IMMUNOPATHOLOGIC AND TOXIC EFFECTS OF ENDOSULFAN IN CHICK EMBRYO

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Thesis submitted in partial fulfilment of the requirement for the degree of

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I hereby declare that this thesis entitled " **IMMUNOPATHOLOGIC AND TOXIC EFFECTS OF ENDOSULFAN IN CHICK EMBRYO** " is a bonafide record of research work done by me during the course of research and that the thesis has not previously formed the basis for the award to me any degree, diploma, associateship, fellowship or other similar title, of any other University or Society.

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Certified that the thesis entitled " **IMMUNOPATHOLOGIC AND TOXIC EFFECTS OF ENDOSULFAN IN CHICK EMBRYO** " is a record of research work done independently by **Dr. P. Kalai Selvan**, under my guidance and supervision and that it has not previously formed the basis for the award of any degree, diploma, fellowship or associateship to him.

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Introduction

1. INTRODUCTION

Pesticide residue in the environment is an ever-growing globalised problem. Pesticides protect crops against pest but in the long process affect man and livestock. To increase agricultural production a spectrum of pesticides was introduced which resulted in the emergence of pests with more resistance. Hence it became inevitable to use excess quantity of pesticides and this in turn led to widespread occurrence of pesticide residues in all agricultural products. Today, hundreds of pesticides are in use by farmers, foresters and in house hold. These pesticides developed during the past three decades, though are useful to the mankind, have led to a plethora of environmental problems.

The most commonly used pesticides are chlorinated hydrocarbons, organophosphorous compounds, carbamates and synthetic pyrethroids. Synthetic pyrethroids have low mammalian toxicity and are considered to be safe. Organophosphrous compounds and carbamates are in general rapidly biodegraded in the environment whereas organochlorine insecticides are known to be ubiquitous in the environment, not easily biodegradable, persist for years and tend to accumulate in the food chain. Because of the high residual effect in soil, vegetables, milk, meat, egg yolk and drinking water produced by most of the organochlorine compounds, they have been banned in many countries.

Human beings interact with their environment every day and are exposed to a spectrum of synthesized chemicals present in the food they eat, the air they breath and the water they drink. Samples of food and water revealed residue levels far exceeding the maximum limits permitted by World health organisation. Recent studies have revealed the presence of significant amount of organochlorine pesticides and its metabolites in the human body fat, blood and milk without causing apparent clinical symptoms (Banerjee *et al.*, 1996).

The toxic chemicals have become an integral part of the ecosystem and the effects of these agents on human health are yet to be satisfactorily explored. Most of the earlier studies are restricted to acute and chronic effects of pesticides in experimental animals. These studies have provided significant information regarding the toxicological properties and pharmacodynamics of pesticides. However the situation in practice is not toxicity resulting from a single or a few large doses of a given pesticide, but due to oral intake of very small quantities over a reasonably long period of time.

Pesticide induced immunomodulation plays a greater role in the pathogenesis and causation of disease. The immune system is more sensitive and reacts more rapidly than other organ systems to the effect of pesticides, even in concentrations of these chemicals lower than those necessary for acute systemic toxicoses (Black *et al.*, 1992).

Immunomodulation by agrochemicals is gaining significance in toxicity evaluations as low-level dietary intake through feed residues might cause break down of immunity following vaccination (Varshenya *et al.*, 1988).

Several of the organochlorine toxic agents like dichlorodiphenyltrichloroethane (DDT), lindane, endosulfan and malathion are immunosuppressive and impair thymus-dependent immunity. The human population is ubiquitously exposed to complex mixtures of these contaminants, generally at much lower levels of exposure than those routinely examined in animal toxicity studies.

Endosulfan, an organochlorine compound belonging to cyclodiene group having low mammalian toxicity and rapid biodegradability, is widely used now as a broadspectrum non-systemic, contact and stomach insecticide against numerous insects and certain mites attacking maize, rice, cotton, vegetables etc.

Endosulfan is the third most commonly used pesticide in India. The presence of endosulfan in the form of residues in the environment poses a great hazard to the health of animals and human beings. Controversies exist about this compound as a cause for human ailments in Kerala. Plantation corporation of Kerala was spraying endosulfan over the cashew plantation area twice in a year for a period of 20 years without pesticide rotation. People belonging to Padre village of Kasargode district were having unusually high incidence of congenital malformations, liver and blood cancer, mental retardation, epilepsy, asthma and infertility. Centre for Science and Environment, a non-governmental organisation found unusually high residue level in the biological samples after endosulfan spraying. It was thought that these medical ailments were due to endosulfan toxicity and the compound was subsequently banned in Kerala. However, no significant residual level in the biological and environmental samples were detected in a study conducted by Kerala Agricultural University.

Elucidation of the effect of endosulfan in suitable model system therefore seems to be important in clearing the controversies to a certain extent. The chick embryo offers a model for understanding the early differentiation of the organ systems and the fundamental process of body formation common to all groups of vertebrates. Unlike in other animals, any agent introduced into the egg system remains there throughout the developmental period and is not lost externally. The effects of the causative agent can be directly seen and assessed and the information can be applied to the development of ameliorative measures.

Therefore, the present study involving chick embryo as a model system has been undertaken with the following objectives

1. To evaluate the toxicity of endosulfan in chick embryos
2. To study the effect of endosulfan on the developing immune system
3. To study the effect of endosulfan on preformed maternal immunoglobulin.

Review of Literature

2. REVIEW OF LITERATURE

Pesticides are a heterogenous group of substances used for preventing, destroying or repelling the pests. They cover large groups of chemicals such as insecticides, herbicides, rodenticides etc. Insecticides used to kill insects are broadly classified as organochlorine compounds, organophosphrous compounds, carbamates and synthetic pyrethroids. Organochlorine compounds include diphenyl aliphatic compounds or DDT group, Aryl hydrocarbons and Cyclodienes (Sandhu and Brar, 2000). The toxicity of endosulfan, an organochlorine compound belonging to cyclodiene group is reviewed.

2.1 ENDOSULFAN

2.1.1 Formulations

Endosulfan was developed and introduced by Farbody weighterke Hoechest AG in 1954 under the trade name Thiodan. Technical endosulfan is a mixture of two stereoisomers (α-endosulfan, β-endosulfan) having approximate ratio of 70:30. The technical material is a 90-95 per cent pure mixture of the two isomers (Canada National Research Council, 1975). The technical endosulfan may also contain endosulfan alcohol and endosulfan ether not exceeding a concentration of two per cent and one per cent respectively. It was formulated as emulsifiable concentrate (EC), wettable powder, dust, granules and ultra volume formulations. It is a non-systemic insecticide with contact and stomach action. It was used against a wide variety of agricultural pests, home garden pests and also used as wood preservative (Canada National Research Council, 1975). Endosulfan was classified as moderately hazardous pesticide (WHO, 1984).

2.1.2 Half life

Aquatic half-life of both isomers of endosulfan ranged from four days in river water, subjected to industrial runoff (Eichelberger and Lichtenberg, 1971), to seven days (Greve, 1971) in normal water with pH 7 and normal oxygen saturation. However, the half-life was profoundly affected by pH and oxygen content. A drop in either of these two parameters inhibited endosulfan degradation. Under anaerobic conditions at pH 7, the half-life increased to approximately five weeks and at pH 5.5, the half-life was nearly five months (Greve, 1971).

Steward and Cairns, (1974) reported that α-isomer had a shorter half-life (60 days) than the β-isomer (900 days) in the soil.

2.1.3 Toxicokinetics

Gupta and Ehrnebo (1979) studied the toxicokinetics of α and β endosulfan in rabbits, following intravenous dosing of two milligram per kilogram of endosulfan containing the isomers in the ratio of 70:30. They reported that the β -isomer was more quickly metabolized and rapidly excreted of the two.

Endosulfan was absorbed after ingestion, inhalation and skin contact. Following oral or parenteral dosing it was rapidly metabolized and excreted via faeces and urine. Following acute over exposure, high endosulfan concentration was found in liver and plasma, followed by a rapid decrease in concentration (WHO, 1984).

Metabolism in animals is by oxidation and hydrolysis. When given to rats by various routes, endosulfan was metabolized to the sulfate, diol, hydroxyether, lactone, ether, hydroxy endosulfan carboxylic acid. Most endosulfan metabolites are polar and yet to be identified (WHO, 1984.).

2.1.4 Mechanism of Action

Chlorinated hydrocarbon insecticides act by altering the electrophysiological and associated enzymatic properties of nerve cell membranes, causing a change in the kinetics of Na^+ and K^+ ion flow through the membrane. Disturbances of calcium transport of Ca^{+2}-ATPase activity may also be involved, as well as phosphokinase activities (Hayes and Laws, 1991). Cyclodiene insecticides bind to a site close to or in the ionic channel on gamma amino butyric acid (GABA) receptors and inhibit the binding of the inhibitory neurotransmitter GABA to the receptor. As a result of this there is no opening through which chloride ions can influx. This results in only partial repolarisation of the neuron and a state of uncontrolled excitation. So cyclodienes cause intense stimulation of the nervous system (Sandhu and Brar, 2000). Endosulfan may inhibit differentiation and proliferation of neuronal stem cells and gap junctional intercellular communication, which play crucial role in the maintenance of cellular homeostasis (Kang *et al.*, 2001).

2.2 TOXICITY OF ENDOSULFAN

2.2.1 Maternal Transfer

Bargar *et al.* (2001) reported that very less metabolically susceptible endosulfan was excreted into the egg, relative to Polychlorinated biphenyls (PCBs), when hens were injected subcutaneously every fourth day, during a period of 21 days with 100 microlitre of dosing solution. Dioxins, PCB, DDT, dichlorodiphenyldichloroethylene (DDE), hexachlorohexane (HCH), chlordanes, endosulfan , phytoestrogens (genistein, daidzein and equol), lead and cadmium were detected in human umblical cord and cord serum (Mori *et al.*, 2001).

2.2.2 Embryo Toxicity

Smith *et al.* (1970) did not find any decrease in hatchability when endosulfan was injected in to egg albumin of fertile eggs prior to incubation, at the dose level of 1.5 mg/egg.

Developmental defects have been observed in rats following oral administration of endosulfan, to pregnant dams during gestation. Daily administration of endosulfan at doses of five or 10 mg/kg/day during sixth to 14^{th} day of gestation produced a statistically significant increase in the percentage of resorption and skeletal abnormalities like absence of sixth sternebrae in the foetuses. A dose-related increase in maternal deaths was observed in both test groups. Thus, embryo toxic effects were observed at doses that also caused maternal toxicity (Gupta *et al.* 1978).

Varnagy and Hadhazy (1981) reported that Thiodan 35 EC produced lower incidence of lesions in the developing chick embryo, when it was inoculated into the air chamber on the 12^{th} day of incubation.

Endosulfan was non-teratogenic on administration into the air space of embryonated Japanese quail eggs, during ninth day of incubation (Varnagy, 1981).

Exposure of rabbits to endosulfan during sixth to 28^{th} day of gestation produced no significant effects on the number of implants, litter size, sex ratio, fetal weight or length or the percentage of live or resorbed fetuses. However, dams treated with 1.8 mg/kg/day exhibited neurotoxic signs such as noisy, rapid breathing, hyperactivity and convulsions (Food Machinery and Chemical Corporation, 1981).

Administration of endosulfan to rats at doses as low as six milligram per kilogram per day, from two weeks prior to mating through weaning, resulted in a significant decrease in mean litter weight during lactation (Hoechst 1982). At eight milligram per kilogram per day in this study, an increase in pup mortality was also

observed. Maternal toxicity characterised by decreased body weight and increased relative liver weight was observed in females at six milligram per kilogram per day and above.

The extent of organochlorine pollutant metabolism in chick embryo was dependent upon liver enzyme induction. Liver mixed-function oxidases were induced by several classes of organochlorines, midway through embryonic development. Avian embryos were particularly at risk from metabolite activation because the metabolite products were not excreted from the egg, but remained in the blood circulation throughout incubation (Michael Fry, 1995).

Lemonica and Takahashi (1996) studied embryo toxicity of endosulfan in malnourished rats by giving endosulfan @ 0.60 mg/kg/day. The malnourished rats presented decreased foetal and placental weight. Endosulfan treatment did not cause a rise in the number of foetal malformation, but caused embryo lethal effect in malnourished mothers.

Pushpanjali *et al.* (1999) found that the possible LD_{50} of α-endosulfan in chick embryo was five microgram in 0.1 ml of five per cent ethanol.

Fox and Grasman (1999) reported that when chicken eggs were injected with PCB 126 at the dose level of 0.051 to 0.80 ng/g egg into the air cell before incubation, thymus mass dropped sharply between 0.13 and 0.32 ng/g and lymphoid cell numbers in the thymus fell sharply between 0.051 and 0.13 ng/g. Bursa mass began to decrease at the lowest dose of 0.051 ng/g and reached a minimum at 0.32 ng/g. The number of viable cells decreased slightly at 0.051 ng/g and reached a minimum at the 0.13 and 0.32-ng/g doses. In general, lymphoid cell numbers were more sensitive to PCB 126 than organ masses and the bursa tended to be more sensitive than the thymus.

Pourmirza (2000) observed that malathion and endosulfan at 1.25 mg/egg caused no pronounced increase in embryo mortality when injected into the yolk sac prior to incubation and noted that increased dose generally resulted in a decrease in embryonic body weight.

The evidence for endosulfan-induced adverse developmental effects in animals was inconclusive (Agency for Toxic Substances and Disease Registry, 2000).

Wiebe *et al.* (2001) studied the alligator egg and maternal concentrations of several persistent pesticides as a pointer of alligator egg quality. Mean pesticide concentrations were highest for egg yolk while maternal blood did not differ. The primary persistent pesticides in eggs were toxaphene, DDE, chlordanes, dieldrin, endosulfan and other DDT metabolites.

Dalsenter *et al.* (2003) observed no reduction in the body weight of the dams, litter size, number of viable pups, body weight of the offspring at birth and weaning, when endosulfan was given to female rats at the dose level of 0.5 or 1.5 mg/kg, 21 days prior to mating, during mating, pregnancy and lactation.

2.2.3 Gross Pathology

2.2.3.1 *Poultry*

Reduction in absolute and per cent organ weights of liver, spleen, kidney, lungs, bursa with atrophy of thymus and bursa were observed when day old White Leghorn (WLH) chicks were fed endosulfan @ eight ppm to 48 ppm for 11 weeks (Kurkure *et al.* 1993). Bhattacharya *et al.* (1993) reported that when WLH birds of either sex weighing 800-1000 g were administered 0.75 mg endosulfan/kg body weight orally for 21 days, the kidney, spleen, liver and heart were enlarged and blackish. Petechial

haemorrhage in the brain, edema and haemorrhagic spots in the intestine were also observed.

Selvaraj *et al.* (2000) observed enlarged and congested liver, engorgement of the gall bladder, splenomegaly, enlarged congested kidneys and mild congestion of brain, when technical grade endosulfan was fed to broiler chicks @ 30, 60 and 120 ppm from day old to eighth week. They did not observe any gross lesions in the bursa.

2.2.3.2 *Cattle*

No gross lesions were seen when four to 11-month- old calves were dusted with four per cent endosulfan (Nicholson and Cooper, 1977). Haemorrhage on the serosa of the visceral organs and lungs, severe edema and emphysema of lungs were observed when cattle were applied topically with endosulfan for ectoparasitic control (Mor and Ozmen, 2003).

2.2.3.3 *Goat*

Das *et al.* (1992) studied endosulfan toxicity in Black Bengal goats, by giving endosulfan @ 0.1 mg/kg body weight intravenously. They observed enlargement and focal areas of hardness in liver, flabby heart, enlarged and edematous lungs with oozing of serous exudate from the cut surface. They also observed rough and thick intestine, enlarged kidneys and thickening of the capsule of the adrenal gland.

2.2.3.4 *Dogs*

No gross lesions were seen in dogs when endosulfan was administered orally at levels of 0.075, 0.25 or 0.75 mg/kg body weight for six days a week, over a one year period (WHO, 1968). Rao and varshneya (1995) reported that a four-year-old male gaddi dog which died after licking empty endosulfan container showed haemorrhages in the oesophagus, entire gastro intestinal tract, peritoneum, heart, lungs and brain.

Abdominal cavity was filled with tarry coloured fluid and the whole respiratory tract was filled with froth.

2.2.3.5 *Laboratory Animals*

Male rats given single oral dose of 200 mg/kg of endosulfan had myocardial haemorrhages and acute emphysema of lungs (Terziev *et al.*, 1974). Gupta and Srivastava (1980) reported that when adult male albino rats were given orally 0.625, 2.5 and five milligram per kilogram body weight of endosulfan for 58 days, the weight of the testes at all dose levels remained unchanged where as at 2.5 and five milligram per kilogram body weight levels the weight of the epididymis, seminal vesicles and ventral prostates were decreased.

Banerjee and Hussain (1986) reported that when endosulfan was administered in the diet of male rats at concentrations ranging from five to 50 ppm for six to 22 weeks resulted no change in spleen and thymus weight in animals treated for six weeks, but a significant decrease in spleen weight was observed at 22 weeks in the 1.8 mg/kg/day dose group.

Yaqoob *et al.* (1995) reported that adult female rabbits fed 1.5 mg/kg of endosulfan for 12 weeks showed, enlarged, fragile liver with congestion and necrotic changes and pale lung with necrotic and haemorrhagic changes. The heart, kidney and adrenal did not show any lesion other than increase in the weight.

Sprague-Dawley rats of six-week age, that were fed endosulfan at three, 7.5, 15 and 75 ppm levels for 104 weeks, showed greater number of enlarged kidneys in females. Aneurysms and enlarged lumbar lymph nodes in males were noticed at 75ppm level (Hack *et al.*, 1995).

Wade *et al.* (2002) reported that when Sprague Dawley rats were administered with complex mixture of persistent contaminants including endosulfan @ one

microliter per kilogram body weight by gavage daily for 70 days, resulted in significant increase in the weight of liver and kidney, but there was no effect on the weight of thymus, adrenal glands and organs of reproductive tract.

2.2.4 Histopathology

2.2.4.1 *Thymus*

Deshmukh *et al.* (1990) reported that when day-old male WLH chicks weighing 35 to 40 g were fed endosulfan at the concentration of two ppm for first two weeks, four ppm during third and fourth week and eight ppm during five to ten weeks, cell population in thymic lobules was reduced.

Increase in the Hassal's corpuscles with hyalinisation of follicles in the thymus was observed when endosulfan was fed @ eight to 48 ppm for 11 weeks to chicks (Kurkure *et al.*, 1993).

2.2.4.2 *Bursa*

Edema of bursa, cell depletion in medullary area, necrosis and atrophy in some follicles were observed when day old male WLH chicks weighing 35 to 40 g were fed endosulfan at the concentration of two ppm for the first two weeks, four ppm during third and fourth week and eight ppm during five to ten weeks (Deshmukh *et al.*, 1990).

Vacuolar degeneration and necrosis of the follicles in the bursa, reticular hyperplasia and proliferation of fibrous connective tissue were observed when endosulfan was fed @ eight to 48 ppm for 11 weeks to chicks (Kurkure *et al.* 1993).

Selvaraj *et al.* (2000) reported lymphoid depletion, hyperplastic and vacuolar degenerative changes in the plical epithelium, when technical grade endosulfan was

fed to broiler chicken daily from day one to eighth week at different dose levels. They also observed reticular hyperplasia, medullary necrosis, heterophilic infiltration, glandular formation and atrophic changes in some of the follicles in the bursa.

2.2.4.3 *Spleen*

Necrotic and hyperplastic changes in lymphoid follicles were seen in spleen when eight-week-old WLH cockerels were fed 25, 50 and 100 ppm endosulfan for 90 days (Varshneya *et al.*, 1988).

Concentric hypertrophy of the arterioles in the spleen and reticular hyperplasia were observed when endosulfan was fed @ eight to 48 ppm for 11 weeks to chicks (Kurkure *et al.*, 1993). Bhattacharya *et al.* (1993) reported hyperplastic changes in germinal centre, diffuse haemorrhage in sinuses and abnormal thickening of blood vessels in the spleen, when endosulfan was fed to WLH birds at the dose level of 0.75 mg/kg for 21 days.

Dwivedi and Singh (1994) reported that when three to four month-old cross-bred, male, bovine calves were drenched endosulfan in water either as a single dose of eight milligram per kilogram or two oral doses of two milligram per kilogram body weight one week apart, spleen revealed congestion, marked lymphoid degeneration and rarefaction.

Selvaraj *et al.* (2000) reported that when 30, 60 and 120 ppm technical endosulfan was fed to broiler chicken daily from day one to eighth week, spleen revealed congestion, lymphoid depletion and reticular hyperplasia, hyperplastic changes in blood vessels and formation of germinal centres.

2.2.4.4 *Liver*

Varshneya *et al.* (1988) reported that eight-week-old WLH cockerels fed with 25, 50 and 100 ppm endosulfan for 90 days showed mild to moderate vascular and sinusoidal congestion and dose dependent degenerative changes, fatty changes and focal areas of hepatocellular necrosis.

Fatty changes in liver and marked connective tissue proliferation in the periportal areas were observed when Black Bengal goats were given 0.1 mg endosulfan/kg body weight intravenously (Das *et al.*, 1992).

