In both F_1 inbreeding and backcrosses, among the larvae that survived, males and females were in 1:1 ratio, which indicated the linkage of the resistant gene to autosomes. In this context it may be mentioned that in *Cx. quinquefasciatus*, sex karyotype of males and females is the same, and the sex is determined by a pair of alleles, *m* and *M*. Females are homozygous for *m* (*m'm*) and males heterozygous (*m/M*). Though the F_1 progeny from the reciprocal crosses exhibited similar responses, to rule out sex linkage, surviving larvae from backcrosses and F_1 inbreeding were examined for sex by dissecting the reproductive organs. If the gene is sex-linked in F_1 inbreeding and in backcrosses, where F_1 males are heterozygous for the resistant gene, depending upon whether the resistant gene is introduced into the crosses along with *M* or *m* gene, the sex of the surviving progeny would respectively be either male or female. However, the recombinants would include the other sex. As in all the crosses males and females are in 1:1 ratio, linkage of resistant gene to sex chromosomes is ruled out.

The study has thus established that the resistance to *B. sphaericus* in *Cx. quinquefasciatus* is recessive, linked to autosome/s and is controlled by more than one gene. No maternal effect was observed in the expression of resistance. Further, the homozygous resistant strain was found fully susceptible to *B. thuringiensis israelensis*, the other commonly used biolarvicide, the LC_{50} and LC_{90} values being similar to those of the susceptible strain.

It is well established that a rare dominant gene is selected more rapidly than a recessive gene[9]. Since the gene for resistance in *B. sphaericus* is recessive, theoretically it should have taken a long time to develop resistance in the field populations. However, resistance appeared shortly after a year of spraying (20–25 rounds) in all sprayed areas. The probable reason for this could be that during larviciding, large population of immatures are continuously exposed, increasing the selection pressure[10].

Contrary to the presumption that insects are unlikely to become resistant to microbial pesticides[11], resistance in lepidopterans to *B. thuringiensis*[12] and now resistance in the mosquito *Cx. quinquefasciatus* to *B. sphaericus* has developed.

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ACKNOWLEDGEMENTS. We are grateful to Drs I Kar (Madras), A Kumar (Panaji) and S. N Sharma (Farrukhabad) for sending field-collected mosquitoes. We express our sincere thanks to the following staff members for their excellent technical assistance Pritam Singh, K. B Masiwal, H D. Joshi, A K. Mukherjee, Uday Prakash, Krishan Gopal, Ram Kanwar and Bhagwan Das

Received 7 April 1995; revised accepted 4 September 1995

Lead impairs hepatic type I-5′-monodeiodinase activity and thyroid function in cockerels

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Chronic exposure of cockerels to 1.5 mg/bird/day of lead nitrate for a period of 30 days impaired thyroid function. Lead decreased serum triiodothyronine (T_3) concentrations, inhibited hepatic type I-5′-monodeiodinase enzyme activity and marginally increased serum thyroxine (T_4) concentrations. Our findings demonstrate the lead-induced inhibition of hepatic type I-5′-monodeiodinase enzyme activity in avian system, leading to decreased production of T_3, the most potent metabolic hormone.

TYPE I-5′-monodeiodinase (5′-D) is a microsomal membrane-bound enzyme[1]. The most potent thyroid hormone, triiodothyronine (T_3), is predominantly produced in extrathyroidal tissues by 5′-monodeiodination of phenolic ring of thyroxine (T_4), the hormone that is known to be the prohormone of the former[2]. Although 5′-D enzyme system is present in almost all tissues, liver and kidney are known to have the highest T_4 to T_3 conversion activity[3]. Regulation of the 5′-monodeiodination in pe-

ripheral tissues is an important control point with respect to thyroid hormone action[4].

Lead is being increasingly recognized as an important environmental contaminant[5,6]. Many animal studies have been carried out on the toxic effects of this metal, particularly on hemopoietic, nervous, renal, cardiovascular and reproductive systems[7–10]. However, its effect on thyroid function has received very little attention of the scientists. That too, they are mostly based on simple thyroidal histology and ^{131}I uptake studies in mammal and fish[11,12]. Until today, not a single report is available on the lead-induced alteration of 5′-monodeiodination in any animal model. In the present investigation an experiment was conducted to reveal lead-induced alteration in hepatic 5′-D activity, if any, in cockerel. In addition, changes in serum T_3 and T_4 concentrations were also studied to reveal the thyroid dysfunction.

