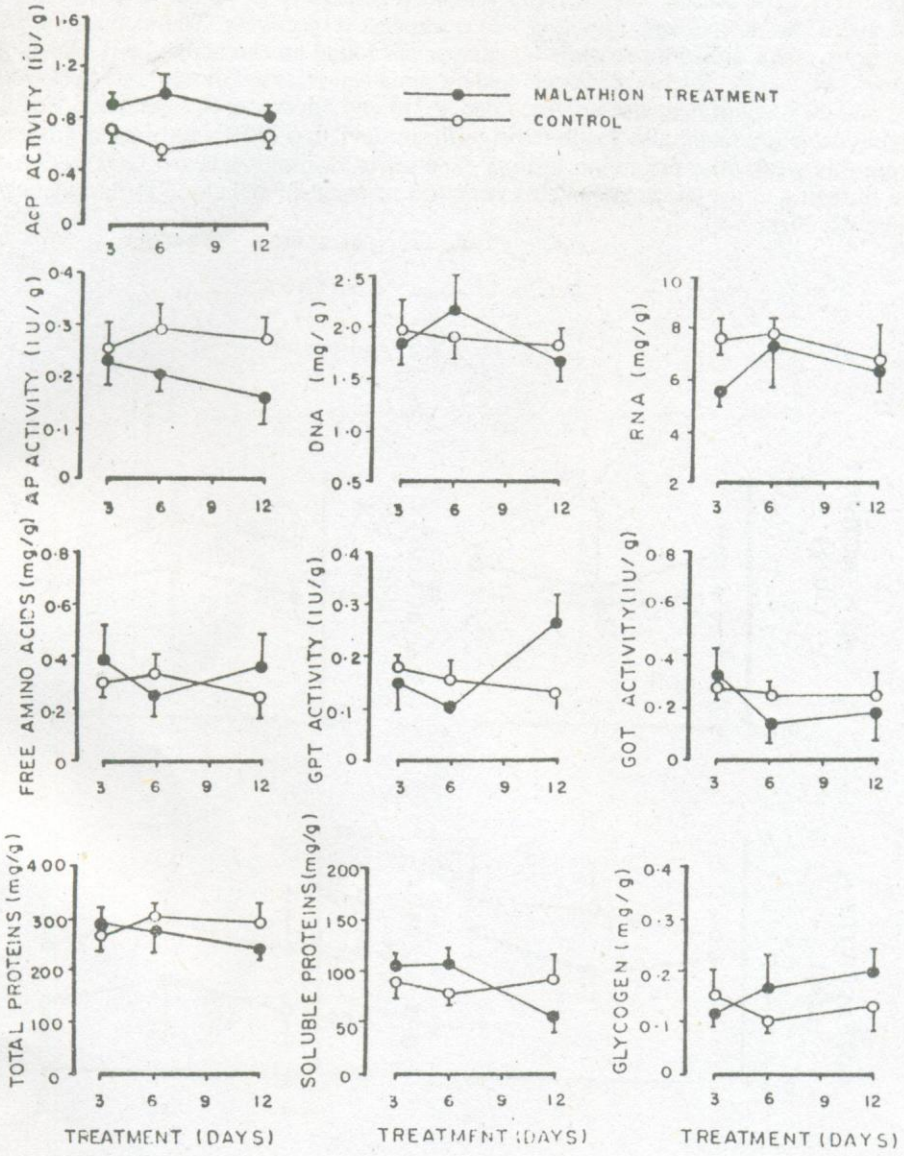

Fig. 2. Effect of malathion (400 mg/kg body weight/day), adminis- tered for a total period of 12 days on some muscle enzyme activities and metabolite concentration in chicks.

AP activity exhibited 38% and 28% fall at 2nd (n=4) and 3rd (n=5) weeks, respectively. The muscle GPT activity remained constantly at higher level *i.e.* (33%, 29% and 33%, at 2, 3 and 4 week (n=4) treatments respectively. The increase in FAA content was 62% at 3rd week while no change was found on extending the treatment for another two weeks. Muscle glycogen content remained resistant during 2nd week while 31% and 20% significant rise was recorded at 3rd and 4th weeks of insecticide feeding. Soluble protein content also exhibited significant deviation (70% increase) at 3rd week during this weak dose malathion feeding experiment. Muscle AcP and GOT activities, total protein and nucleic acid contents remained resistant to malathion during the study (Table III; Figs. 3-4).