Kurkure *et al.* (1993) reported that day-old WLH chicks fed endosulfan at the dose level of eight to 48 ppm for 11 weeks showed granular degeneration of hepatocytes. Bhattacharya *et al.* (1993) observed haemorrhages, coagulative necrosis in the liver and aggregation of leucocyte in periportal areas when endosulfan was fed to WLH birds weighing 800 to 1000 g at the dose level of 0.75 mg/kg for 21 days.

Dwivedi and Singh (1994) reported that when three to four-month-old cross bred, male, bovine calves were drenched endosulfan in water, either as a single dose of eight milligram per kilogram or two oral doses of two milligram per kilogram body weight one week apart, liver showed congestion, degeneration and necrosis.

Rao and Varshneya (1995) found fatty changes in liver of a four-year-old male gaddi dog that died after endosulfan intoxication.

Selvaraj *et al.* (2000) observed that when 30, 60 and 120 ppm technical endosulfan was fed to broiler chicken daily from day one to eighth week, showed congestion, dilatation of sinusoidal spaces from second week, haemorrhage, hydropic and fatty changes from fourth week and periductular mononuclear cell infiltration from sixth week in 30 ppm group. Mononuclear cell collections were noticed from second week onwards and perihepatitis from fourth week onwards in 120ppm group.

Hypertrophy and fatty change were seen in hepatocytes, when male rats were exposed to complex mixture of persistent contaminants including endosulfan, at the dose rate of one microliter per gram body weight by gavage daily for 70 days (Wade *et al.*, 2002).

Lipofuschin was accumulated in 69 per cent of hepatocytes in fresh water catfish exposed to endosulfan at the dose level of one microgram per litre (Nowak and Kingsford, 2003).

2.2.4.5 ***Kidney***

Congestion and focal degeneration in the epithelial lining of kidney tubules were observed in male rats treated with doses of 10 mg/kg/day for 15 days (Gupta and Chandra 1977).

Consumption of technical-grade endosulfan for 78 weeks at doses of 20 mg/kg/day to male rats and 11.1 mg/kg/day to female rats resulted in toxic nephropathy, characterized by degenerative changes in the proximal convoluted tubules at the junction of the cortex and medulla. Cloudy swelling, fatty degeneration and necrosis of the tubular epithelium were also evident (National Cancer Institute, 1978).

Microscopically, no adverse effects were observed in the kidney when endosulfan was fed at the dose level of 7.3 mg/kg/day to male rats and 7.52 mg/kg/day to female rats for 13 weeks (Hoechst 1984).

Yellow protein aggregates in the lumen and eosinophilic droplets in the cells of some proximal convoluted tubules were observed in rats following consumption of a diet that provided 3.9 mg/kg/day of technical endosulfan for 13 weeks (Hoechst, 1985).

No adverse histopathological effects were observed in the kidneys and urinary bladder when endosulfan was given to male and female dogs at the dose level of 2.9 and 2.6 mg/kg/day respectively for 146 days (Hoechst, 1989a).

Das *et al.* (1992) observed marked connective tissue proliferation in the intertubular spaces of kidneys when Black Bengal goats of either sex weighing 10 to 14 kg were given 0.1 mg endosulfan/kg body weight.

Kurkure *et al.* (1993) observed swelling of tubular epithelial cells of the kidneys when day old WLH chicks were fed endosulfan at the dose level of eight to 48 ppm for 11 weeks.

Bhattacharya *et al.* (1993) reported extensive haemorrhages in the kidney, coagulative necrosis in the tubules and the glomeruli with mononuclear cell infiltrations, when endosulfan was fed to WLH birds at the dose level of 0.75 mg/kg for 21 days.

Extensive glomerular and tubular degeneration and desquamation of tubular epithelium with severe nephritis were observed, when three to four-month-old cross bred, male, bovine calves were drenched endosulfan in water either as a single dose of eight milligram per kilogram or two oral doses of two milligram per kilogram body weight, one week apart (Dwivedi and Singh, 1994).

Rao and Varshneya (1995) observed acute tubular degeneration in the kidney of a four-year-old male gaddi dog, which died after endosulfan poisoning.

Hack *et al.* (1995) reported a minimal incidence of progressive glomerulonephrosis and an increased incidence of aneurysms in the kidneys of male rats fed 75 ppm of technical endosulfan for 24 months.

Selvaraj *et al.* (2000) reported that when technical endosulfan was fed @ 30, 60 and 120 ppm to broiler chicken daily from day one to eighth week, showed diffuse hyperemia, swelling of the tubular epithelial cells and occasional mononuclear cell infiltration in the interstitium. Lumen of most of the tubules contained eosinophilic cellular casts at 30 ppm level. Tubular epithelial necrosis, multifocal mononuclear cell infiltration, focal areas of fibrosis, hypercellularity in the glomerulus and marked thickening of the capsule with adhesion to the cortex were noticed in 60 ppm group. Atrophic changes in the glomerulus, tubular epithelial necrosis, mononuclear cell infiltration and interstitial fibrosis were observed in 120 ppm group.

2.2.4.6 *Lungs*

Local inflammation of the lungs and dilated alveoli were observed in rats administered 10 mg/kg/day of endosulfan in peanut oil by gavage for 15 days (Gupta and Chandra, 1977)

Das *et al.* (1992) observed pneumonic changes, emphysema and marked connective tissue proliferation in the interalveolar spaces in lungs when endosulfan was given at the dose level of 0.1 mg/kg body weight intravenously to Black Bengal goats.

Dwivedi and Singh (1994) reported that when three to four-month-old cross bred, male, bovine calves were drenched endosulfan in water, either as a single dose of eight milligram per kilogram or two oral doses of two milligram per kilogram body weight one week apart, lungs showed congestion and serofibrinous pneumonia.

2.2.4.7 *Heart*

Long-term exposure of animals to sub lethal concentrations of endosulfan has not resulted in gross or microscopic evidence of cardiovascular toxicity (Hoechst 1984). However, male rats that consumed 2.9 mg/kg/day for two years had an

increased incidence of aneurysms in blood vessels. Female rats were not similarly affected at doses up to 3.8 mg/kg/day for two years (Hoechst 1989b).

Anand *et al.* (1990) reported that when albino rabbits were exposed to 2.5 to five milligram per kilogram HCH or 2.5 to five milligram per kilogram endosulfan intraperitoneally twice a week for 12 months, showed muscle fiber degeneration with vacuolization and leukocytic infiltration in the hearts of rabbits receiving five milligram per kilogram HCH or endosulfan for 12 months. Some of the muscle fibers in the subendocardial region were fragmented and revealed loss of striations with the high HCH dose.

Das *et al.* (1992) observed fatty changes and coagulative necrosis in the myocardium of Black Bengal goats, following intravenous dosing of endosulfan at 0.1 mg /kg.

Bhattacharya *et al.* (1993) reported that when WLH birds of either sex weighing 800 to 1000 g were administered 0.75 mg endosulfan/kg body weight orally for 21 days, heart muscle showed fragmentation and occasional coagulative necrosis. The coronary vessels were engorged with red blood cells and the space between the myofibrils showed infiltration of red blood cells and mononuclear cells.

2.2.4.8 *Intestine*

Das *et al.* (1992) observed alteration and disruption of architecture of intestine when Black Bengal goats were dosed with endosulfan at 0.1 mg/kg body weight intravenously. Marked connective tissue proliferation in the lamina propria of large intestine and hyperactivity and hypercellularity in the peyer's patchs were also observed.

2.2.4.9 *Brain*

Encephalitis, meningitis and edema with infiltration of eosinophilic granule cells in the brain of the fish were seen when endosulfan was sprayed at the concentration of six to 12 g/hectare for tsetse fly control in Botswana (Matthiessen and Roberts, 1982).

Bhattacharya *et al.* (1993) reported that when WLH birds of either sex weighing 800 to 1000 g were administered 0.75 mg endosulfan/kg body weight orally for 21 days, brain showed colliquative necrosis.

Degenerative and necrotic changes along with infiltrated phagocytic cells in the brain were the lesions observed by Mahipal *et al* (1995) when chicks were fed 60 ppm of endosulfan.

Selvaraj *et al.* (2000) reported that when 30, 60 and 120 ppm technical endosulfan was fed to broiler chicken daily from day one to eighth week, brain showed congestion, spongiosis, focal neuronal degenerative change, satellitosis, neuronophagia, meningeal thickening, focal areas of gliosis and swollen meningeal vessels. Atrophy and focal degenerative changes of Purkinje cells were also noticed in the cerebellum.

2.2.4.10 *Adrenal Gland*

Degenerative changes in adrenal cortex were observed when eight-week-old cockerels were fed 25, 50 or 100 ppm endosulfan for a period of 90 days (Varshneya *et al.*, 1988).

Thickened capsules and hyperplasia of cortical cells were seen in the adrenal gland of Male albino rabbits exposed to 2.5 to five milligram per kilogram HCH or

2.5 to five milligram per kilogram endosulfan intraperitoneally twice a week for 12 months (Anand *et al.*, 1990).

Fatty changes and marked connective tissue proliferation in the capsular areas of adrenal gland were observed when Black Bengal goats were given endosulfan 0.1 mg/kg body weight intravenously (Das et *al.*, 1992).

2.2.4.11 ***Immunotoxicity***

No specific immunotoxic effects were found when endosulfan was fed at the dietary concentration of 20, 100 or 250 ppm for three weeks, to male weanling Wistar rats. The most sensitive parameter for the toxicity of endosulfan was a reduction in body weight gain, which was observed at 100 ppm (Vos *et al.*, 1982).

Male albino rats were given a diet containing five, 10 or 20 ppm endosulfan for eight to 22 weeks and immunized with tetanus toxoid in Freund's complete adjuvant subcutaneously. Antibody titre was significantly decreased in endosulfan-exposed rats at 10 and 20 ppm levels. Rats in the 10 and 20 ppm dose groups had significantly depressed leukocyte migration inhibition and macrophage migration inhibition responses. Results obtained in this study revealed marked suppression of the humoral and CMI responses in rats administered with sub chronic doses of endosulfan (Banerjee and Hussain , 1986).

Endosulfan was administered to male Wister rats at dietary doses of up to 50 ppm for six weeks. Significant decrease in total serum antibody titre to tetanus toxoid was seen at 30 and 50 ppm. A decrease in both IgM and IgG in the total γ-globulin content of rat serum was observed at 50 ppm. There was also significant decrease in inhibition of leukocyte and macrophage migration in a dose dependent pattern (Banerjee and Hussain, 1987).

White leghorn cockerels of four to eight week-old were fed with 400, 800 and 1600 ppm of malathion and 25, 50 and 100 ppm of lindane and endosulfan. The cockerels receiving lindane at 50 and 100 ppm and endosulfan at 25 ,50 and 100 ppm had significantly ($p<0.05$) lower haemagglutinin titres. Highest decline was observed in cockerels receiving lindane at 100 ppm. Endosulfan produced immunosuppressive effect even at the lowest dietary concentration of 25 ppm. Malathion however produced only a slight non-significant decrease ($p>0.05$) in haemagglutinin titres at 1600 ppm (Varshenya *et al.*, 1988).

Kurkure *et al.* (1993) reported that when day-old WLH chicks were fed with endosulfan at the dose level of eight parts per million to 48 ppm for 11 weeks and vaccinated against New castle disease on third day and at eighth week with lasota strain, the haemagglutination inhibition titres were lowered in a dose dependent manner. The cell mediated immune responses studied by skin sensitivity test using hapten -1-chlor -2, 4-D-nitrobenzene (DNCB) was also found to be suppressed.

Koner *et al.* (1998) studied the relationship between oxygen free radical (OFR) generation and immunotoxicity of pesticides by giving DDT at the dose level of 100 and 200 ppm, lindane at the dose level of 40 and 80 ppm in the diet for eight weeks to male wistar rats. They stated that increased thiobarbituric acid reactive substance (TBARS) in the serum in a dose dependent manner indicated an increased oxygen free radical generation by these chemicals. There was increase in super oxide dismutase activity in the erythrocytes. The antibody titre against sheep red blood cells was also reduced. They suggested that organochlorine induced immunosuppression might be due to OFR generation.

Khurana *et al.* (1998) reported significant depression in the number of functional macrophages ($P<0.05$) following endosulfan treatment at the dose level of 30 ppm to broiler chicks for eight weeks.

Pistl *et al.* (2003) noticed decreased index of metabolic activity when sheep leucocytes were treated with one per cent solution of 10^{-1} to 10^{-6} *M* endosulfan. They also observed decrease in spontaneous migration of leukocytes at the concentration of $10^{-1}M$ and decrease in lymphocyte activation with phytohaemagglutinine at the concentration of 10^{-2} to$10^{-3}M$.

2.2.4.12 *Immunostimulation*

Vijayan *et al.* (1990) observed stimulation of humoral immune response in ducks administered aqueous solution of furadan at 0.25 mg/kg body weight on alternative days for two months.

2.2.4.13 *Endocrine toxicity*

Endosulfan significantly inhibited testicular androgen biosynthesis in adult rats, when fed at 7.5 and 10 mg/kg body weight orally, consecutively for 15 and 30 days. Profound decrease in the levels of plasma gonadotrophins (FSH and LH) along with plasma testosterone and testicular testosterone were observed. No appreciable alterations were apparent in body weight and testicular weights (Singh and Pandey, 1990).

The dietary exposure to endosulfan at the dose levels of 12.5 and 37.5 mg/250g daily to female goats for 90 days caused a significant decrease in urinary 17-ketosteroid level. The decrease in level was associated with decreased hormone synthesis and hypofunction of the adrenal and ovarian glands (Bose *et al.*, 1991).

Wade *et al.* (2002) studied the thyroid toxicity due to sub chronic exposure to a complex mixture of 16 organochlorines (including endosulfan), lead and cadmium, by exposing at the dose level of 1X, 10X, 100X and 1000X of their daily safe or no effect levels, to male Sprague Dawley rats for 70 days. They observed significantly reduced circulating T_4 levels only in response to 1000x treatment while T_3 levels were

increased in 100X and 1000X animals. They also observed reduced amount of colloid within follicles, increased size and vacuolization of follicular epithelium during exposure to highest dose of the mixture.

2.2.4.14 *Reproductive effects*

Chlordane and endosulfan at the dose level of 0.41 ng/ml or 0.41ppb strongly inhibited the acrosome reaction of sperm initiated by progesterone. Inhibitory concentration of these cyclodienes were well within the range detected in human and wildlife tissue and fluids as a result of environmental contamination (Turner *et al.*, 1997).

Male rats of pubertal age were orally administered endosulfan at a dose of one milligram per kilogram body weight for 30 days. Decrease in the testicular lactate and pyruvate activities and reduction in the testicular DNA and RNA concentrations and increase in the testicular protein concentration were the changes observed (Chitra *et al.*, 1999).

Dalsenter *et al.* (1999) reported that when female rats were treated orally with 1.5 or 3.0 mg endosulfan/kg from day 15 of pregnancy to postnatal day 21 of lactation, the daily sperm production in the male offsprings was permanently decreased in the highest dose group. The dose of three milligram per kilogram induced a decrease in maternal body weight during pregnancy. Daily sperm production was the most susceptible endpoint in the male offspring exposed to endosulfan during pregnancy and lactation. However pre and postnatal exposure to low doses of endosulfan (0.5 and 1.5 mg/kg) did not induce significant adverse effects in the reproductive system of male offspring Wistar rats at adulthood (Dalsenter *et al.*, 2003)

2.2.4.15 *Carcinogenicity*

Flodstrom *et al.* (1988) opined that endosulfan was a potential inhibitor of intercellular communication, a property of many tumor-promoting agents.

Hack *et al.* (1995) reported that treating six week old Sprague-Dawley rats and four week old NMRI mice with endosulfan at doses of three, 7.5, 15 or 75 ppm for 104 week and two, six or 18 ppm for 24 months respectively, showed no carcinogenic potential.

2.3 MATERNAL IMMUNOGLOBULIN Y (IgY) LEVEL IN THE EGG YOLK AND ITS QUANTIFICATION

2.3.1 Passive Immunity in the Chick

Antibodies were transmitted in increasing amounts from 11^{th} day of incubation to hatching (Buxton, 1952). Antibodies were secreted into the yolk sac from the secretory follicles in the epithelial lining of the oviduct during maturation of the egg (Solomon, 1971).

Yamamoto *et al.* (1975) found the presence of IgY, IgM and IgA in concentrated preparation from chicken egg yolk, white and oviduct washings of chicks.

Serum immunoglobulins were readily transferred from hen serum to the yolk while the egg was still in the ovary. IgY was found at levels equal to those in hen serum. As the egg passes down the oviduct IgM and IgA from oviduct secrections are acquired with the albumin. As the chick embryo develops, it absorbs some of the yolk IgY. The newly hatched chick does not absorb its entire yolk sac antibody until about 24 h after hatching (Tizard, 2000).

2.3.2 Persistence of Antibody in the Egg Yolk During Storage and Incubation

Brandly *et al.* (1946) found the titre of antibody to Newcastle disease virus to be well maintained in the eggs that had been stored for six months at 6^0C.

Antibody in yolk appears to be remarkably stable and persistent, although proteolytic enzymes are known to be present. Although the quantity of antibody in the yolk sac presumably declines with the quantity of yolk, the concentration in the residue does not decline appreciably (Brambell, 1970).

2.3.3 IgY Quantification

A variety of methods have been employed for the quantification of avian serum proteins, which include salting out with sodium sulphate, electrophoresis, polyacrylamide gel and radial immunodiffusion. Of these, the single radial immunodiffusion method developed by Mancini *et al.* (1965) had proved valuable for quantification of individual immunoglobulins.

IgY was easily reduced and its antibody activities including precipitation could be diminished or eliminated by mild reduction with two-mercaptoethanol or dithioerythritol (Szendberg *et al.*, 1965).

By radial immunodiffusion test, Lerner *et al.* (1971) found that at hatching, chicks had about 150 mg per cent circulating IgY, derived from the yolk. The amount of IgY in yolk was reported to be 20-25 mg/ml in hen's egg (Rose and Orlans, 1981).

Akitha and Nakai (1992) applied radial immunodiffusion assay to quantify IgY concentration in the egg yolk.

Akitha and Nakai (1993) opined that IgY concentration in egg yolk was about 15-25 mg/ml.

3. MATERIALS AND METHODS

3.1 PESTICIDE

Technical grade endosulfan of 95 per cent purity was procured from Hindustan Insecticides Ltd., Alwaye, Kerala.

3.2 FERTILE CHICKEN EGGS

Three hundred and thirty fertile chicken eggs obtained from University Poultry Farm, Mannuthy, were used for the study.

3.3 PESTICIDE SOLUTION

Technical grade endosulfan dissolved in five per cent ethanol was used for egg dipping and inoculation.

3.4 EXPERIMENTAL DESIGN

The fertile eggs were randomly divided in to 11 groups of 30 eggs each and designated as group I to group XI.

3.4.1 Egg Dipping

Group I eggs were cleaned with antiseptic lotion, dried and fumigated. Prior to incubation the eggs were dipped for 15 minutes in distilled water containing 25 ppm endosulfan dissolved in five millilitre of five per cent ethanol. The eggs were allowed to dry and incubated in automatic incubator at 37^0C, with relative humidity ranging from 60-70 per cent. All the eggs were candled on fourth day and the infertile eggs were removed.

3.4.2 Egg Inoculation

The remaining eggs belonging to other groups were also incubated. The infertile eggs were removed on the fourth day of incubation and replaced with four-day-old embryonated eggs of equal size and weight. These embryonated eggs were used for egg inoculation.

3.4.2.1 Air Cell Route

Four day old embryonated eggs of group II, III, IV and V were inoculated with endosulfan at the dose level of two, four, eight and 12 microgram respectively through air cell route. Prior to inoculation the eggs were candled, cleaned with rectified spirit, drilled and 0.1 ml of the dosing solution was inoculated through air cell route and sealed by paraffin wax and incubated.

3.4.2.2 Yolk Sac Route

Embryonated eggs of group VI, VII, VIII and IX were inoculated with endosulfan during fifth day of incubation at the dose level of two, four, eight and 12 microgram respectively through yolk sac route and sealed with paraffin and incubated.

3.4.2.3 Control Groups

Group X eggs were inoculated with 0.1 ml of ethanol alone through yolk sac route and incubated. Group XI eggs were incubated without any treatment.

All the embryos were candled daily and the dead embryos were removed weighed, examined in detail for abnormalities and the tissue samples were collected for histopathological studies.

3.5 PATHOANATOMICAL STUDIES

Six eggs from each group were collected for embryopathic studies on the ninth day, 14 th day, 19 th day and all the remaining eggs on the 21st day. The embryos were weighed after gross examination. Whole embryos during ninth day and thymus, spleen, bursa, liver, kidney and brain during 14 th day, 19 th day and 21st day were collected and fixed in 10 per cent neutral buffered formalin. Sections were cut at three-micron thickness and stained with routine Haematoxylin and Eosin staining (Sheehan and Hrapchak, 1980). Weight of spleen, bursa, and thymus were recorded for chicks collected at 21st day.

3.6 QUANTIFICATION OF MATERNAL IgY IN THE POOLED YOLK SAMPLES

3.6.1 Biologicals

Chicken IgY was obtained from Bangalore Genei chemicals, Bangalore. Swine antichicken IgY was procured from Veterinary Institute, Czeck Republic.