One-day-old broiler chicks were purchased from a local supplier and were acclimatized to standard laboratory conditions (14L:10D, 27 ± 1°C) for seven days. They were provided with commercial grower feed and water *ad libitum* throughout the experiment.

The birds were divided into two groups of eight birds each. Group I birds, receiving 0.1 ml of distilled water served as control. Group II birds were administered lead nitrate (1.5 mg/bird/day) subcutaneously and the treatment was continued for 30 days. The dose and the number of days were chosen from the earlier studies[13–15]. On the last day, blood was collected and the serum stored at –20°C for the estimation of T_3 and T_4 concentrations. They were estimated by the radioimmunoassay (RIA) method as described earlier, with little modification[16]. RIA kits were purchased from Bhabha Atomic Research Centre, Bombay. The lowest detection limit for T_3 assay was 0.05 ng/ml and for T_4 0.01 ng/ml. Interassay coefficient of variation was less than 5% for both the hormones.

5′-D was estimated by the method of Decuypere *et al.*[17] with little modification. The birds were sacrificed and the liver of each animal was removed and homogenized in 4 volumes (wt/vol) of phosphate buffer (0.15 M, pH 6.5, with 0.25 M sucrose and 5 mM EDTA). After centrifugation at 2000 *g* for 30 min at 4°C, the supernatant was incubated with T_4 (400 µM) and dithiothreitol (DTT, 4 mM) for 60 min. The reaction was stopped by the addition of 95% ethanol. The amount of T_3 generated was measured by RIA. Generation of T_3 was expressed as ng of T_3 generated per hour of incubation per mg protein. Liver protein was estimated by the method of Lowry *et al.*[18].

The data were expressed as mean ± SE. Significance level between two groups was calculated using Student's *t* test.

The results (Figure 1) clearly indicate lead-induced inhibition of hepatic 5′-D activity. Lead also decreased serum T_3 and marginally increased serum T_4 concentrations.