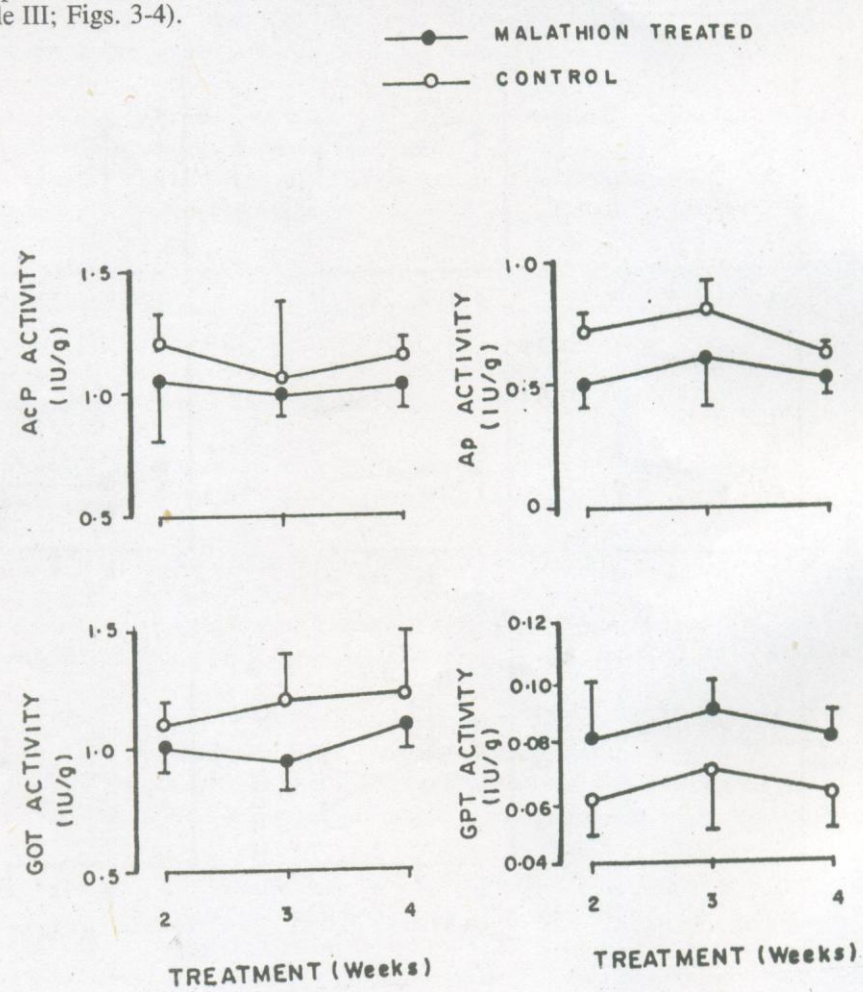

Fig. 3. Effect of malathion (250 mg/kg body weight/day) adminis- tered for a total period of 4 weeks, on some muscle enzyme activities in chicks.