3.6.2 Reagents and buffers

3.6.2.1 *Phosphate Buffered Saline (PBS) [10x Stock Solution]*

Sodium chloride	80 g
Potassium chloride	2 g
Disodium hydrogen phosphate (Na_2HPO_4. 12 H_2O)	11.32 g
Potassium dihydrogen phosphate (KH_2PO_4)	2 g
Distilled water	1000 ml

pH was adjusted to 7.4 by 1*N* NaOH and sterilised by autoclaving at 121°C for 15 min at 15 lbs pressure.

3.6.2.2 ***PBS (1x Working Solution)***

PBS (10x)	100ml
Distilled water to make	1000 ml

pH adjusted to 7.4 using 1 *N* HCl.

3.6.2.3 ***Gel for Single Radial Immunodiffusion (SRID) test***

Agarose	0.75 g
Sodium azide	0.01 g
Phosphate buffer saline	100 ml

To dissolve the agarose in PBS, the solution was boiled for five minutes.

3.6.2.4 ***Stain for SRID test***

Amidoblack 10B	0.1 g
Sodium chloride	0.85 g
Distilled water	100 ml

3.6.2.5 ***Decolouriser I***

Methanol	120 ml
Acetic acid	30 ml
Distilled water	30 ml

3.6.2.6 ***Decolouriser II***

Absolute alcohol	140 ml
Acetic acid	20 ml
Distilled water	40 ml.

3.6.3 Method

Pooled yolk samples were collected from each group on the ninth, 14th, 19th and 21st day and stored at -80°C for maternal IgY estimation. IgY levels in the pooled yolk samples were determined by single radial immunodiffusion assay developed by Mancini *et al* (1965) with minor modification.

3.6.3.1 *Pre Coating of Slides*

Clean glass slides were coated by smearing 0.75 per cent melted agarose in distilled water and dried in air by keeping the slides horizontally over glass rods.

3.6.3.2 *Preparation of SRID Slide*

Agarose gel (0.75 percent) in PBS was melted and kept at 56°C in a water bath. Swine antichicken IgY antiserum was warmed to 56°C, one millilitre of antiserum was added to eight millilitre of agarose gel and mixed well. These mixtures in three millilitre quantities were over layered on precoated slides. After solidification, wells of three-millimeter diameter were punched out at a distance of 12 mm between the wells.

3.6.3.3 *Calibration of Standard Curve*

Ten milligram of chicken IgY was dissolved in one millilitre of 0.15*M* NaCl solution and double diluted with the same solution to get a concentration of 10, 5, 2.5 and 1.25 mg/ml. Ten microlitre of diluted samples were charged in the antigen wells in the SRID slide and incubated at 37°C for 24 h in humid chamber. Antigen - antibody precipitation rings formed around the wells were observed and the diameter was measured. A calibration curve was obtained by plotting the diameter of the

precipitation rings after 24 h against the IgY concentration (Graph 1). IgY concentration in pooled yolk samples was determined by reference to this curve.

3.6.3.4 *Estimation of IgY Level in the Pooled Yolk Sample*

Pooled yolk samples were triturated well and one gram of yolk sample was diluted four times with PBS. Ten microlitre of diluted yolk samples were used to fill the antigen well for quantification of IgY. The diameter of the precipitation rings was measured and compared with the standard calibration curve to get the IgY concentration of the diluted sample. This value was multiplied by four to get the final maternal IgY level in the pooled yolk sample.

3.6.3.5 *Staining of the SRID slides*

The slides were rinsed first in two changes of normal saline for 24 h each and then in distilled water for another 24 h to remove the unreacted proteins. Then they were dried slowly, stained with amidoblack 10B for 15 min and decolourised for 20 min each in decolouriser I and II. The decolourised slides were dried and mounted in DPX (Fig. 1).

3.7 Statistical analysis

The weight of the embryos and lymphoid organs were subjected to statistical analysis as per the method of Rangaswamy (1995).

4. RESULTS

4.1 PATHOANATOMICAL STUDIES

4.1.1 Mortality Pattern

No significant mortality could be observed in the endosulfan treated groups. One dead in shell embryo was observed during examination on ninth day in group VIII. One embryo died on 10^{th} day in group IX. During 12^{th} day of incubation one embryo each died in group VII, VIII and group IX. Two dead in shell embryos were observed during 21^{st} day in groups V, VI, VII and IX respectively. The mortality in groups V, VI and VIII was 6.6 per cent, where as in groups VII and IX it was 10 and 13.3 per cent respectively.

4.1.2 Teratological Abnormalities

Agenesis of beak with retarded growth in an embryo of group VII, shorter lower beak (Fig. 2) in an embryo of group IX, crossed beak (Fig. 3) in an embryo of group VIII were the abnormalities observed during ninth day of examination. Eventration of viscera with curled toe (Fig. 4) was observed in an embryo of group VIII during 14^{th} day of examination. Torticollis with partial paralysis of legs in a chick of group III and curled toe (Fig. 5) in a chick of group VI was observed after hatching. Teratological abnormalities noticed in Group III, VI, VII and IX were 3.3 per cent where as 6.6 per cent abnormality was observed in group VIII. Control groups did not reveal any malformations.

4.2 MEAN EMBRYO AND LYMPHOID ORGAN WEIGHT

4.2.1 Mean Embryo Weight

The average weight of the embryos on ninth, 14^{th}, 19^{th} and 21^{st} day of incubation are presented in Table 1. The mean embryo weight of endosulfan treated groups did not differ significantly with that of the control group ($p>0.05$) as shown in the graph 2.

4.2.2 Mean Weight of the Thymus, Spleen and Bursa on 21^{st} day of Incubation

The mean weight of the thymus, spleen and bursa on 21^{st} day are presented in Table 2. The average weight of these lymphoid organs of endosulfan treated groups did not differ significantly with that of control group ($p>0.05$) as shown in the graph 3.

4.3 GROSS PATHOLOGY

4.3.1 Ninth Day of Incubation

Group I, Group II embryos did not reveal any marked changes.

4.3.1.1 *Group III (Air cell, Four microgram)*

Petechiae on the head, neck and left lateral aspect of the abdomen were observed (Fig. 6)

4.3.1.2 *Group IV (Air cell, Eight microgram)*

Petechiae on the head, neck, periorbital area and ecchymoses on the thorax were evident (Fig. 7).

Table-1 Mean embryo weight (value in gm ± S.E)

Route	Group	Mean embryo weight (gm)			
		9^{th} day	14^{th} day	19^{th} day	21^{st} day
Dipping	I	1.78 ± 0.03	8.50 ± 0.11	25.69 ± 0.28	33.60 ± 0.66
Aircell	II	1.75 ± 0.03	8.36 ± 0.11	25.24 ± 0.28	34.38 ± 0.66
	III	1.77 ± 0.03	8.30 ± 0.11	25.36 ± 0.28	33.74 ± 0.66
	IV	1.77 ± 0.03	8.36 ± 0.11	25.35 ± 0.28	32.66 ± 0.66
	V	1.76 ± 0.03	8.36 ± 0.11	25.16 ± 0.28	33.02 ± 0.66
Yolk sac	VI	1.77 ± 0.03	8.63 ± 0.11	25.13 ± 0.28	33.57 ± 0.66
	VII	1.75 ± 0.03	8.56 ± 0.11	25.28 ± 0.28	33.60 ± 0.66
	VIII	1.77 ± 0.03	8.37 ± 0.11	24.98 ± 0.28	33.47 ± 0.66
	IX	1.74 ± 0.03	8.33 ± 0.11	24.78 ± 0.28	33.43 ± 0.66
Control	X	1.79 ± 0.03	8.77 ± 0.11	25.76 ± 0.28	34.37 ± 0.66
	XI	1.78 ± 0.03	8.83 ± 0.11	25.75 ± 0.28	34.39 ± 0.66

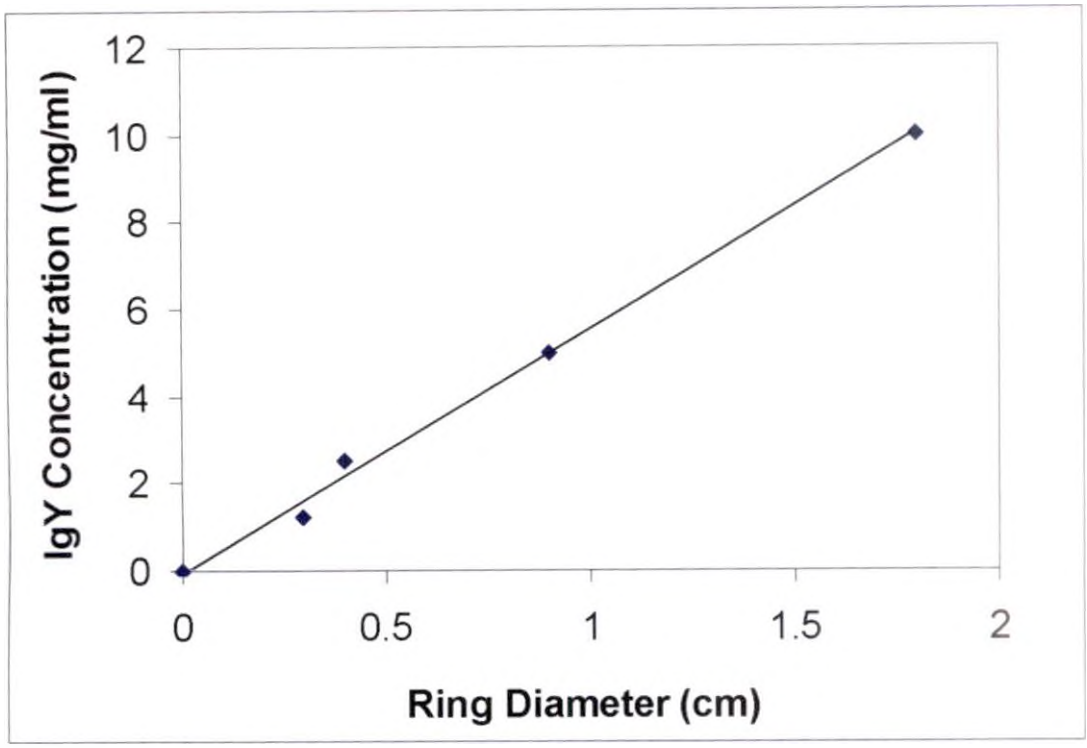
$P > 0.05$

Table -2 Mean weight of lymphoid organs on 21^{st} day (value in mg ± S.E)

Route	Mean weight of lymphoid organs (mg)			
	Group	Thymus	Spleen	Bursa
Dipping	I	37.28 ± 0.23	17.35 ± 0.53	55.55 ± 1.23
Air cell	II	37.20 ± 0.23	17.17 ± 0.53	55.37 ± 1.23
	III	37.23 ± 0.23	16.21 ± 0.53	55.48 ± 1.23
	IV	37.06 ± 0.23	16.22 ± 0.53	54.33 ± 1.23
	V	37.24 ± 0.23	16.67 ± 0.53	55.56 ± 1.23
Yolk sac	VI	37.22 ± 0.23	17.26 ± 0.53	55.57 ± 1.23
	VII	37.20 ± 0.23	17.14 ± 0.53	55.46 ± 1.23
	VIII	37.05 ± 0.23	16.94 ± 0.53	54.73 ± 1.23
	IX	36.87 ± 0.23	16.65 ± 0.53	54.63 ± 1.23
Control	X	37.38 ± 0.23	17.58 ± 0.53	55.68 ± 1.23
	XI	37.43 ± 0.23	17.44 ± 0.53	55.68 ± 1.23