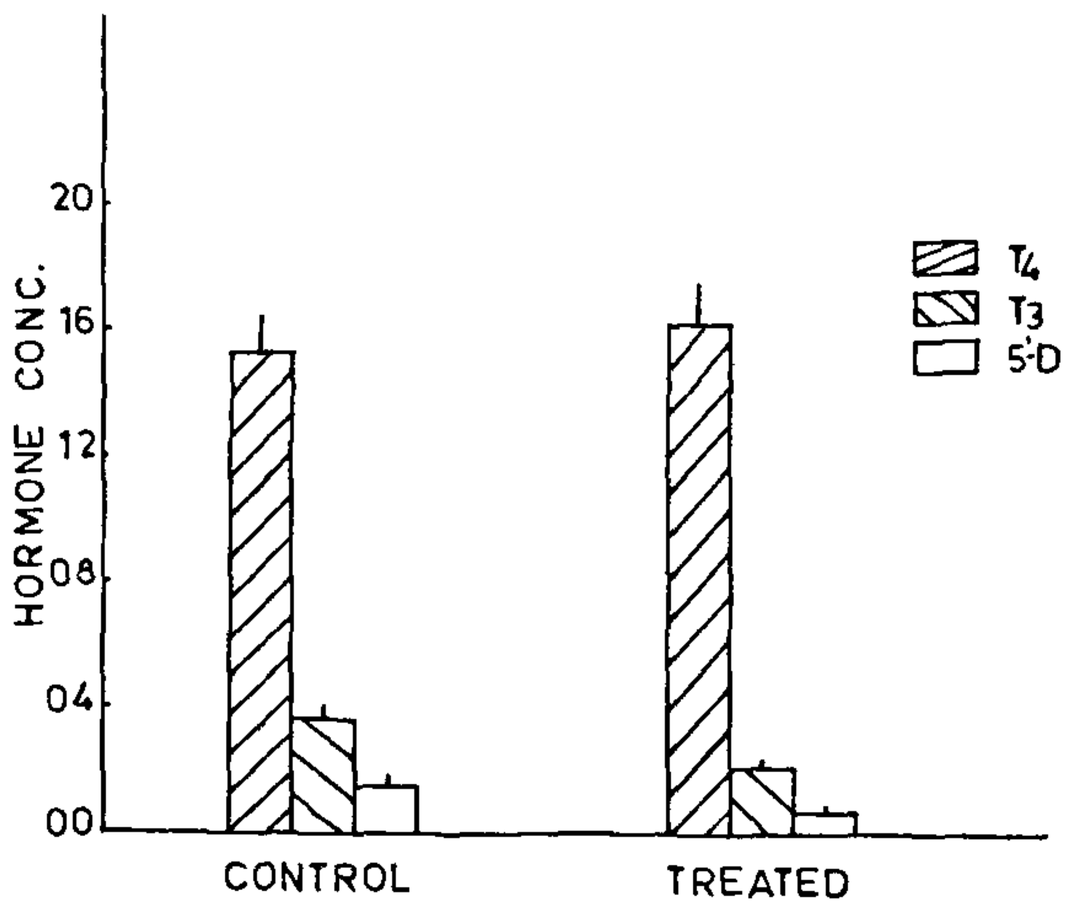

Figure. 1. Effects of lead nitrate (1.5 mg/bird/day) for 30 days on serum T_3, T_4 concentration (ng/ml) and on hepatic type I-5′-D enzyme activity (ng T_3 generated/h/mg protein) in cockerel Vertical lines indicate the standard error of the means.

Earlier report in rat also shows a slight increase in serum T_4 concentration but a significant decrease in serum T_3 following the treatment of another heavy metal, i.e. cadmium[19]. Lead is known to induce inhibition of thyroidal iodine uptake in rats[20] and in adult patients[21]. Although at present, few reports are available on the effects of lead on thyroid function in mammals[22,23], not a single report is available on lead-induced alteration in 5′-D activity in any of the animal models. Particularly in birds, practically nothing is known on the lead-induced alterations in thyroid metabolism.

In the present study, a marginal increase in serum T_4 and a significant decrease in serum T_3, the most potent metabolic hormone, was observed following lead treatment in cockerel. The marginal increase in the serum T_4 concentration may be due to the inhibited 5′-monodeiodination (catabolism) of T_4 to T_3. This increase was not significant probably because T_4 was utilized in the other metabolic pathways[24–26]. The lead-induced decrease in serum T_3 concentration was further supported by the inhibition of 5′-D enzyme activity that is responsible for the conversion of T_4 to T_3. Earlier reports on cadmium- and mercury-induced inhibition of 5′-D in rat also support our findings[19,27].

Type I-5′-D is a sulphydryl (–SH) group-bearing enzyme and contains selenocysteine at its active site[28]. Lead has been found to inhibit a number of enzymes by either binding to its active site or to the free –SH groups of the enzyme[29,30]. Therefore, in the present study two possibilities exist for the metal-induced inhibition of 5′-D enzyme activity. Either lead replaces selenium in the enzyme or it combines with the free –SH groups, which are essential for the enzyme function, thus making it inactive.

In the present experiment, whatever may be the mechanism of action of lead on hepatic type I-5′-D enzyme, our findings for the first time reveal clearly lead-induced inhibition of the aforesaid enzyme in an avian model.

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ACKNOWLEDGEMENT. We thank UGC, New Delhi, for financial support.

Received 29 May 1995, revised accepted 2 September 1995.

MEETINGS/SYMPOSIA/SEMINARS

Workshop on Geology and Exploration of Platinum Group, Rare Metal and Rare Earth Elements

Date: 6–7 February 1996
Place: Calcutta

Topics include: Geological settings and metallogeny of the above elements; Geochemistry and mineralogy of the ores (including analytical techniques); Exploration and exploitation, including mining and benefication.

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Second Asian and Oceanic Congress of Andrology

Place: Chandigarh
Date: 16–20 November 1996

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