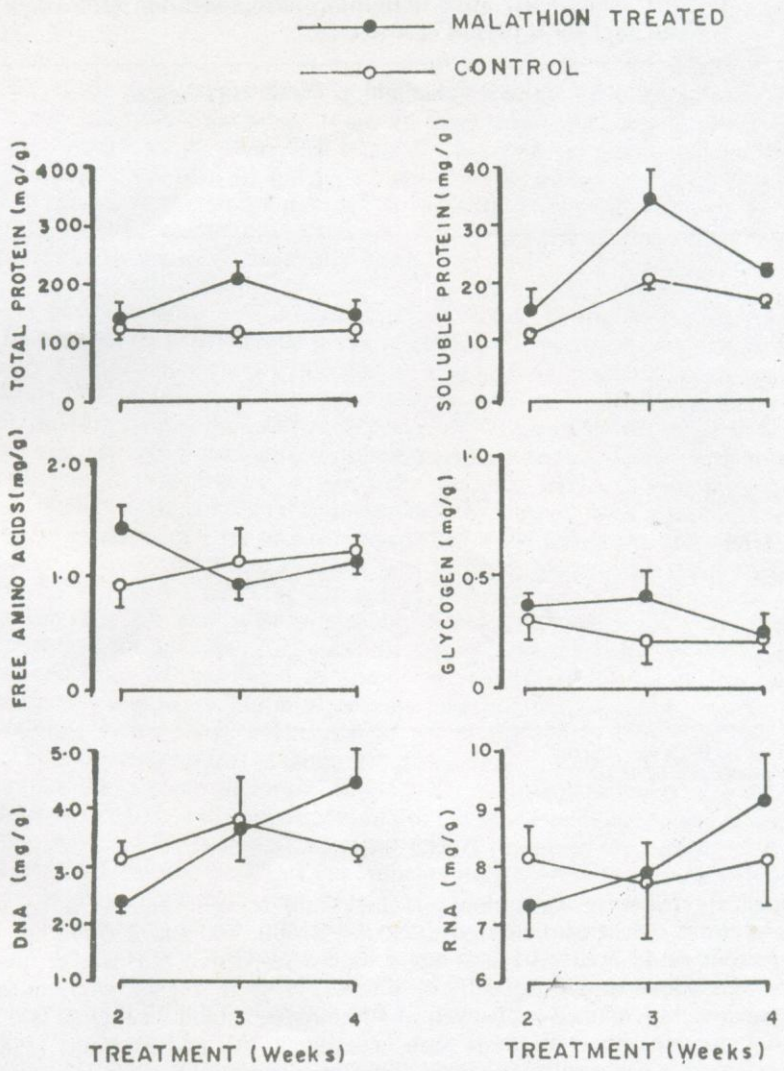

Fig. 4. Effect of malathion (250 mg/kg body weight/day) adminis- tered for a period of 4 weeks, on some muscle metabolite cocentration in chicks.

Table III: Percent increase (+) or decrease (-) from control values in some muscle enzyme activities and metabolite concentrations of chick (*Gallus domesticus*) after malathion administration (250 mg/kg body weight/day) for a period of 4 weeks.

Parameters[a]	Malathion Treatment (Weeks)		
	2 (n=4)	3 (n=5)	4 (n=4)
AcP activity	-11.76	-7.69	-10.53
AP activity	-37.84*	-28.21*	-5.88
GOT activity	-8.85	-18.26	-9.32
GPT activity	+33.33*	+28.57*	+33.33*
Free amino acids	+61.90*	-16.81	-5.83
Glycogen content	+9.68	+31.03*	+20.00*
Soluble proteins	+30.32	+69.94*	+32.36
Total proteins	+8.46	+29.50	+18.13
DNA content	-24.52	-1.07	+34.66
RNA content	-10.59	+0.52	-7.00

Student's 't' test, *$P<0.05$.
[a]For abbreviations, see Table I.

DISCUSSION

Biochemical effects of malathion on chick muscle were found to be mild to moderate intensity, when administered to chicks @ 600, 400 and 250 mg/kg /day for the total periods of 40 hours, 12 days and 4 weeks, respectively. However, maximum alterations were found in 400 mg daily dose level (Table II, Fig.2), while most of the analysed parameters remained unchanged in 40 hours malathion feeding @ 600 mg/kg, once during the experiment in short term experiment. Ali and Shakoori (1981) also reported that rabbits, generally remained resistant to malathion when administered @ 95 mg/kg body weight/day unintrruptedly for 15 months alongwith feed, except some significant decrease in cholinesterase (28%) and GPT (36%) activities, and raised AcP activity (55%). However hepatic GPT activity showed considerable (47%) inhibition during this study.

On comparing the effects of three doses of malathion for three different durations, it was observed that in most cases, the biochemical changes in chick muscle were not

induced immediately after administration of insecticide. However, the FAA and glycogen content seemed quite sensitive which showed significant increase within 5 hours of malathion feeding in short term experiment but normalized after 10 hours (Table I, Fig. 1).

Same is true in case of 400 mg/kg malathion feeding daily for 12 days. Only AcP is the exception which was increased within three days of insecticide feeding (Table II, Fig. 2). Moreover, the dose and treatment duration, at which maximum alterations were observed , was 400 mg/kg/day administered for a total period of 12 days. Various biochemical parameters showed a sort of recovery or normalization at the end of long term (4 week) malathion treatment (250 mg/kg/day) indicating induction of detoxification processes in the treated chick.