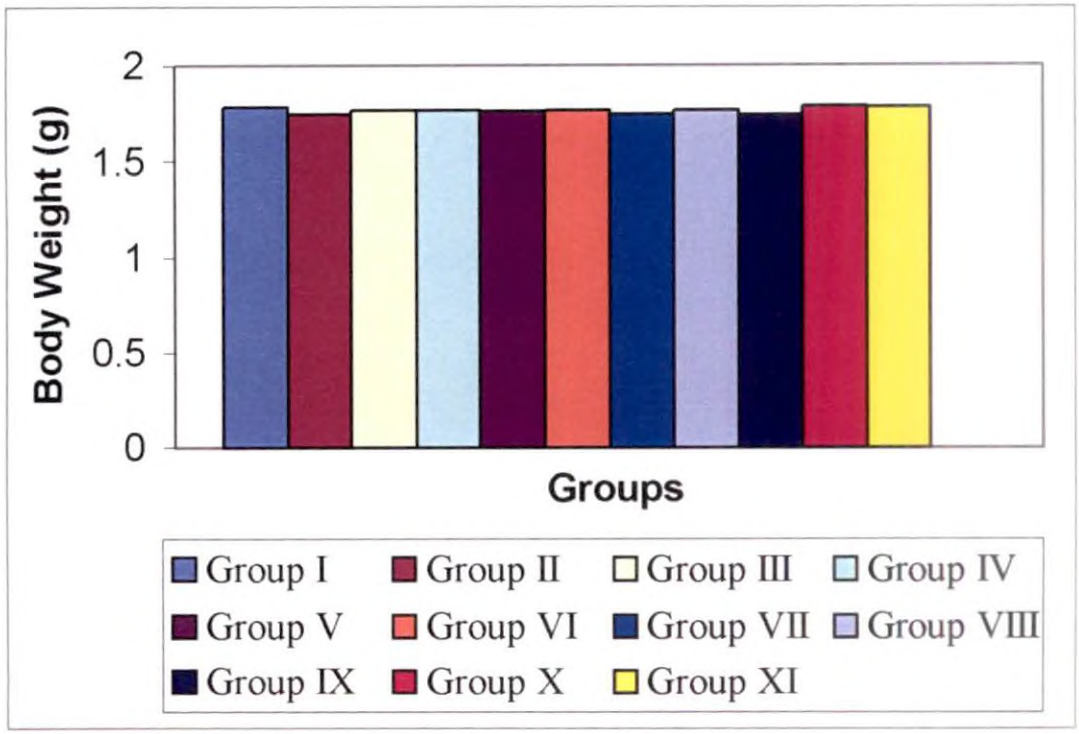
$P > 0.05$

Graph 1- Standard curve for IgY estimation

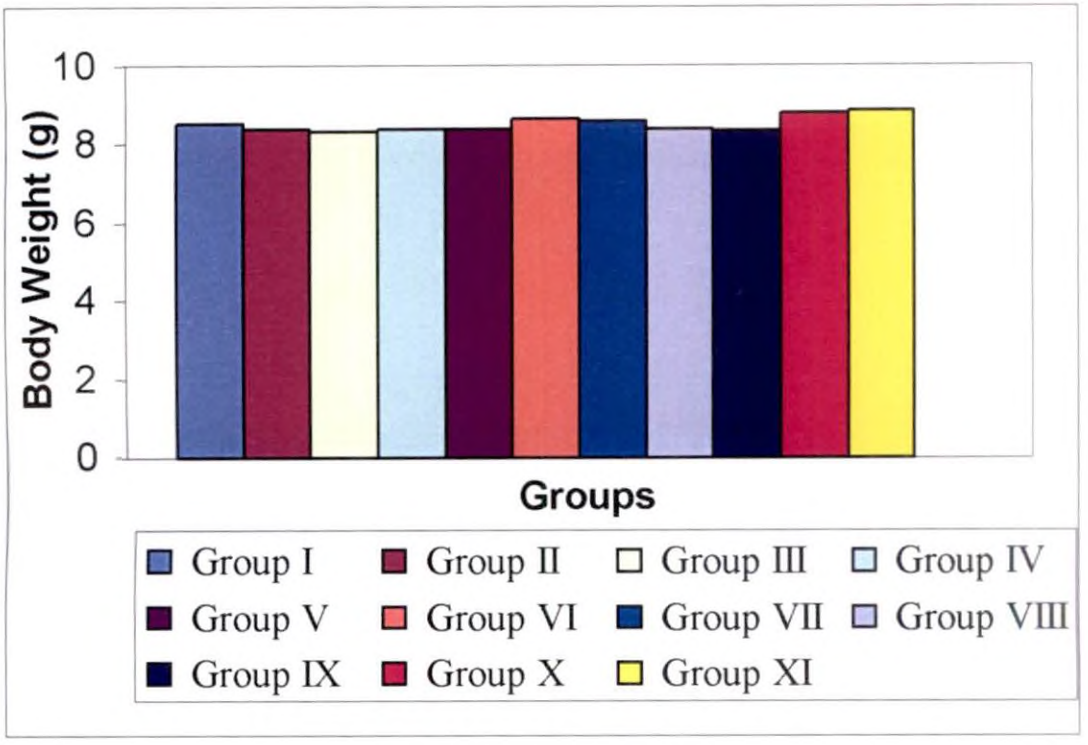


Graph 2 - Mean weight of the embryos

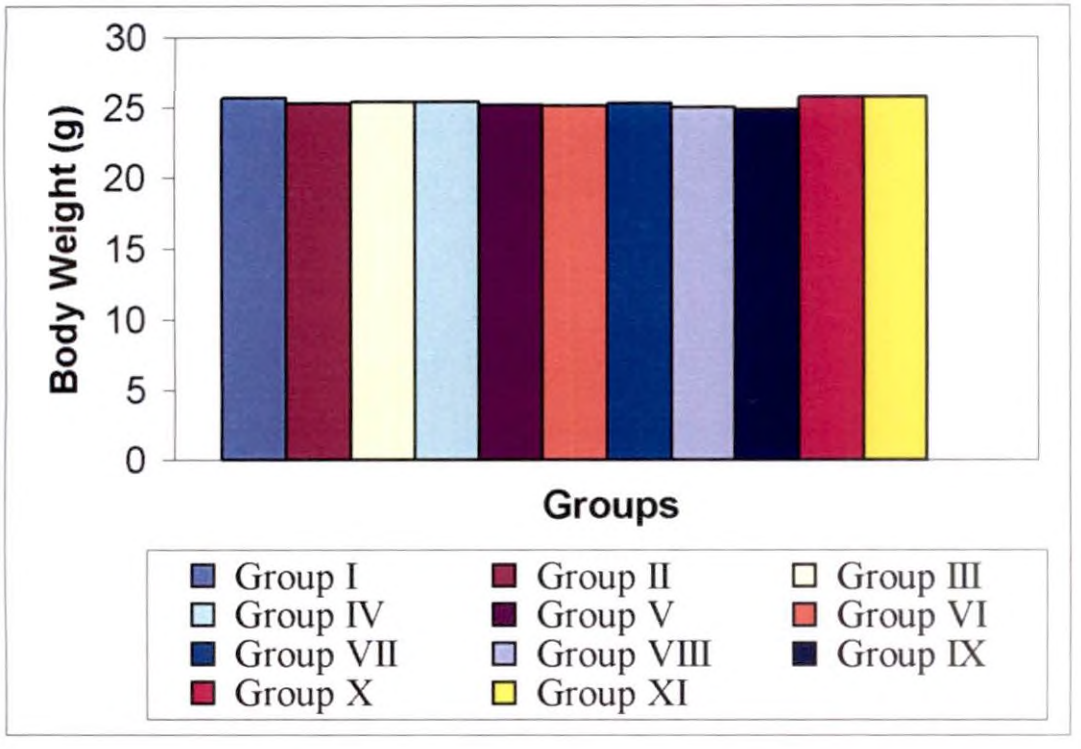
2.1 9th day of incubation



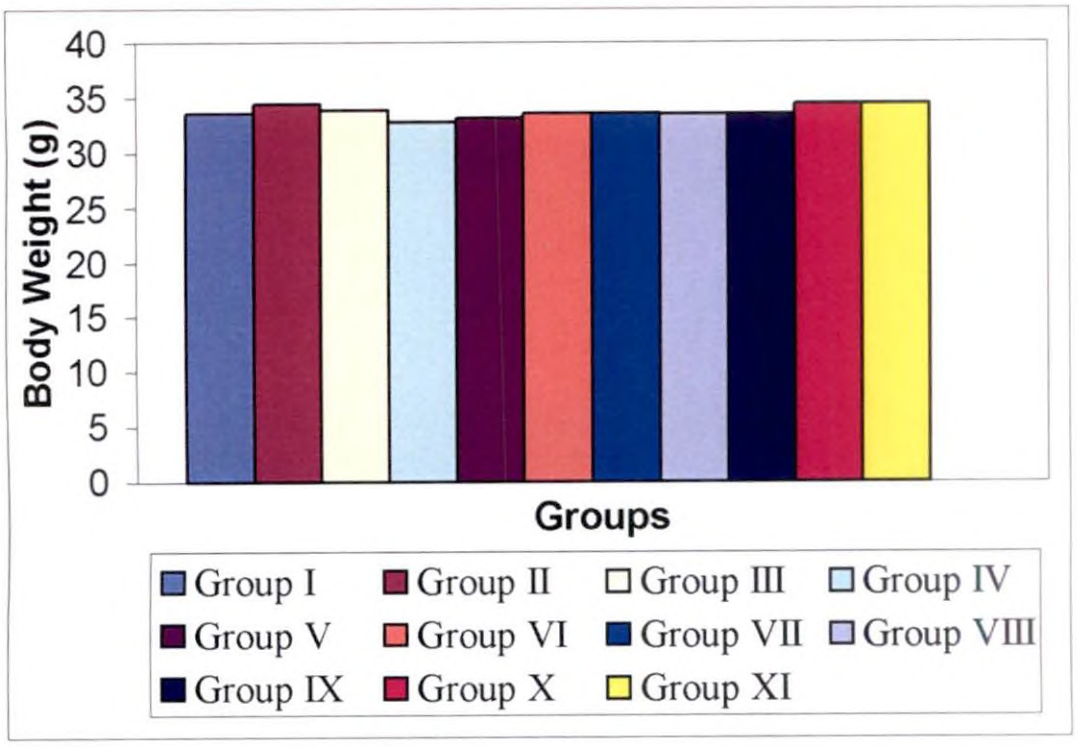
2.2 14th day of incubation



2.3 19th day of incubation

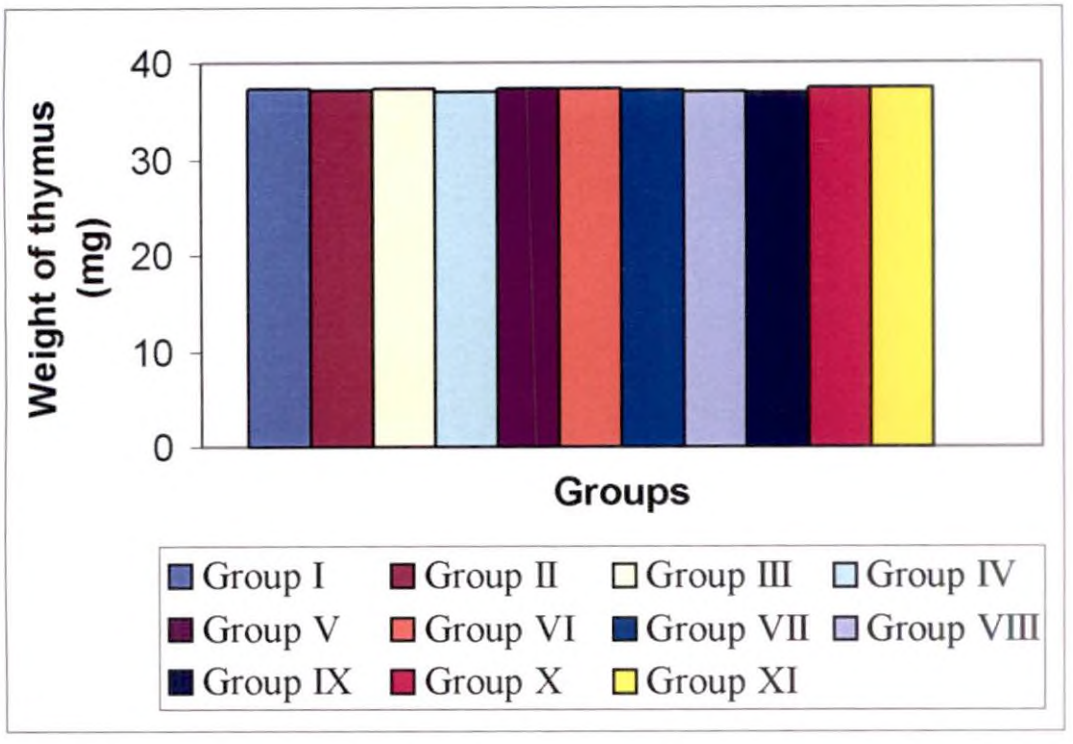


2.4 21st day of incubation

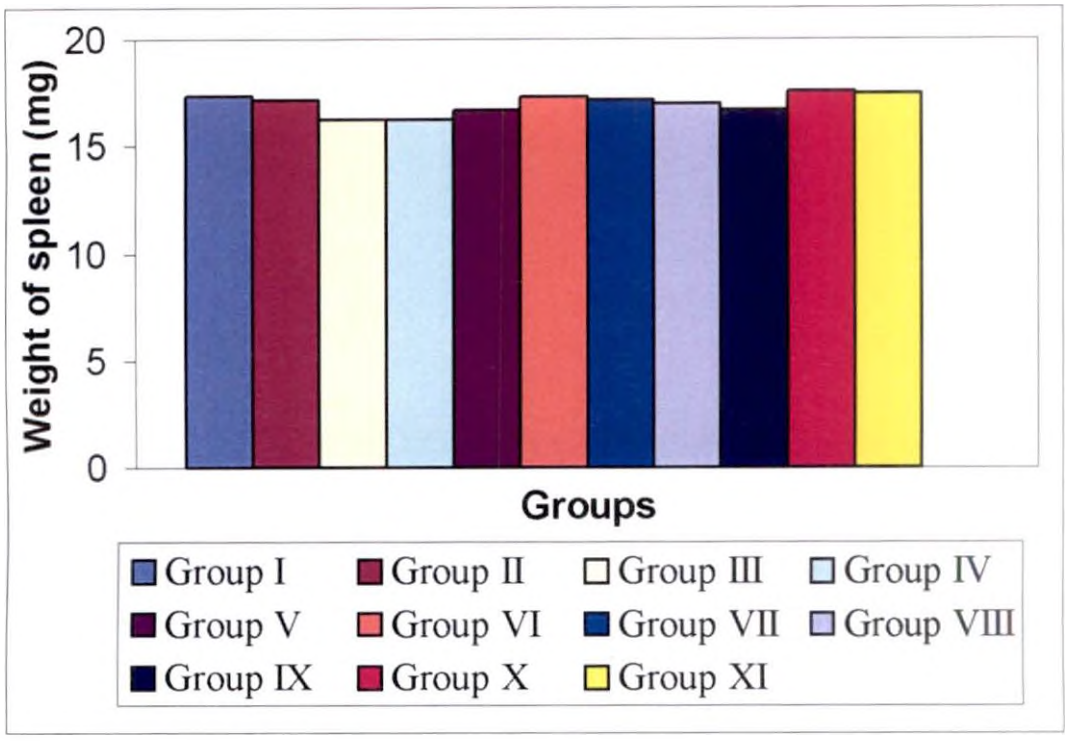


Graph 3 - Mean weight of the lymphoid organs

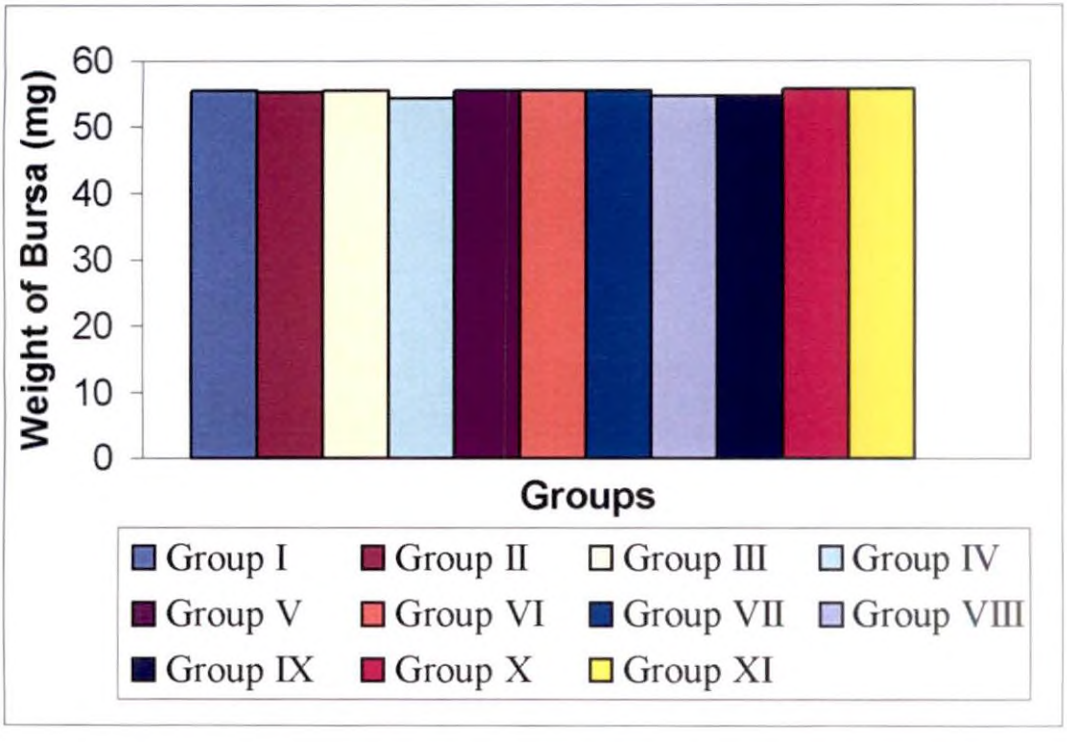
3.1 Mean weight of thymus



3.2 Mean weight of Spleen



3.3 Mean weight of bursa



4.3.1.3 *Group V (Air cell route, 12 µg endosulfan)*

There was ecchymoses on the head and back region.

4.3.1.4 *Group VI (Yolk sac route, Two microgram endosulfan)*

Petechiae on the head and neck were seen.

4.3.1.5 *Group VII (Yolk sac route, Four microgram endosulfan)*

Petechiae on the head, neck and ecchymoses on the left lateral abdomen (Fig.8) were observed.

4.3.1.6 *Group VIII (Yolk sac route, Eight microgram endosulfan)*

There were petechiae on the head, thigh and back region (Fig. 9).

4.3.1.7 *Group IX (Yolk sac route, 12 µg endosulfan)*

Ecchymoses at the base of the lower beak, thigh and in the back region were noticed. Petechiae below the right ventral aspect of the eye and retarded growth in an embryo was also seen.

4.3.1.8 *Control Groups*

Group X and group XI also revealed haemorrhagic lesions in the body but it was less extensive when compared to the treatment groups.

4.3.2 14 th Day of Incubation

Endosulfan treated and control groups did not reveal any gross lesions on examination during 14th day of incubation. All the organs appeared apparently normal.

4.3.3 21st Day of Incubation

Thymic haemorrhages were observed in group V, VIII and IX embryos (Fig. 10). The internal organs did not reveal any gross changes.

4.4 HISTOPATHOLOGY

4.4.1 Group I (Dipping, 25 ppm endosulfan)

4.4.1.1 *Ninth day of incubation*

Kidney revealed vacuolar degeneration of the tubular epithelial cells. Swelling of hepatocytes, mild vacuolation, central venous congestion and diffuse sinusoidal dilatation were observed in the liver (Fig. 11 and 12). Generalised vascular congestion was evident in the lungs. Spleenic vessels were engorged. All the vessels along the para vertebral areas were congested (Fig. 13). No characteristic lesion could be observed in the bursa and thymus except mild congestion.

4.4.1.2 *14th day of incubation*

Thymic follicles appeared well developed and compact. Bursa revealed developing follicles in the cortex. Spleen did not reveal any characteristic lesion. Focal degeneration of tubular epithelium was observed in the kidney. Liver showed focal degeneration of the hepatocytes. Capillary congestion was evident in focal areas in the cerebrum.

4.4.1.3 *19th day of incubation*

Thymus, bursa, spleen and brain did not reveal any characteristic lesions. Kidney showed moderate degeneration of the tubular epithelium. There was moderate vacuolation in the liver.

4.4.1.4 *21 st day of incubation*

Thymic follicles showed widening of the medullary zone (Fig.14), which contained the immature and proliferating cells. These cells were larger and lightly stained. Intrafollicular haemorrhage (Fig. 15) was also noticed in the thymus. Occasional follicles in the bursa showed congestion with diffuse degenerative changes of the lymphocytes. Spleen and the brain were apparently normal. Diffuse degeneration of the renal tubular cells and diffuse vacuolation of the hepatocytes were the other lesions observed.

4.4.2 Group II (Air cell route, Two microgram endosulfan)

4.4.2.1 *Ninth day of incubation*

Active haemopoiesis was observed in the intertubular areas of the kidney. This was characterised by the presence of greater collections of erythrocytes in the intertubular area. The tubules appeared intact. There was mild capillary congestion of glomerulus. The other organs did not reveal any lesions.

4.4.2.2 *14 th day of incubation*

Thymic follicles were compact and well developed. Bursa showed large number of developing follicles when compared to that of control (Fig. 16a and 16b) Mild degenerative changes were observed in the renal tubules. Liver, spleen and brain did not reveal any lesions.

4.4.2.3 *19 th day of incubation*

There was widening of medullary zone in the thymus. There were no microscopic lesions in the bursa, spleen and brain. Occasional mesangial proliferation was seen in few glomeruli. Mild vacuolation of the hepatocytes and prominent Kupffer cells were seen in the liver.

4.4.2.4 *21 st day of incubation*

There was mild degeneration of lymphocytes in the thymus. Bursa revealed mild congestion in the follicles. No lesions were observed in the spleen and brain except mild congestion. Kidney revealed less cellular and shrunken glomeruli along with degeneration of the tubular epithelial cells (Fig. 17). Liver showed extensive congestion and dilatation of hepatic sinusoids. Diffuse hepatic degeneration characterised by pyknotic nucleus and large vacuolation of the hepatocytes were also observed.

4.4.3 Group III (Air cell route, Four microgram endosulfan)

4.4.3.1 *Ninth day of incubation*

Kidney showed mesangial proliferation of the glomeruli (Fig. 18a and 18b). There was marked haemopoiesis in the intertubular area of the kidney. Cloudy swelling, detachment of tubular epithelial cells and tubular dilatation were also evident (Fig. 19). Liver showed mild Cloudy swelling of the hepatocytes. The lymphoid organs and the brain did not reveal any characteristic lesions.

4.4.3.2 *14th day of incubation*

In the bursa, the plical epithelial cells and the plical stroma were prominent. Developing follicles were seen budding off from the plical epithelial cells (Fig. 20). The number of developing bursal follicles were found increased when compared to that of control (as in Fig. 16). Thymus was apparently normal. There was marked haemopoiesis in the spleen. Mild degeneration of the renal tubules was evident. Liver showed occasional vacuolations.

4.4.3.3 *19th day of incubation*

There was widening of medullary zone in the thymus. Bursa, spleen and the brain appeared normal. Diffuse tubular degeneration and increased cellularity in the glomeruli were seen in the kidney. Dissociation and diffuse vacuolation of the hepatocytes along with prominent Kupffer cells were observed in the liver (Fig. 21).

4.4.3.4 *21st day of incubation*

Thymus revealed mild degeneration of the lymphocytes. There was mild congestion of the bursal follicles. Spleen showed active haemopoiesis. Kidney showed diffuse tubular degeneration. Reduced cellularity along with clumping of the cells was evident in some of the glomeruli. There was moderate vacuolation in the hepatocytes.

4.4.4 Group IV (Air cell route, Eight microgram endosulfan)

4.4.4.1 *Ninth day of incubation*

Lymphoid organs did not reveal any marked lesions. Liver showed hepatocyte degeneration with mild vacuolation and focal sinusoidal dilatation. Kidney showed focal mesangial proliferation of the glomeruli.

4.4.4.2 *14th day of incubation*

Congestion, lymphoid depletion and increase in the size of Hassal's corpuscles were observed in the thymus. Decrease in the number of developing follicles were seen in the bursa. Kidney showed cystic dilatation of the tubules with focal mesangial proliferation in the glomerulus. There was moderate vacuolation in the hepatocytes. Congestion and numerous budding capillaries were seen in the cerebrum.

4.4.4.3 *19th day of incubation*

There was interfollicular congestion along with mild lymphoid depletion in the bursa (Fig. 22). Thymus showed mild degenerative changes along with increase in the size of the Hassal's corpuscles (Fig. 23). Moderate congestion and diffuse tubular degeneration were seen in the kidney. Liver showed diffuse vacuolation with central venous congestion.

4.4.4.4 *21st day of incubation*

Thymus showed widening in the zone of Hassal's corpuscles, which were extended towards the cortex. Bursa revealed focal lymphocyte degeneration. In the spleen there was sub capsular congestion (Fig. 24). Vacuolar degeneration with diffuse necrosis of tubular epithelial cells (Fig. 25) and clumping of cells in the glomeruli (Fig. 26) were the lesions observed in the kidney. There was diffuse vacuolar degenerations in the liver.

4.4.5 Group V (Air cell route, 12 µg endosulfan)

4.4.5.1 *Ninth day of incubation*

There was no marked lesions in the thymus, spleen, bursa and brain. Diffuse vacuolar degeneration was observed in the tubular epithelial cells of the kidney. Active haemopoiesis and engorged blood vessels were seen in the spleen. All the capillaries along the para vertebral areas were congested. Central venous congestion and sinusoidal dilatation were evident in the liver. There was mild vacuolation of the hepatocytes along with active haemopoiesis (Fig. 27).

4.4.5.2 *14th day of incubation*

Thymus showed less cellularity of the follicles. Depletion of lymphocytes was also evident. The Hassal's zone appeared prominent. There was decrease in the number of developing follicles in the bursa when compared to that of control. Spleenic blood vessels were engorged. There were mild degenerative changes in the tubular epithelial cells of the kidney. Cystic dilatation of the tubules were also observed in certain areas. Mild hepatic degeneration with moderate vacuolations were seen in the hepatocytes. Brain did not reveal any characteristic lesions.

4.4.5.3 *19th day of incubation*

Mild degenerative changes in the lymphoid follicles of the thymus and bursa were observed. Sub capsular congestion was seen in the spleen. Kidney showed moderate degeneration of tubular epithelial cells. There was mild degenerative changes along with focal widening of sinusoids in the liver. Brain appeared apparently normal.

4.4.5.4 *21st day of incubation*

Widened zone of Hassal's corpuscle and interlobular haemorrhages were noticed in the thymus. There was interfollicular haemorrhage in the bursa with mild degeneration of the lymphocytes. Spleen revealed marked haemopoiesis and engorged blood vessels. Kidney showed reduced cellularity and shrinkage of few glomeruli along with diffuse degeneration of the tubular epithelium. There was moderate sinusoidal dilatation and congestion with diffuse hepatic degeneration in the liver. Capillaries in the brain were congested.

4.4.6 Group VI (Yolk sac route, Two microgram endosulfan)

4.4.6.1 *Ninth day of incubation*

Intervertebral haemorrhage and generalised congestion of all the organs were evident. Lymphoid organs and brain appeared normal. Kidney showed occasional tubular degeneration, mesangial proliferation and glomerular haemorrhage. Dilated tubules along with active intertubular haemopoiesis in the kidney were evident (Fig. 28). There was focal cloudy swelling of hepatocytes in the liver.

4.4.6.2 *14th day of incubation*

There was increase in the number of developing follicles in the bursa. Spleen revealed active haemopoietic zones. Diffuse congestion of the vessels and mild tubular degeneration were seen in the kidney. Liver revealed mild vacuolation of the hepatocytes. Thymus and brain appeared normal.

4.4.6.3 *19th day of incubation*

There was mild intra follicular congestion in the bursa. Thymus showed widening of medullary area. Spleen and brain showed no characteristic lesions. Kidney revealed mild tubular degeneration. Liver showed moderate vacuolation.

4.4.6.4 *21st day of incubation*

Bursa showed diffuse lymphoid depletion, hypo cellularity of the follicles and interfollicular haemorrhage (Fig. 29). Thymus revealed moderate degenerative changes in the follicles. There was mild sub capsular congestion in the spleen. Brain was apparently normal. There was mild tubular degeneration in the kidney. Clumping of glomerular cells with hyalinisation and shrinkage were observed in

occasional glomeruli. Dilatation of the central vein with diffuse hepatocyte vacuolation was evident in the liver.

4.4.7 Group VII (Yolk sac route, Four microgram endosulfan)

4.4.7.1 *Ninth day of incubation*

Thymus, spleen, bursa and brain did not reveal any marked lesions. Kidney showed desquamation of tubular epithelial cells and mesangial proliferation. Hepatic degeneration with dilated sinusoids were seen in the liver.

4.4.7.2 *14th day of incubation*

There was focal lymphocyte degeneration in the thymus and bursa. Spleen and brain were apparently normal. Kidney revealed mild tubular degeneration. There was central venous congestion, diffuse dilatation and congestion of the sinusoids with focal bile duct proliferation in the liver (Fig. 30).

4.4.7.3 *19th day of incubation*

In the bursa there was mild degeneration of the lymphocytes, mild follicular haemorrhage and moderate infiltration of plasma cells in the interfollicular area (Fig. 31). Thymus revealed focal degeneration of the lymphocytes. Spleen and brain did not reveal any characteristic lesions. There was mild tubular degeneration in the kidney. Kupffer cells were prominent in the liver. Extensive vacuolation of hepatocytes with loss of normal architecture were also seen in the liver.

4.4.7.4 *21st day of incubation*

Increase in Hassal's zone and vacuolar changes in the cells surrounding Hassal's corpuscles were seen in the thymus (Fig. 32). Bursa revealed moderate

lymphocyte degeneration. Congestion and vascular sclerosis were evident in the spleen. There was no marked lesion in the brain. Renal tubular epithelial cells were swollen with granular cytoplasm (Fig. 33). Kidney also showed hyalinisation of the tubules with glomerular tuft degeneration. Liver showed moderate vacuolation of the hepatocytes.

4.4.8 Group VIII (Yolk sac route, Eight microgram endosulfan)

4.4.8.1 *Ninth day of incubation*

The lymphoid organs and the brain did not reveal any marked lesions. Glomerular haemorrhage (Fig. 34) and diffuse degeneration of the tubules were observed in the kidney. Liver showed diffuse sinusoidal congestion and dilatation of the central vein. Diffuse muscular haemorrhage along the cervical region (Fig. 35) and congestion of the lungs were also evident.

4.4.8.2 *14th day of incubation*

Congestion, mild lymphoid depletion and focal degeneration of lymphocytes were seen in the thymus (Fig. 36). There was a reduction in the number of developing follicles in the bursa (Fig. 37). Kidney revealed diffuse degeneration of the tubular epithelium. Moderate hepatocyte vacuolation and moderate sinusoidal congestion were observed in the liver.

4.4.8.3 *19th day of incubation*

Mild degenerative changes in the thymocytes along with moderate lymphoid depletion were observed in the thymus. Interfollicular congestion with focal lymphoid degeneration was observed in the bursa. Spleen and brain were apparently normal. Kidney showed diffuse degeneration of the tubular epithelium. Occasional

degeneration of the hepatocytes and mild dilatation of the sinusoids were evident in the liver.

4.4.8.4 *21st day of incubation*

Increase in the zone of Hassal's corpuscle and focal lysis of lymphocyte in the cortex were observed in the thymus (Fig. 38). Interlobular haemorrhage was also evident in the thymus. There was diffuse lymphoid depletion in the bursal follicles with interfollicular haemorrhage in the bursa (Fig. 39). Congestion and haemorrhage in the sub capsular area were seen in the spleen. Extensive swelling, degeneration and necrosis of tubular epithelium were seen in the kidney (Fig. 40). Focal coagulation necrosis, moderate vacuolation of hepatocytes and central venous congestion were the lesions observed in the liver. Brain was apparently normal.

4.4.9 Group IX (Yolk sac route, 12 µg endosulfan)

4.4.9.1 *Ninth day of incubation*

Thymus, bursa, spleen and brain did not reveal any characteristic lesion. Diffuse sinusoidal congestion, dilatation of the central vein and diffuse degeneration of hepatocytes were observed in the liver. Kidney showed mesangial proliferation, glomerular congestion and diffuse tubular degeneration.

4.4.9.2 *14th day of incubation*

Bursa showed moderate hyalinisation of the developing follicles (Fig. 41). Thymus showed mild degenerative changes in the follicles. Active haemopoiesis was seen in the spleen. There was moderate vacuolation in the renal tubular epithelial cells. Liver showed moderate vacuolation of the hepatocytes.

4.4.9.3 *19th day of incubation*

There were moderate degenerative changes in the thymic follicles. Interlobular congestion in the bursa and moderate vacuolation of the hepatocytes in the liver were also evident. Vacuolar degeneration of the tubules was seen in the kidney. Spleen and brain were apparently normal.

4.4.9.4 *21st day of incubation*

Diffuse depletion and degeneration of the lymphocytes was observed in the bursal follicles along with interfollicular haemorrhage (Fig. 42). Increase in the zone of Hassal's corpuscle and lymphocyte degeneration around this zone was observed in the thymus. Diffuse degeneration and necrosis of tubular epithelium with cystic dilatation of the tubules were observed in the kidney (Fig. 43). Extensive hepatic vacuolation, haemorrhage, edema (Fig. 44), central venous congestion and focal areas of coagulation necrosis were observed in the liver. Spleen showed congestion and haemorrhage in the sub capsular area. Diffuse degeneration of purkinje cells in the cerebellum (Fig. 45), perivascular and perineuronal edema in the cerebrum were evident.

4.4.10 Control Groups

Liver revealed mild vacuolation during ninth day. The size and number of the vacuoles increased progressively and reached their maximum during 21st day of incubation. Renal tubules were dilated during ninth day of incubation. Apart from this there was no marked lesions in any of the other organs in the control groups.

4.5 Maternal IgY level in the egg yolk

Maternal IgY level in the pooled egg yolk sample collected during ninth, 14th, 19th and 21st day of incubation is presented in table 3. One millilitre of egg yolk is

approximately equal to one gram of yolk. IgY level in the pooled yolk samples of endosulfan treated groups varied from 15.2 mg/g to 17.6 mg/g, which did not differ significantly to that of control (15.2 to 16.4 mg/g) and standard (15 to 25 mg/g).

Table-3 Maternal IgY level in the Yolk

Group	IgY concentration (mg/g)			
	9^{th} day	14^{th} day	19^{th} day	21^{st} day
I	15.2	15.2	15.2	15.2
II	15.2	16.4	15.2	15.2
III	16.4	16.4	15.2	16.4
IV	16.4	16.4	16.4	15.2
V	17.6	17.6	15.2	17.6
VI	16.4	17.6	15.2	16.4
VII	16.4	15.2	15.2	15.2
VIII	16.4	15.2	15.2	15.2
IX	15.2	16.4	15.2	16.4
X	15.2	15.2	15.2	15.2
XI	16.4	15.2	15.2	16.4

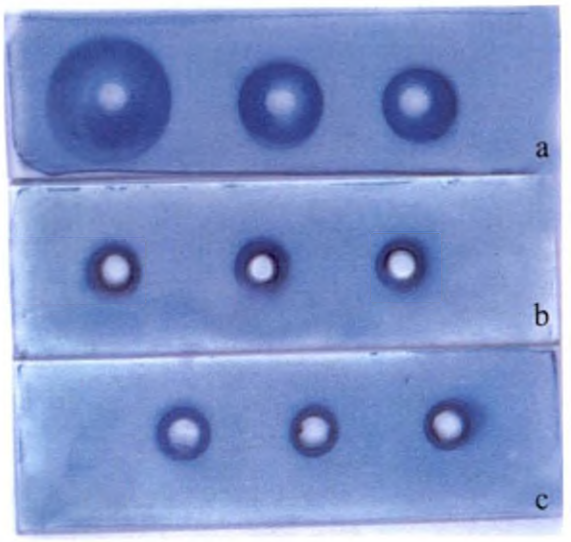


Fig. 1

Fig. 2

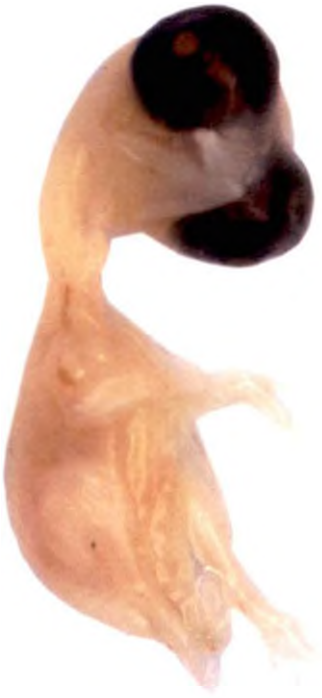


Fig. 3

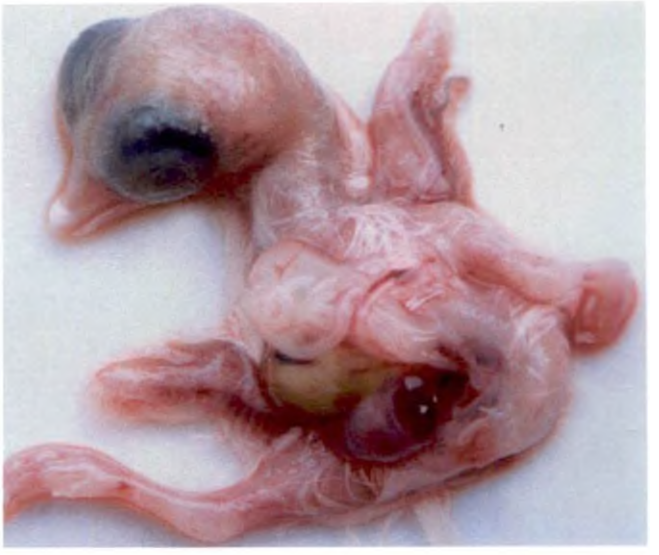


Fig. 4

Figure-1
Single radial immunodiffusion test - stained slides
a-standard, b-control,c-treatment

Figure-2
Embryo showing short lower beak -Yolk sac, 12 µg endosulfan

Figure-3
Embryo with crossed beak -Yolk sac, 8 µg

Figure-4
Eventration of viscera with curled toe -Yolk sac, 8 µg

Fig. 5

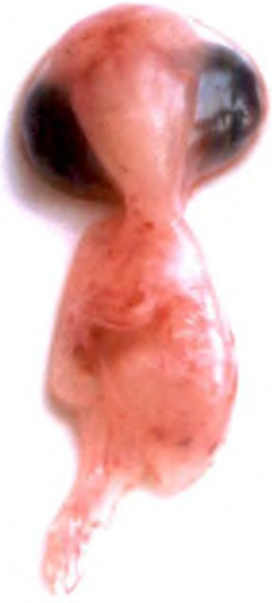


Fig. 6

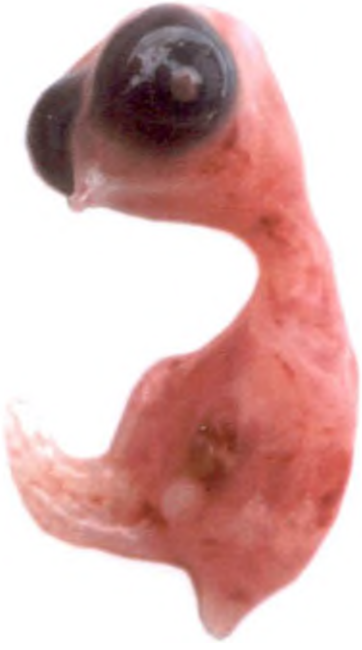


Fig. 7

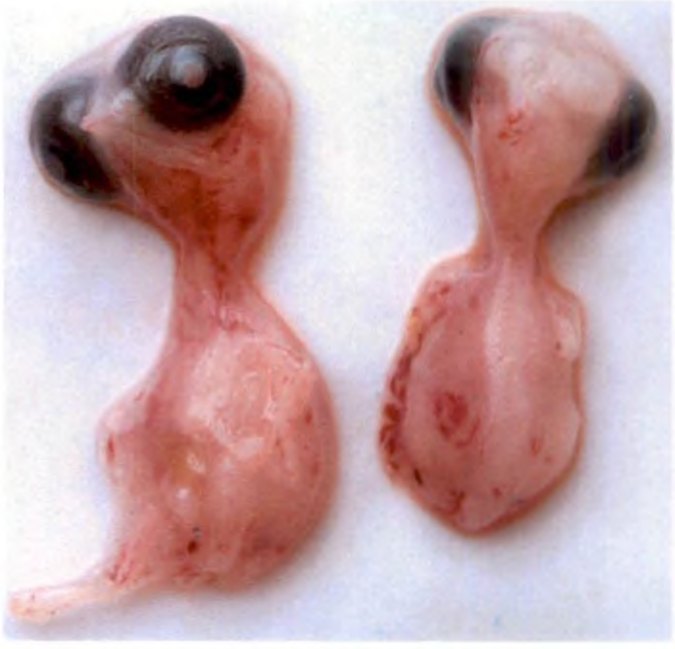


Fig. 8

Figure- 5

Newly hatched chick with curled toe-Yolk sac, 2 µg

Figure- 6

Petechiae on the head, neck and left lateral aspect of the abdomen - Air cell, 4 µg

Figure- 7

Petechiae on the head, neck, periorbital area and ecchymoses on the thorax - Air cell, 8 µg

Figure- 8

Petechiae on the head, neck and ecchymoses on the left lateral abdomen -Yolk sac, 4 µg

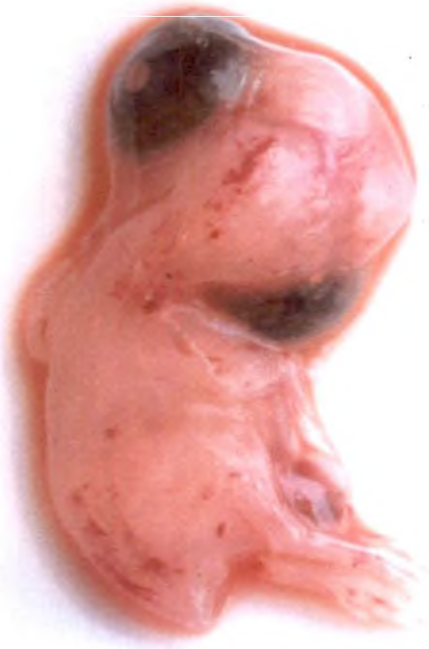


Fig. 9

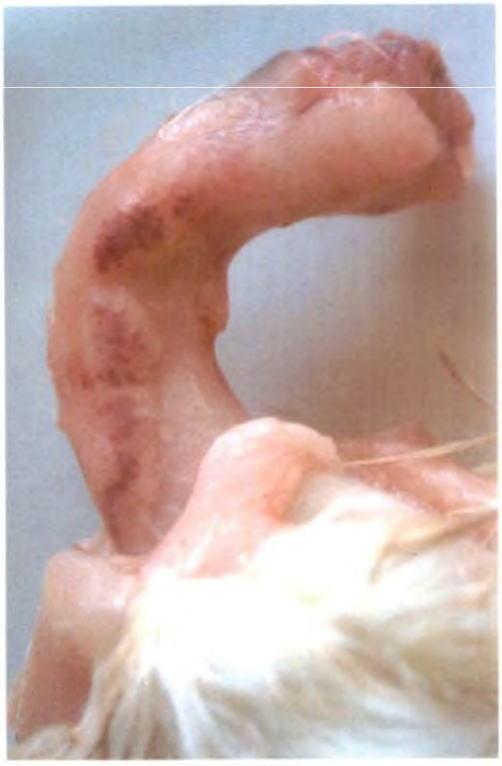


Fig. 10

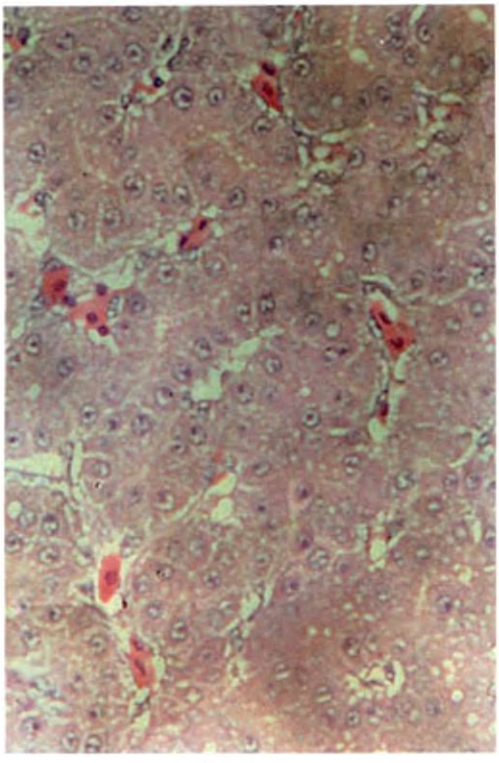


Fig. 11

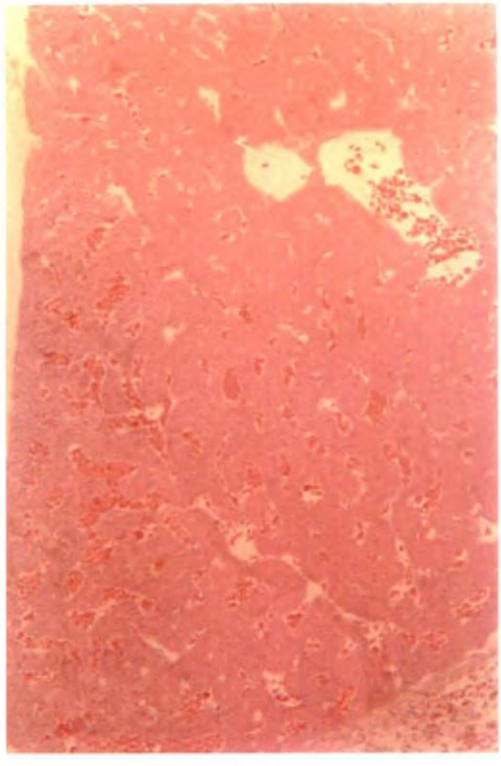


Fig. 12

Figure-9

Petechiae on the head, thigh, and back region - Yolk sac, 8 µg

Figure-10

Newly hatched chick showing thymic haemorrhages

Figure-11

Liver - Dipping, 9th day - Swollen hepatocytes and mild vacuolation of the hepatocytes- H & E x 400

Figure-12

Liver - Dipping, 9th day - Diffuse sinusoidal dilatation and active haemopoiesis - H & E x 160

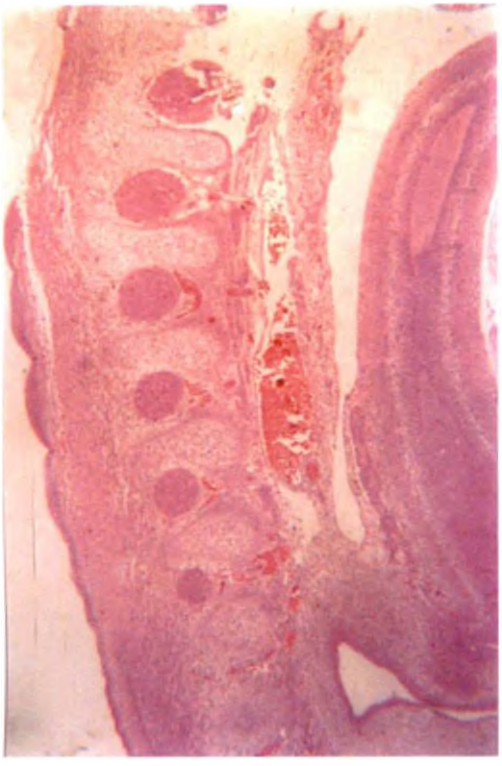


Fig. 13

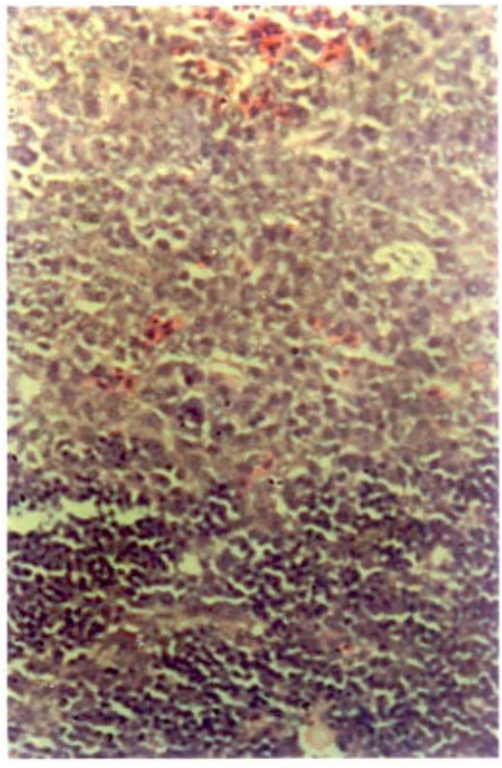


Fig. 14

Fig. 15

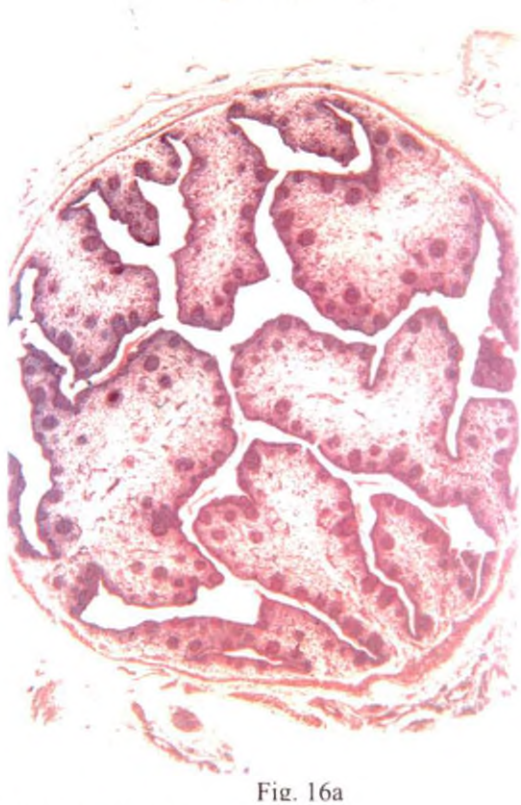


Fig. 16a

Figure-13

Congestion of vessels along the para vertebral areas - Dipping, 9th day - H & E x 63

Figure-14

Thymus - Dipping, 21st day - Widening of the medullary zone with proliferating lymphocytes in thymic follicles - H & E x 400

Figure-15

Thymus - Dipping, 21st day - Intrafollicular haemorrhage in the thymic follicles - H& E x 400

Figure-16a

Bursa - Air cell, 2µg, 14th day - Large number of developing follicles - H & E x 160