Acid phosphatase is a hydrolytic enzyme, found in the lysosomal fraction of the cell. Increase in its activity may be an indication of increased break down activities of waste cellular components produced as a result of insecticidal toxicity. Another possibility for increased AcP activity may be the increased biosynthesis of enzyme protein due to elevated demands in the cells. AP is generally regarded as liver function enzyme but it is also found in plentiful amounts in intestine, kidney and bone tissue. Its main function is to hydrolyse various monophosphate esters to split phosphoric acid, a reaction which is of considerable importance in various body processes. It is mainly rich in the tissues which are actively engaged in active transport. The rise in AP activity at 10 and 20 hour malathion feeding, was probably due to increased active transport process for supply of nutrients for energy generation to counter the toxic effects of insecticide. The AP activity showed significant decrease when the insecticide treatment was extended for another 20 hours, in strong dose experiment. In remaining two experiments, the AP exhibited a significant inhibition. It is not clear whether this inhibition was due to inhibition of enzyme protein biosynthesis or some other factor is responsible for this change. The normal pH in the cell is slightly alkaline (approx., 7.3). This enzyme requires alkaline pH in the range of 10.5. It can be suspected that malathion induces some alterations in the cell's environment which may inhibit this pH shift from 7 to 10.5, with consequent inhibition of AP activity. Muscle GPT activity is generally required for transamination purposes. The rise in the activity of this enzyme, at the end of LT-I and in LT-II experiments may be due to increased transamination process which is a primary step in utilization of amino acids for energy generation, required to detoxify the foreign toxic compounds. The elevated activities of AcP, AP, GOT and GPT have also been reported in another study (Awal and Malik, 1992) after administering a single oral dose of phosphamidon (an organophosphate pesticide) @ 20 and 40 g/kg body weight in *Bubalus bubalis*.

Amongst other biochemical components of muscle, FAA contents generally increased at the durations where rise in glycogen was also observed *i.e.*, at 40 hours and 12 days malathion feeding study. This indicates that during these periods, glucose was not available for energy generation in muscle, rather amino acids, which were available in higher amounts, were being utilized for various activities of the muscle tissue. Most probably the glucose was being routed towards glycogen biosynthesis. Normally, amino acids are utilized as fuel in the muscle cells for energy production when glucose and glycogen sources are not available. In this study, it was noticed that

FAAs are being utilized in the presence of glycogen reserves (Tables I-III), the condition which indicated the possible inhibition of glycogen utilization system. Lal *et al.* (1986) showed fall in liver and muscle glycogen content with simultaneous rise in plasma glucose in 16 day malathion treated fish @ 8 mg/l of water.

In weak dose (long term) insecticide feeding study, case was somewhat different. The FAA contents exhibited 62% rise after 2 weeks of insecticide treatment while glycogen was raised after 3 and 4 weeks. However, the percent increase was reduced to 31 and 20, respectively, which indicated the trends towards normalization. The normal FAA pattern during 3rd and 4th weeks may be due to re-availability of glucose for energy generation processes in the muscle which was supported by the decreased accumulation of glycogen. The 42% decline in soluble proteins may be due to some inhibition in protein biosynthetic pathway or it may be due to increased breakdown of proteins to amino acids which showed 54% rise during the experiment.

It is important to note that at the end of weak and strong dose experiments, almost all parameters, except few, showed recovery (Tables I,III). Moreover, the results in this study indicated, that the effects of malathion feeding to on chicks muscle biochemical components were in the range of mild to moderate as for as their intensity is concerned. This was, probably, due to the induction of drug metabolizing and other xenobiotic degrading enzymes in the liver because liver is considered as the major site for all types of biotransformations of the foreign toxic compounds. As a result, these pesticides may be transformed into various less toxic or non-toxic metabolites and excreted before reaching the other organs or tissues.

However, many workers have reported some metabolic changes in the muscle of different animals after insecticide exposure. Acetylcholine esterase (AChE) activity of the fish muscle showed significant depression at 5.88 ppm malathion concentration in 96 hours. According to this report, the activity was not fully recovered even six days after transferring the fish to pesticide free environment (Sulaiman *et al.*, 1989). Bashamohideen and Sailbala (1989) however, reported that inhibition of AChE activity was greater in white muscle as compared to red muscle. Significant fall in RNA and protein was observed by Jyoti *et al.* (1989) after malathion treatment in *Channa punctatus* but glycogen showed variations in different tissues. Similarly Husain *et al.* (1987) showed a significant increase in AP, GOT and GPT activities of liver, kidney and brain tissues of malathion treated (55 and 137.5 mg/kg orally for 32 days) rats.

It was concluded that malathion at the dose levels used in this experiment probably showed a moderate toxicity as for as biochemical parameters of chick muscle are concerned when compared with liver which is a primary target of all foreign toxic compounds (Gupta and Paul, 1977; Ali and Shakoori, 1981; Thompson *et al.*, 1991; Ali and Ali, 1992).

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