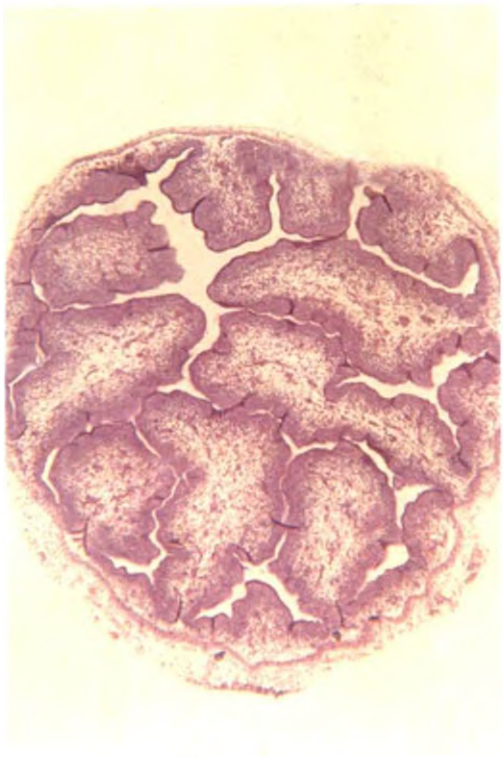
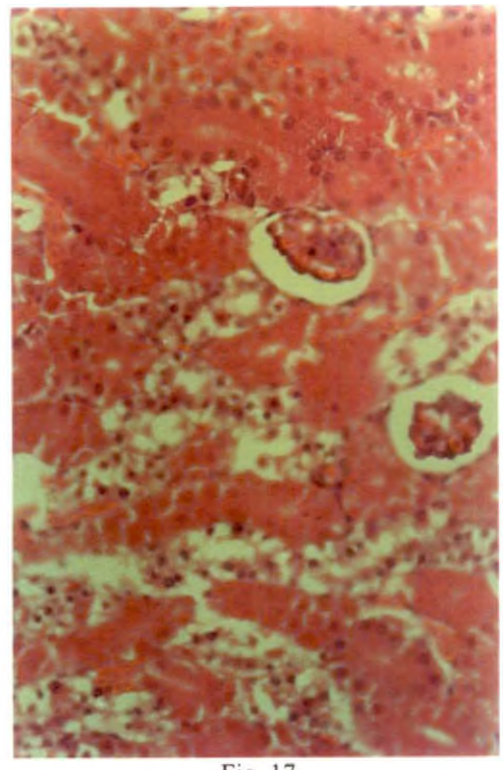


Fig. 16b



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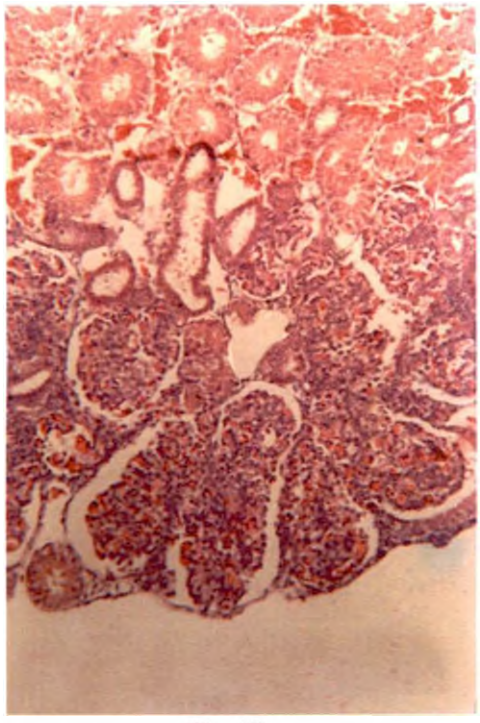


Fig. 18a

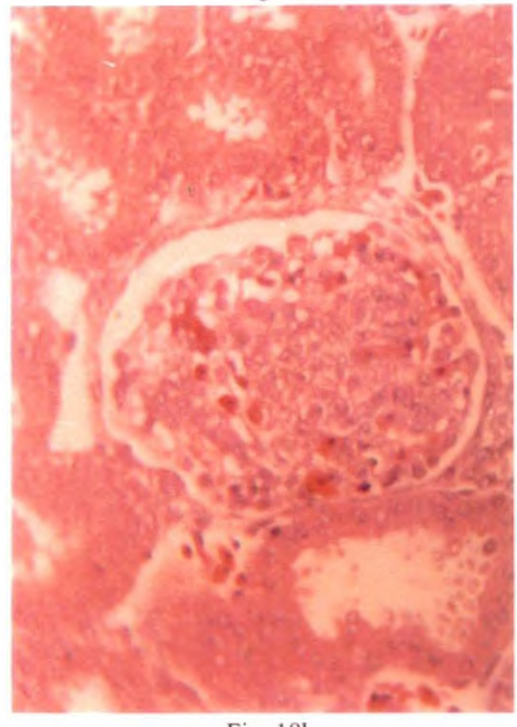


Fig. 18b

Figure-16b

Control bursa showing comparatively less number of developing follicles - H & E x 160

Figure-17

Kidney - Air cell, 2 µg, 21st day - Degeneration of the tubular epithelium and shrunken glomeruli - H & E x 400

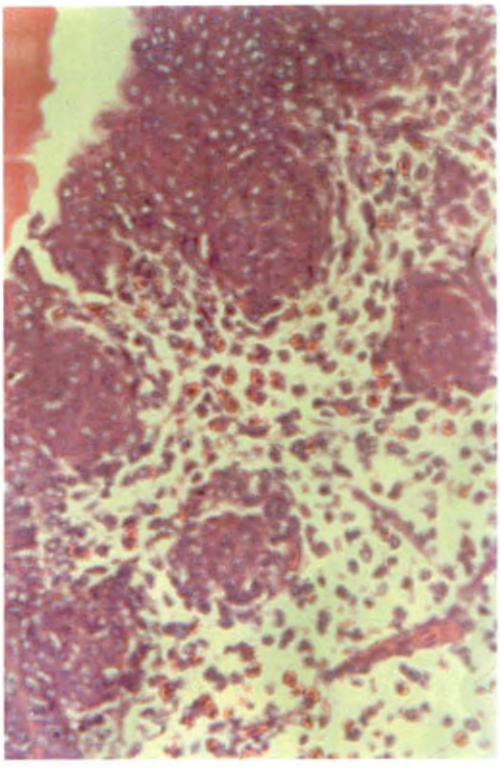
Figure-18a

Kidney - Air cell, 4 µg, 9th day - Mesangial proliferation of the glomeruli - H & E x 100

Figure-18b

Kidney - Air cell, 4 µg, 9th day - Mesangial proliferation of the glomeruli - H & E x 400

Fig. 19



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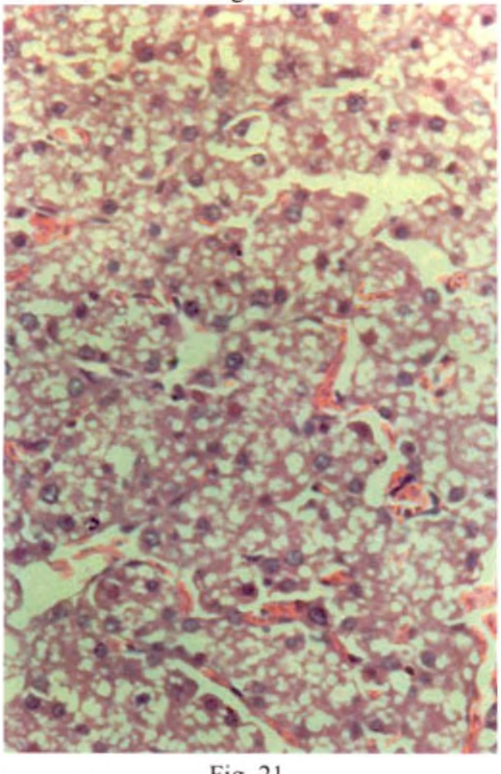

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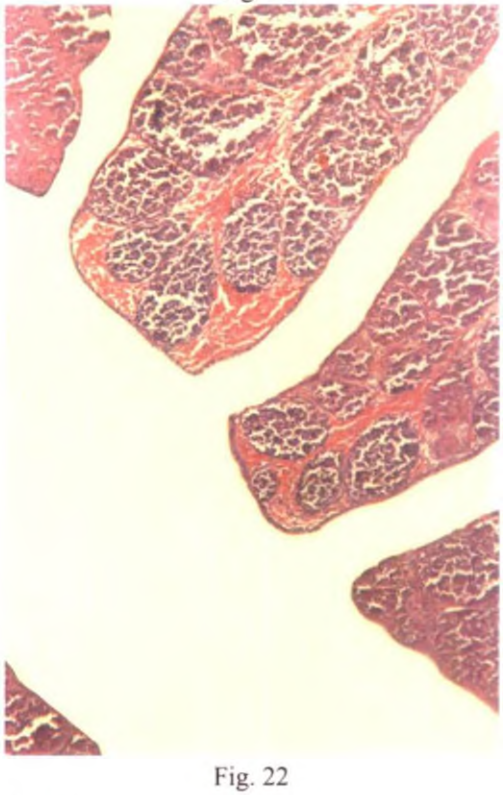


Fig. 22

Figure-19

Kidney - Air cell, 4 µg, 9th day - Tubular dilatation - H & E x 160

Figure-20

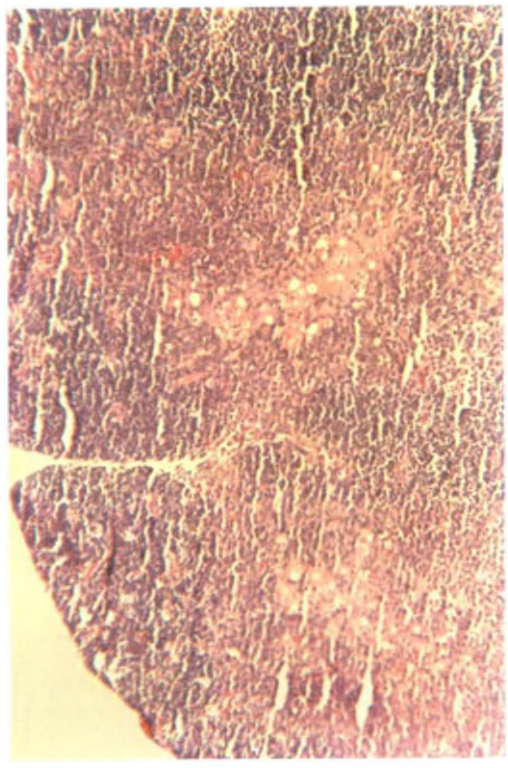
Bursa - Air cell, 4 µg, 14th day - Budding of developing follicles from plical epithelial cells - H & E x 400

Figure-21

Liver - Air cell, 4 µg, 19th day - Diffuse vacuolation of the hepatocytes with prominent Kupffer cells - H & E x 400

Figure-22

Bursa - Air cell, 8 µg, 19th day - Interfollicular haemorrhage with mild lymphoid depletion- H & E 160



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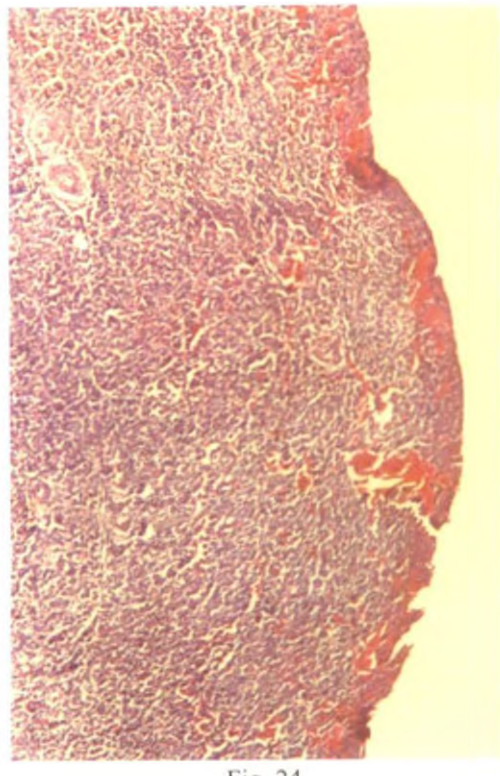

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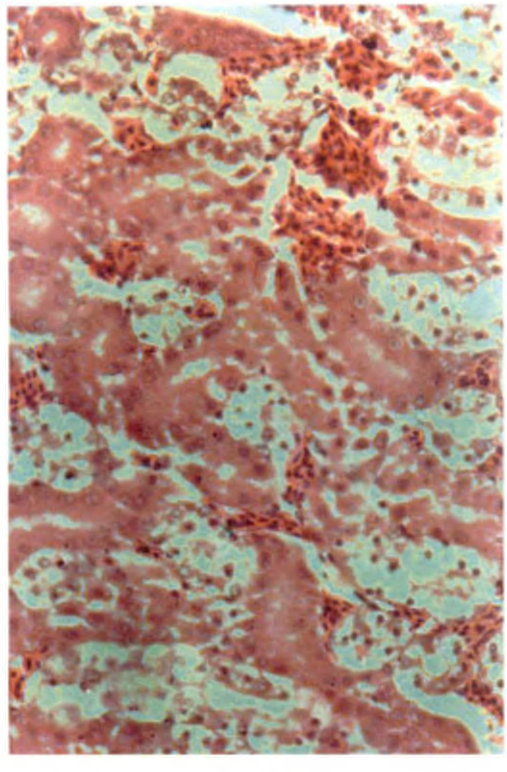

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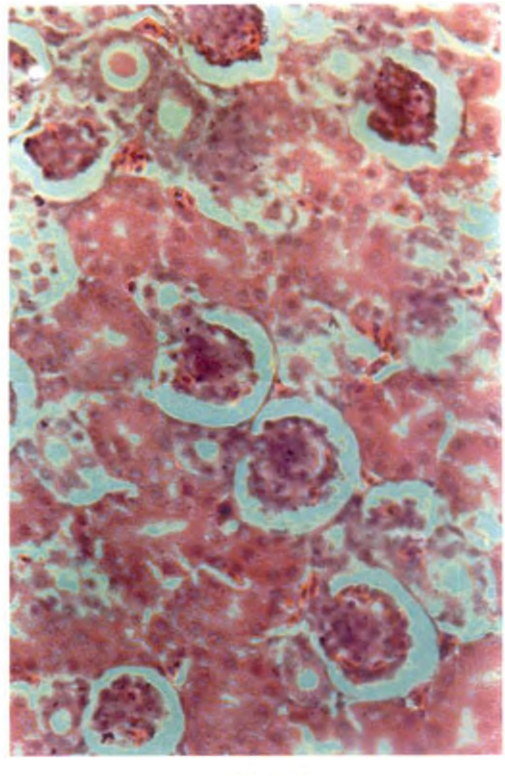


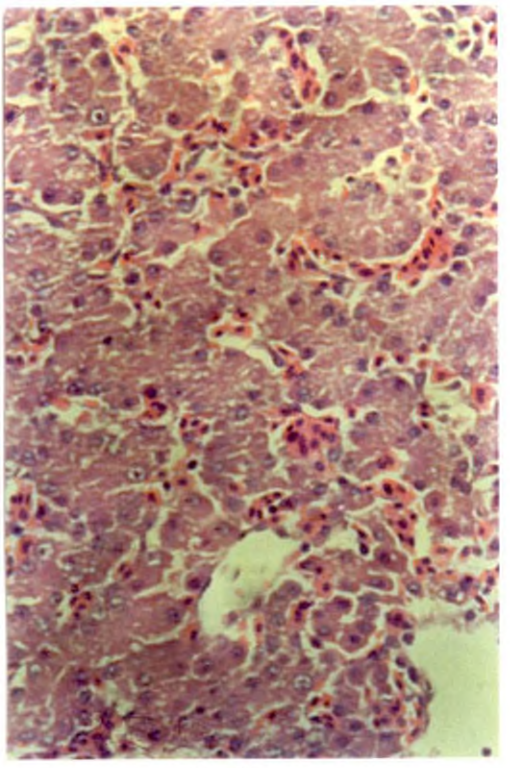
Fig. 26

Figure-23
Thymus - Air cell, 8 µg, 19th day - Degenerative changes with increase in the size of Hassal's corpuscles- H & E x 250

Figure-24
Spleen - Air cell, 8 µg, 19th day - Sub capsular congestion - H & E x 160

Figure-25
Kidney - Air cell, 8 µg, 21stday - Vacuolar degeneration with diffuse necrosis of tubular epithelial cells - H & E x 400

Figure-26
Kidney - Air cell, 8 µg, 21st day - Degeneration and clumping of cells in the glomeruli with tubular degeneration - H & E x 400



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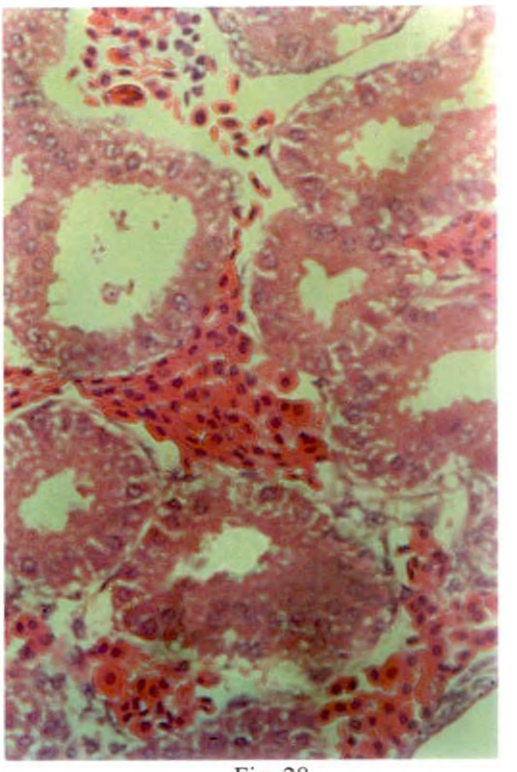

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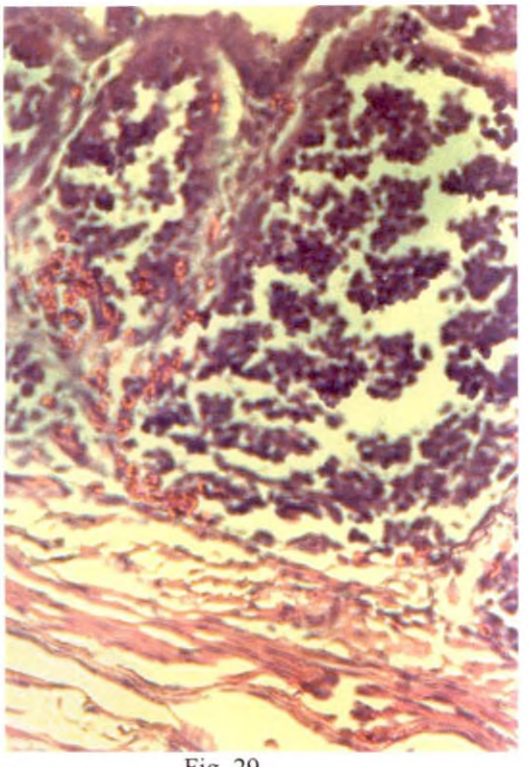

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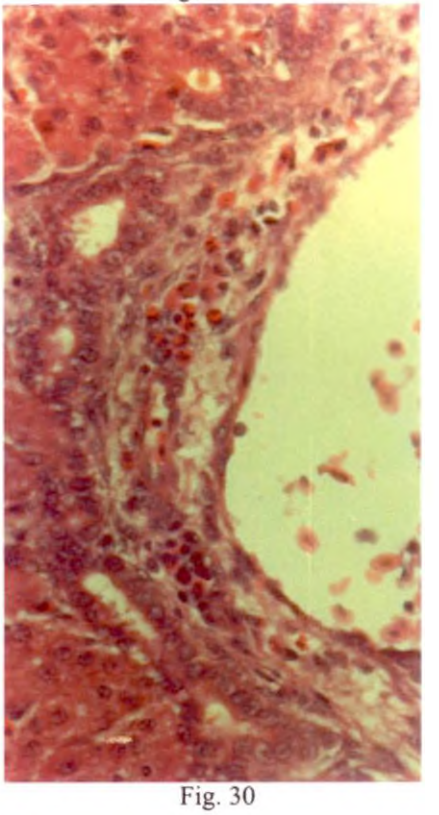


Fig. 30

Figure-27

Liver - Air cell, 12 µg, 9th day - Mild vacuolation of the hepatocytes with active haemopoiesis - H & E x 400

Figure-28

Kidney - Yolk sac, 2 µg, 9th day - Dilated tubules with active haemopoietic interstitium - H & E x 400

Figure-29

Bursa -Yolk sac, 2 µg, 21st day - Diffuse lymphoid depletion, hypo-cellularity of the follicles and interfollicular haemorrhage - H & E x 400

Figure-30

Liver - Yolk sac, 4 µg, 14thday - Bile duct proliferation - H & E x 400

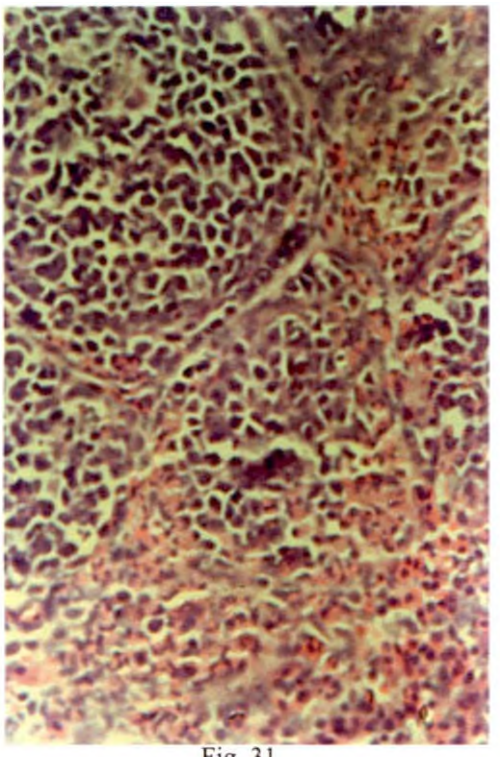
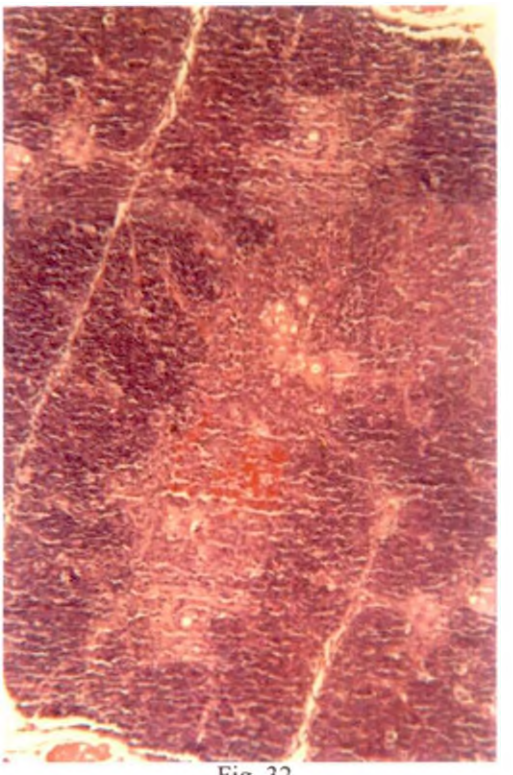


Fig. 31



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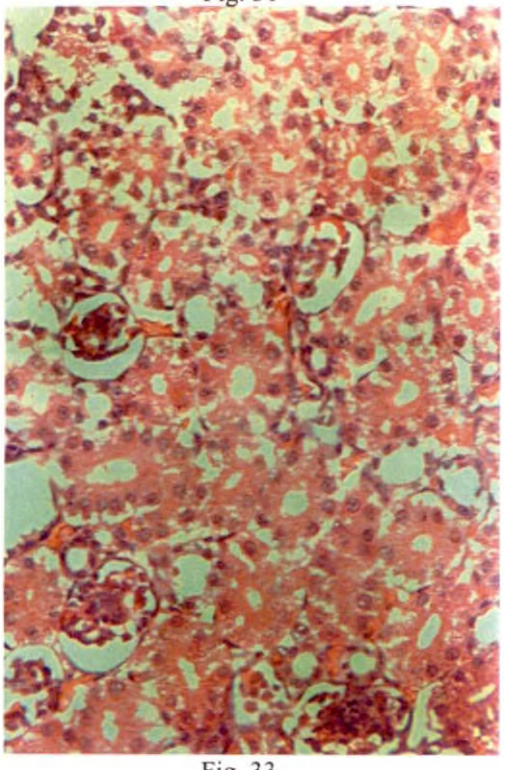

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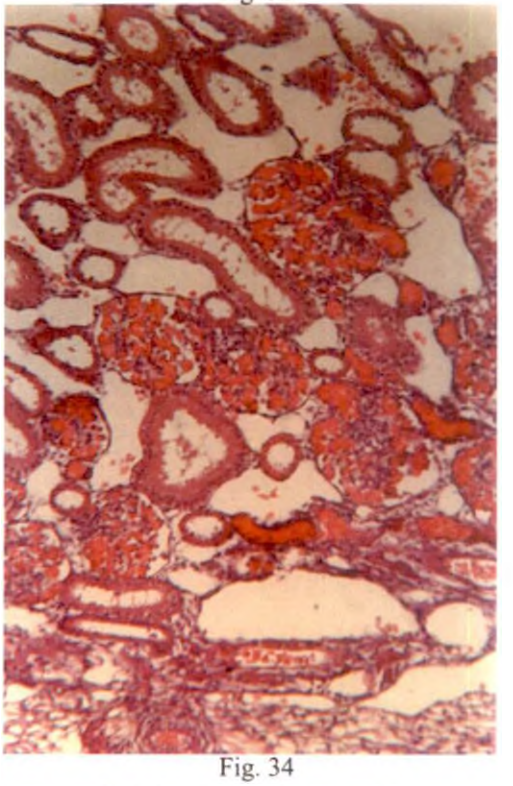


Fig. 34

Figure-31

Bursa - Yolk sac, 4µg,19thday - Degeneration of the lymphocytes, follicular haemorrhage and infiltration of plasma cells in the interfollicular area - H & E x 400

Figure-32

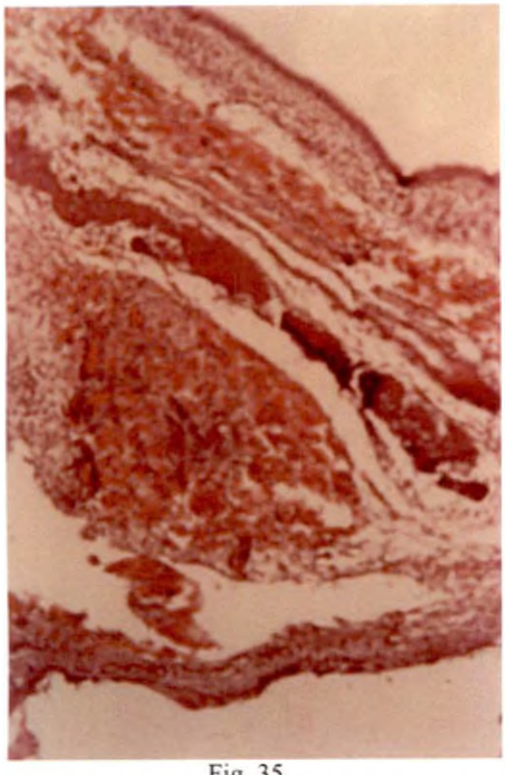
Thymus -Yolk sac, 4 µg, 21st day - Increase in Hassal's zone and vacuolar changes in the cells surrounding Hassal's corpuscles - H & E x 160

Figure-33

Kidney -Yolk sac, 4 µg, 21st day - Tubular epithelial cell swelling, granularity of the cytoplasm and partial occlusion of the lumen - H & E x 400

Figure-34

Kidney - Yolk sac, 8 µg, 9th day - Glomerular haemorrhage - H & E x 160



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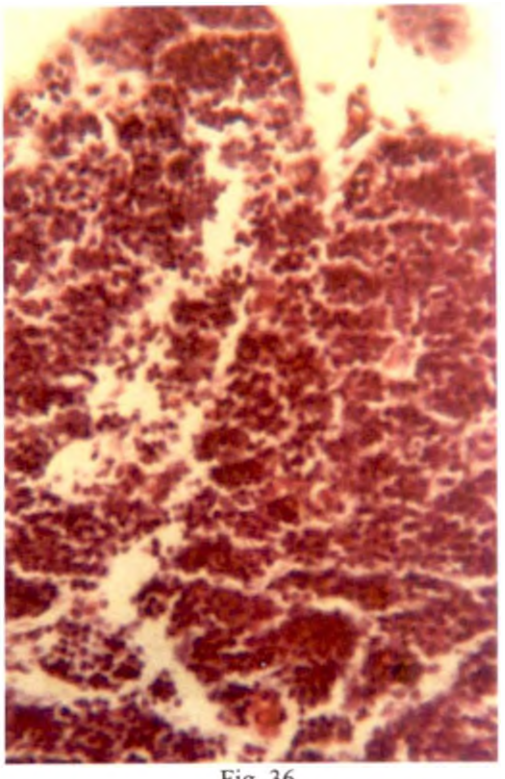

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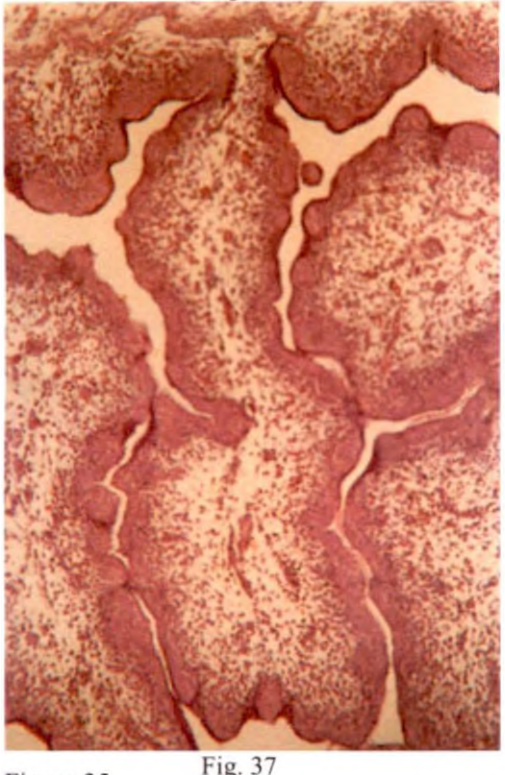

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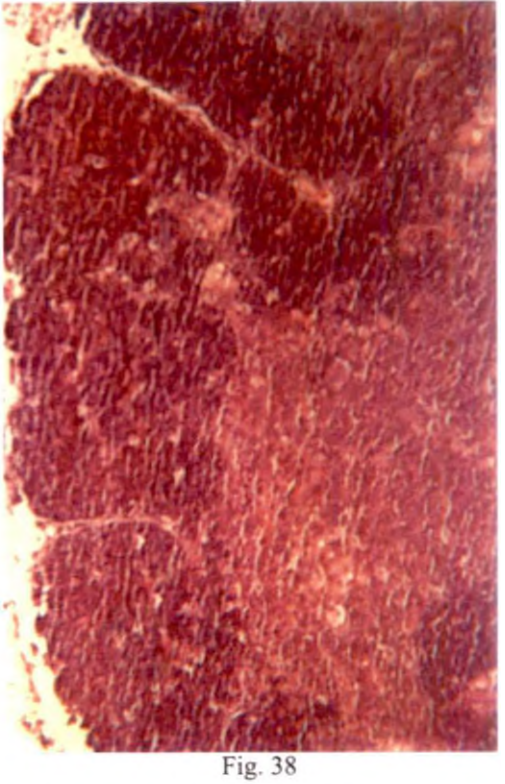

Fig. 38

Figure-35

Diffuse muscular haemorrhage in the cervical region - Yolk sac, 8 µg, 9th day - H & E x 100

Figure-36

Thymus - Yolk sac, 8 µg, 14th day - Mild lymphoid depletion and focal degeneration of lymphocytes - H & E x 160

Figure-37

Bursa -Yolk sac, 8 µg, 14th day - Reduced number of developing follicles - H & E x 160

Figure-38

Thymus - Yolk sac, 8 µg, 21st day - Focal lysis of lymphocytes in the cortex -H & E x 160

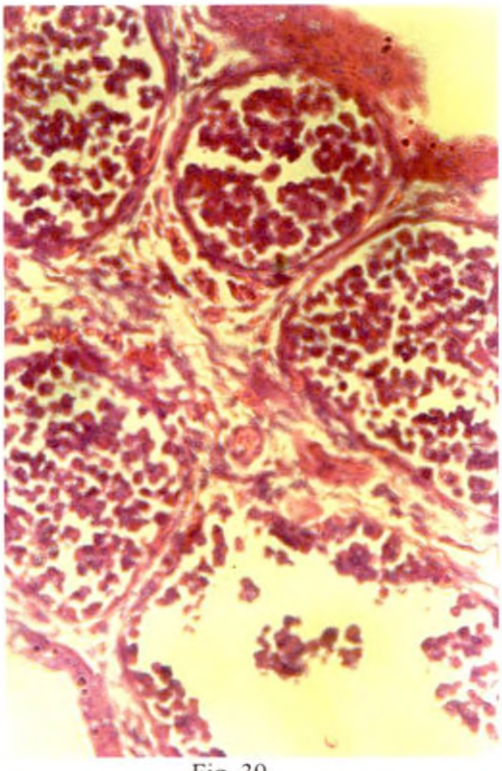

Fig. 39

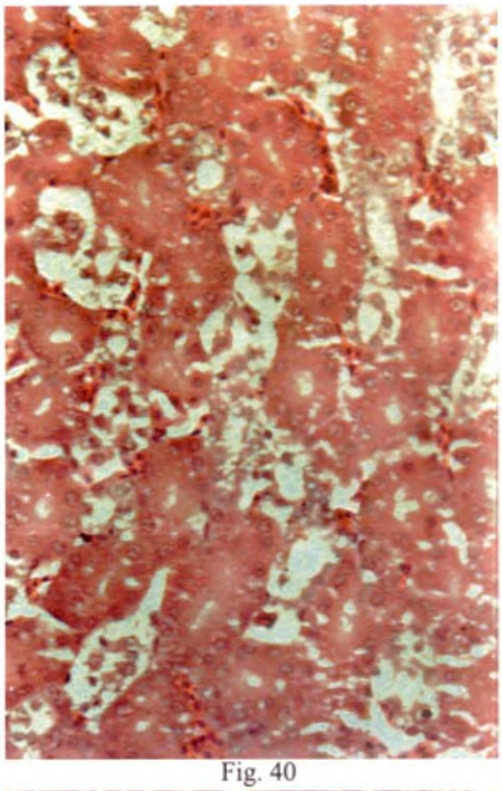

Fig. 40

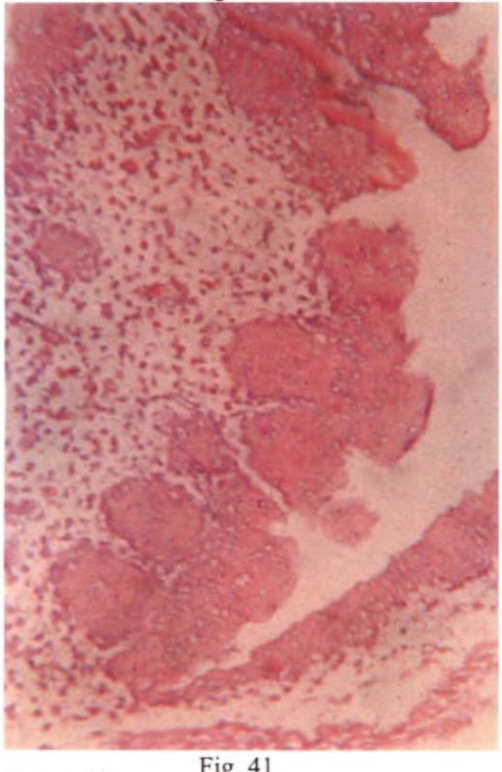

Fig. 41

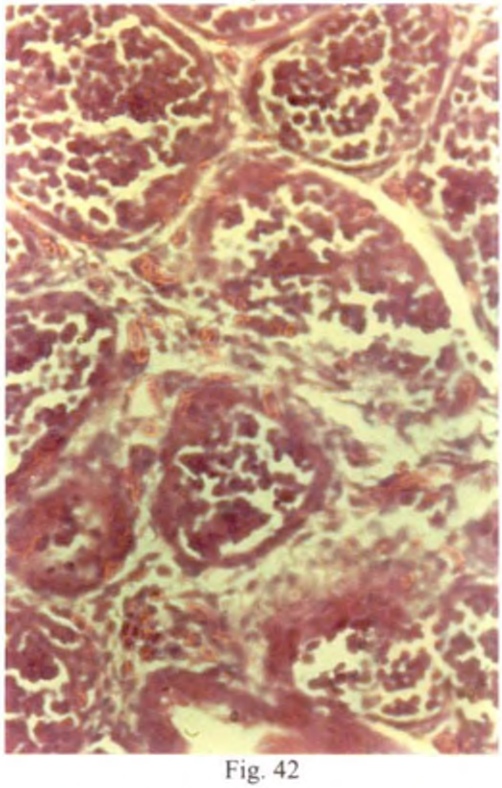

Fig. 42

Figure-39

Bursa - Yolk sac, 8 µg, 21st day - Diffuse lymphoid depletion in the bursal follicles with interfollicular haemorrhage - H & E x 400

Figure-40

Kidney - Yolk sac, 8 µg, 21st day - Tubular epithelial degeneration and necrosis - H & E x 400

Figure-41

Bursa - Yolk sac, 12 µg, 14th day - Moderate hyalinisation of the developing follicles - H & E x 250

Figure-42

Bursa -Yolk sac, 12 µg, 21st day - Diffuse depletion and degeneration of the lymphocytes in the bursal follicles with interfollicular haemorrhage - H & E x 400

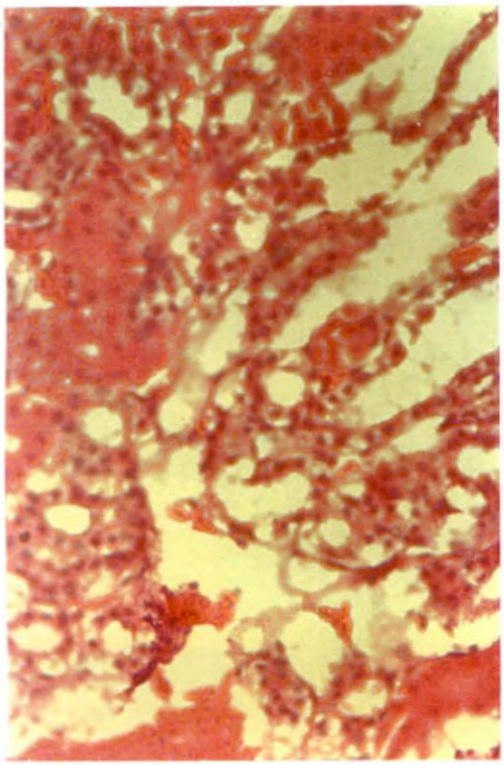

Fig. 43

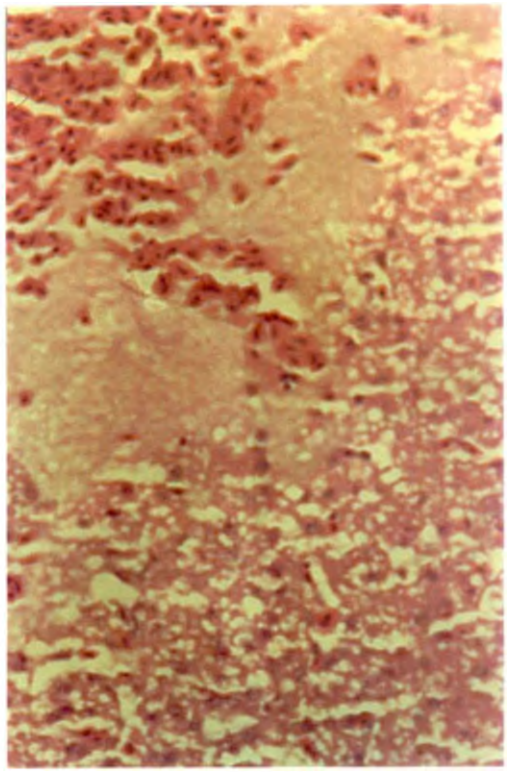

Fig. 44

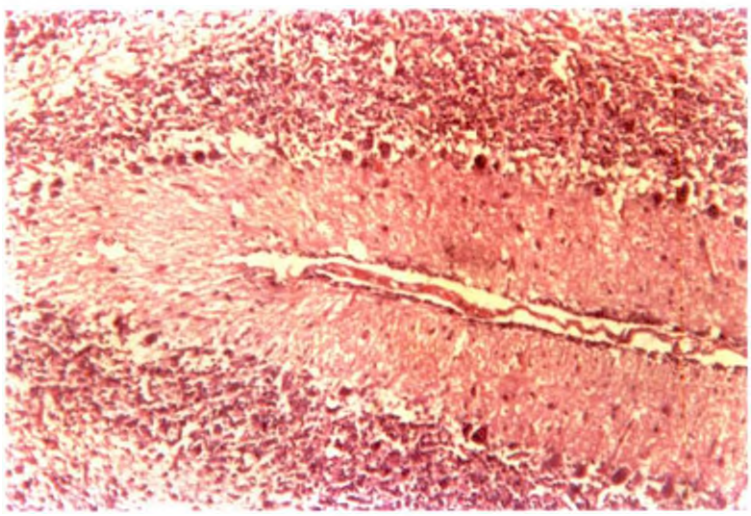

Fig. 45

Figure- 43

Kidney - Yolk sac, 12 µg, 21st day - Diffuse degeneration and necrosis of tubular epithelium with cystic dilatation of the tubules - H & E x 400

Figure- 44

Kidney - Yolk sac, 12 µg, 21st day - Liver - Hepatic vacuolation, haemorrhage and edema - H & E x 400

Figure- 45

Cerebellum - Yolk sac, 12 µg, 21st day - Degeneration of Purkinje cells - H & E x 250

Discussion

5. DISCUSSION

The presence of endosulfan residue in the environment due to widespread and indiscriminate use of this pesticide resulted in great health hazard to human and animals. A number of studies have demonstrated the acute and chronic toxic effects of endosulfan on various laboratory animals. Controversies still exist about its teratogenicity. Endosulfan was found to be excreted in egg yolk and transmitted via the human placenta (Bargar *et al.*, 2001; Mori *et al.*, 2001). Therefore it is very likely that the developing embryos may be exposed to subtle quantities of endosulfan. Various embryo toxicity studies have been conducted earlier but none of them explain the immunotoxic effect on the developing embryo. Hence to elucidate the developmental immunotoxicity and general embryotoxicty a study has been undertaken by exposing the chick embryos to endosulfan at various dose levels and through different routes.

5.1 PATHOANATOMICAL STUDIES

5.1.1 Mortality Pattern

Significant embryo mortality was not encountered in this study, irrespective of the dose and routes of exposure. Smith *et al.*, (1970) and Pourmirza, (2000) did not observe any significant mortality when endosulfan was inoculated either in to the egg albumin or in to the yolk sac prior to the incubation. Similar was the observation made by Dalsenter *et al.* (2003) wherein they did not find any reduction in the number of viable pups when female rats were exposed to endosulfan prior to mating, during mating and pregnancy. However, Lemonica and Takahashi (1996) observed embryo lethal effect in malnourished pregnant female rats, indicating the potential toxicity of this compound to the embryos of malnourished mothers. Guptha *et al.* (1978) also observed the embryo toxic effects at doses that also cause maternal

toxicity. In the present study only 13.3 per cent mortality was observed even when 12 µg of technical grade endosulfan, which contained about 70 per cent of α-endosulfan, was inoculated through yolk sac route on day five of incubation. This is in contrast to the findings of Pushpanjali *et al.* (1999) who observed 50 per cent mortality when five microgram per egg of α-endosulfan was inoculated into the chorio-allantoic membrane on day 12 of incubation and they suggested that this dose as the LD_{50} dose. Lalithakunjamma (1987) observed 16.63 per cent embryo mortality during routine incubation of eggs. In this context the embryo mortality observed in the present study does not reflect much of endosulfan toxicity rather than a routine event.

5.1.2 Teratological Abnormalities

The teratological abnormalities observed when eight microgram of endosulfan was inoculated in to the yolk sac route (group VIII) was 6.6 per cent. In Group III, VI, VII and IX the malformations observed was 3.3 per cent. The various anomalies observed in the different groups included agenesis of beak, crossed beak, short lower beak, eventration of viscera, torticollis and curled toe. This indicates that endosulfan was teratogenic and the teratogenicity was predominant when endosulfan was inoculated into the yolk sac. Byerly (1972) observed 9.5 per cent of spontaneous malformation in the chick embryo. However the teratological effects observed in the present study could be due to endosulfan induced genotoxicity, since none of control group embryos showed malformations. This observation is in contrast with Varnagy (1981) who observed that endosulfan was non teratogenic on administration into the air space of embryonated Japanese quail eggs during ninth day of incubation. Lemonica and Takahashi (1996) also observed that endosulfan treatment did not cause a rise in the number of foetal malformations when malnourished pregnant rats were exposed to endosulfan. On the other hand, Guptha *et al.* (1978) observed statistically significant increase in the per cent of skeletal abnormalities such as absence of sixth sternebrae in the foetuses of pregnant rats, which were exposed to

endosulfan during gestation. Malformations observed in the present study, though limited in number have to be viewed seriously and further systematic work on this direction is warranted.

5.2 MEAN EMBRYO AND LYMPHOID ORGAN WEIGHT

5.2.1 Mean Embryo Weight

The administration of endosulfan at different doses and through different routes did not significantly decrease the mean embryo weight during ninth, 14^{th}, 19^{th} and 21^{st} day of incubation. Dalsenter *et al.*, (2003) also observed no reduction in the body weight of the pups when endosulfan was given to female rats during pregnancy. No significant effects on foetal weight was observed when rabbits were exposed to endosulfan during sixth to 28^{th} day of gestation (Food and Machinery Corporation, 1981). However, Lemonica and Takahashi (1996) found decrease in foetal weight when malnourished rats were given endosulfan at a dose rate of 0.60 mg/kg/day. Pourmirza (2000) also observed decrease in embryonic body weight when malathion and endosulfan were injected into the yolk sac at a dose level of 1.25 mg/egg, prior to incubation.

5.2.2 Mean Weight of Thymus, Spleen and Bursa on 21^{st} day of Incubation

The mean weight of the Thymus, spleen and bursa of the embryos collected during 21^{st} day was not affected by the endosulfan treatment at the given dose level. Eventhough mild to moderate lymphocyte depletion and degeneration was observed in the thymus and bursa of endosulfan treated groups, the process was not found reflected in the weight and size of the lymphoid organs. This finding is in agreement with Banerjee and Hussain (1986). They observed that when endosulfan was administered in the diet of male rats at concentrations ranging from five to 50 ppm for six to 22 weeks, it resulted in no change in spleen and thymus weight in animals treated for six weeks, but a significant decrease in spleen weight was observed at 22

weeks in the 1.8 mg/kg/day group. Hack and Leist (1988) also observed no reduction in the thymus and spleen weight when technical endosulfan of 96 per cent purity was administered to female Wister rats at doses of 0.5, 1.5, or 4.5 mg/kg body weight for nine days. Vos *et al.*, (1982) also did not find significant change in spleen and thymus weight when endosulfan was given to Wister rats in the diet at concentrations of 20, 100 or 250 ppm for three weeks. However, Kurkure *et al.*, (1993) observed reduction in absolute and per cent organ weight of bursa, spleen, thymus, liver, kidney and lungs when day-old white leghorn chicks were fed with endosulfan at the dose rate of eight ppm to 48 ppm for 11 weeks.

5.3 GROSS PATHOLOGY

Varying degrees of haemorrhage were seen throughout the body in both treatment and control groups, during ninth day of incubation. However, in the control groups the haemorrhage was less extensive. No gross lesions were observed during 14^{th}, 19^{th} and 21^{st} day of incubation, except thymic haemorrhages at the 21^{st} day of incubation in the higher dose group of both air cell and yolk sac route. However the haemorrhage observed in the treatment groups during ninth day of incubation could not be correlated with the toxicity of the compound at the given dose levels as it was equally seen in the control groups too. At the same time the haemorrhages seen in the thymus indicated a direct toxic effect of the compound on the developing thymus. Gross lesions in the exposed embryos were not reported in the various embryo toxicity studies except the teratological effects. However the reported gross lesions in animals exposed during postnatal period were plenty. Atrophy of bursa and thymus were observed when day-old white leghorn chicks were fed endosulfan at the concentration of eight to 48 ppm for 11 weeks (Kurkure *et al.*, 1993). However Selvaraj *et al.* (2000) did not observe any gross lesions in the bursa when day-old broiler chicks were fed endosulfan at a concentration of 30, 60 and 120 ppm for eight weeks. They also observed enlarged and congested liver with scattered cyanotic

areas, splenomegaly, enlarged congested kidneys and mild congestion of brain. Haemorrhage on the serosa of the visceral organs and lungs, severe edema and emphysema of lungs were observed when cattle were applied topically with endosulfan for ectoparasitic control (Mor and Ozmen, 2003). Das *et al.* (1992) observed enlargement and focal areas of hardness in liver, flabby heart, enlarged and edematous lungs with oozing of serous exudate from the cut surface, when endosulfan was given to Black Bengal goats at the dose rate of 0.1 m/kg body weight. They also observed rough and thick intestine, enlarged kidneys and thickening of the capsule of the adrenal gland. However, no gross lesions were recorded by other authors (WHO, 1968; Nicholson and Cooper, 1977).

5.4 HISTOPATHOLOGY

5.4.1 Thymus

Effect of endosulfan on developing lymphoid organs were of mild to moderate intensity. Widening of medullary zone observed in-group I, II, III and VI may be due to stimulatory effect of endosulfan on the proliferating lymphoblasts. Apart from this, mild to moderate lymphoid depletion and degeneration, increase in the number and size of Hassal's corpuscles, occassional lysis of thymocytes, interfollicular congestion and haemorrhage were the main lesions observed in thymus. This finding was in agreement with Deshmukh *et al.* (1990) and Kurkure *et al.* (1993) who studied the effect on thymus of chicks exposed during postnatal period. Koner *et al.* (1998) suggested that the organochlorine induced immunosuppression might be due to generation of oxygen free radical.

5.4.2 Bursa

During 14th day of incubation, there were increase in the number of budding follicles in the bursa of Fabricious of groups dosed with two and four microgram

through air cell route and two microgram through yolk sac route. This indicated that endosulfan at the lower dose levels stimulated the formation of the budding follicle during the early development process. There was widening of medullary zones of the thymus in the above said groups during 19th day of incubation. In this context it is worth mentioning the finding of Vijayan *et al.* (1990). They observed stimulation of humoral immune response in ducks administered aqueous solution of furadan at 0.25 mg/kg body weight on alternative days for two months. However, moderate degeneration of lymphoid elements were seen in both thymus and bursa of the above said groups during 21st day. This may be due to prolonged persistence of endosulfan and its metabolites in the foetal circulation. A delay in the development of the bursal follicles was observed during 14th day of incubation in the groups dosed with eight and 12 µg through both air cell and yolk sac routes. Bursa also revealed mild to moderate lymphoid depletion and degeneration, reduced cellularity, interfollicular haemorrhage and follicular congestion in different groups at various dose levels. The histopathological studies therefore confirmed the susceptibility of the immune system of the embryo to endosulfan. The degenerative and lytic changes observed in the thymus and bursa at the highest dose levels will lead to immunosuppression and consequent increase in the susceptibility to infections. This necessitates a systematic study for monitoring the immunological status of chicks, which were exposed to endosulfan during embryonic life. Deshmukh et *al.* (1990) reported edema of bursa, cell depletion in medullary area, necrosis and atrophy in some follicles when day-old white leghorn chicks were fed endosulfan at the concentration of two ppm for the first two week, four ppm during third and fourth week and eight ppm during five to ten week. Vacuolar degeneration and necrosis of the follicles in the bursa, reticular hyperplasia and proliferation of fibrous connective tissue were observed when endosulfan was fed @ eight to 48 ppm for 11 weeks to chicks (Kurkure *et al.* 1993). Selvaraj *et al.* (2000) reported lymphoid depletion, hyperplastic and vacuolar degenerative changes in the plical epithelium, when technical grade endosulfan was fed to broiler chicken daily from day one to eighth week at different dose levels.

They also observed reticular hyperplasia, medullary necrosis, heterophilic infiltration. glandular formation and atrophic changes in some of the follicles in the bursa.

5.4.3 Spleen

Characteristic lesions were not seen in the spleen except mild congestion in the sub capsular area and vascular sclerosis in few cases. Postnatal exposure of endosulfan resulted in definite lesions in the spleen as reported by many authors. Kurkure *et al.* (1993) observed concentric hypertrophy of the arterioles in the spleen and reticular hyperplasia when endosulfan was fed @ eight to 48 ppm for 11 weeks to chicks. Necrotic and hyperplastic changes in lymphoid follicles were seen in spleen when eight-week-old white leghorn cockerels were fed 25, 50 and 100 ppm of endosulfan for 90 days (Varshneya *et al.*, 1988). Selvaraj *et al.* (2000) also reported congestion, lymphoid depletion and reticular hyperplasia, hyperplastic changes in blood vessels and formation of germinal centres in the spleen when 30, 60 and 120 ppm of technical endosulfan was fed to broiler chicken daily from day one to eighth week.

5.4.3 Kidney

From this investigation it was seen that endosulfan was nephrotoxic, which was characterised by varying degrees of tubular dilatation, degeneration and necrosis of tubular epithelium and mesangial proliferation followed by glomerular shrinkage. All these lesions indicated the progressive pathological changes induced by endosulfan. This may be due to the persistence of metabolised xenobiotic in the egg system and its continuous excretion and subsequent irritation of the tubular epithelium. The pesticide, which is metabolised to water-soluble compound, may be excreted via the kidney into the allantoic sac and may be reabsorbed. Metabolised products like endosulfan sulphate are equally toxic as that of the parent compound (WHO, 1984). The presence of endosulfan and its metabolites throughout the

embryonic period might result in the predominant renal lesions. Of all the metabolites of endosulfan, endosulfan sulfate appears to be the one that accumulates predominantly in the liver and kidneys (Hoechst 1989b). Endosulfan was found to accumulate within the proximal convoluted tubules, which imparted yellow discoloration to the cytoplasm (Agency for Toxic substances and Disease Registry, 2000). Braun and Lobb, 1976 also noted that kidney lesions observed in endosulfan toxicity might be due to accumulation of endosulfan and its metabolites in the kidney tissue. However the tubular dilatation observed during ninth day incubation was considered normal to the developing embryo since it was also observed in the control groups. Available literature did not reveal any information on the nephrotoxicity of endosulfan in the developing embryo. Most of the literature describes the postnatal toxicity lesions. Degeneration of the proximal convoluted tubules, cloudy swelling and necrosis of the tubular epithelium were the lesions seen when rats were fed with endosulfan for 78 weeks (National Cancer Institute, 1978). Kurkure *et al.* (1993) observed cell swelling in the tubular epithelium of kidneys when day-old white leghorn chicks were fed endosulfan at the dose level of eight to 48 ppm for 11 weeks. Extensive haemorrhages in the kidney, coagulative necrosis in the tubules and the glomeruli, with mononuclear cell infiltrations were observed when endosulfan was fed to white leghorn birds at the dose level of 0.75 mg/kg for 21 days (Bhattacharya *et al.* (1993). Das *et al.* (1992); Dwivedi and Singh, 1994; Rao and Varshneya (1995) and Selvaraj *et al.* (2000) also found similar lesions. However, no microscopically evident adverse effects were observed in the kidney when endosulfan was fed at the dose level of 7.3 mg/kg/day to male rats and 7.52 mg/kg/day to female rats for 13 weeks (Hoechst 1985). A chronic toxicity study conducted by Hoechst, (1989a) also did not find any adverse histopathological effects in the kidneys and urinary bladder when endosulfan was given to male and female dogs at the dose level of 2.9 and 2.6 mg/kg/day respectively for 146 days.

5.4.4 liver

Liver of nine-day-old embryo revealed marked dilatation of the sinusoids, which got considerably reduced when age advanced. Vacuolation of the hepatocytes markedly increased from ninth day and attained maximum size during 21st day of incubation. This could be due to accumulation of yolk lipids in the developing embryo. Dilated sinusoids, hepatic vacuolation and Kupffer cell prominence were consistently seen in all the endosulfan treated groups. Romanoff (1960) observed changes like this in the normal developing chick embryo. Apart from this there was mild to moderate degenerative changes of the hepatocytes, focal bile duct proliferation, haemorrhage and coagulation necrosis in some of the treated groups, especially the groups treated with the highest dose, that too through yolk sac route. This indicates the hepatotoxic effect of endosulfan to the developing embryo. Varshneya *et al.* (1988) observed mild to moderate vascular and sinusoidal congestion and dose dependent degenerative changes, fatty changes and focal areas of hepatocellular necrosis in white leghorn cockerels fed with 25, 50 and 100 ppm of endosulfan for 90 days. Granular degeneration of the hepatocytes was observed when day-old white leghorn chicks were fed endosulfan at the dose level of eight to 48 ppm for 11 weeks (Kurkure *et al.* 1993). Haemorrhages, coagulative necrosis in the liver and aggregation of leucocyte in periportal areas were observed when endosulfan was fed to white leghorn birds at the dose level of 0.75 mg/kg for 21 days (Bhattacharya *et al.* 1993).

5.4.5 Brain

Brain showed mild congestion of the capillaries, perivascular and perineuronal edema and Purkinje cell degeneration in 12 µg, yolk sac route group. Except this there were no marked lesions in the brain. Though brain is one of the target organs in acute and chronic toxicity, in the present study the developing brain was not affected

markedly by endosulfan and the said lesion may be due to direct neurotoxicity of endosulfan or due to its accumulation in the brain tissue (Nicholson and Cooper, 1977). Mahipal *et al* (1995) reported degenerative and necrotic changes along with infiltrated phagocytic cells in the brain of the chicks, which were fed 60 ppm endosulfan. Bhattacharya *et al.* (1993) reported colliquative necrosis in the brain of white leghorn birds fed 0.75 mg endosulfan/kg body weight for 21 days. Selvaraj *et al.* (2000) reported that when 30, 60 and 120 ppm technical endosulfan was fed to broiler chicken daily from day one to eighth week, brain showed congestion, spongiosis, focal neuronal degenerative change, satellitosis, neuronophagia, meningeal thickening, focal areas of gliosis and swollen meningeal vessels. They also observed atrophy and focal degenerative changes of Purkinje cells in the cerebellum.

5.5 MATERNAL IgY LEVEL IN THE EGG YOLK

Developing embryo derives its passive immunity from the maternal immunoglobulins present in the egg yolk and albumin. IgY is the major immunoglobulin present in the egg yolk. In order to clarify whether there is any reduction in the maternal IgY level as a result of endosulfan treatment, a study was conducted to evaluate the maternal IgY level in the egg yolk. It was seen that the treatment did not cause any depletion of the IgY level in the yolk. Szendberg *et al.* (1965) noted that IgY was easily reducible and its antibody activities including precipitation could be diminished or eliminated by mild reduction with two-mercaptoethanol or dithioerythritol. It seems that endosulfan did not have such reducing ability in the given dose levels. This study forms the first of its kind to see whether endosulfan has any effect in the preformed immunoglobulins.

Erythropoiesis in the embryo is mainly carried out by the liver, with other organs like the kidney and spleen also playing a lesser role. In the endosulfan treated

groups, the extra medullary haemopoietic zones remained active, which indicated that the endosulfan treatment did not affect the normal erythropoiesis.

From the present investigation it was seen that the exposure of endosulfan through different routes resulted in almost similar effects in the developing embryo. This indicates the potential toxicity of the compound irrespective of the routes of administration. The toxicity was more predominant in the higher dose groups.

The systematic investigation conducted brought to the light that endosulfan is embryotoxic at higher doses and it was found to affect the embryogenesis leading to abnormalities. Histopathological studies made in the embryos revealed that endosulfan is both nephrotoxic and hepatotoxic. Though the preformed maternal immunoglobulin (IgY) was not affected by endosulfan treatment, it was found to have an adverse effect on the developing immune system as revealed by lesions in the bursa, thymus, which will in turn result in immunosuppression, leading to increased susceptibility to infections. This needs to be investigated by monitoring the immune status of chicks, which were exposed to endosulfan during embryonic life, by challenging with suitable antigens and evaluation of antibody titre. Further investigation on its embryotoxicity and teratogenicity involving a large population of model systems with varying doses is necessary before extrapolating the information to human toxicity problems.

Summary

6. SUMMARY

The study was designed with the objective of assessing the embryotoxic and immunopathologic effects of endosulfan in chick embryo along with its influence on the preformed maternal immunoglobulin (IgY).

The embryos were exposed to endosulfan through different routes at different dose rates. In Group I, the fertile eggs were dipped in 25ppm of endosulfan before incubation. Group II to V embryos were inoculated with endosulfan at the dose level of two, four, eight and 12 microgram through air cell route in day four of incubation. Group VI to IX embryos were inoculated with the same dose levels through yolk sac route on day five of incubation. The pathological effects in the developing embryos were studied during ninth, 14^{th}, 19^{th} and 21^{st} day. The parameters studied included embryo mortality pattern, gross pathological changes, histopathological examination of liver, kidney, brain, bursa, thymus and spleen, weight of the embryo and lymphoid organs and IgY level in the pooled yolk utilising single radial immunodiffusion method.

The study did not reveal significant embryo mortality in the treatment groups.

Endosulfan was found to be teratogenic as abnormalities were seen in the treatment groups. The various anomalies observed in the different groups included agenesis of beak, crossed beak, short lower beak, eventration of viscera and curled toe. Control groups did not reveal any abnormalities.

Varying degrees of haemorrhage during ninth day and thymic haemorrhage during 21^{st} day were the main lesions observed and the other internal organs did not reveal any gross changes.

There was no significant reduction in the embryonic as well as lymphoid organ weight as compared to the control.

Widening of medullary zone, increase in the size and number of Hassal's corpuscles, mild to moderate degree of lymphoid degeneration, focal lympholysis, hypocellularity and interfollicular congestion were the lesions observed in the thymus.

In the lower dose group, bursa revealed increase in the number of developing follicles during 14^{th} day and at higher dose groups there was a delay in the development and differentiation of follicles from the follicular epithelium.

Bursa also showed mild to moderate lymphiod degeneration, depletion, focal lympholysis, moderate hypo-cellularity of follicles and inter and intra follicular haemorrhage in higher dose groups.

Spleen did not show any characteristic lesion except sub capsular congestion and vascular sclerosis.

Brain of the embryo was not found to be affected considerably by the endosulfan treatment except for mild congestion, perivascular and perineuronal edema and Purkinje cell degeneration.

The endosulfan was found to be both nephrotoxic and hepatotoxic to the developing embryo. Varying degrees of tubular degeneration and necrosis, glomerular haemorrhage, mesangial proliferation and glomerular shrinkage were the characteristic lesions in the kidney.

Liver revealed hepatocyte degeneration, haemorrhage, coagulation necrosis and focal bile duct proliferation in a dose dependent manner.

The extramedullary haemopoiesis in the kidney, liver and spleen of the embryos was not found to be influenced or affected by endosulfan treatment.

The preformed maternal immunoglobulin Y concentration in the yolk was not seen influenced by the endosulfan treatment in any of the dose levels as the IgY level did not differ significantly from that of control.

The result of the study indicated teratogenic, nephrotoxic and hepatotoxic effects of endosulfan in chick embryo. Histogenesis of the lymphoid organs was affected moderately indicating that endosulfan is immunosuppressive which will ultimately increase the susceptibility of chicks to infections.

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IMMUNOPATHOLOGIC AND TOXIC EFFECTS OF ENDOSULFAN IN CHICK EMBRYO

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ABSTRACT

The present study was undertaken to investigate the immunopathologic and toxic effects of endosulfan in chick embryo. The embryos were exposed to endosulfan at various dose levels through different routes.

The weight of the embryo, bursa, thymus and spleen, histopathology of the lymphoid organs, liver, kidney and brain and quantification of the preformed maternal IgY level in the egg yolk were the parameters analysed to study the effects.

Endosulfan was found to be teratogenic at the given dose levels. The abnormalities observed were agenesis of beak, crossed beak, short lower beak, eventration of viscera and curled toe.

Endosulfan did not cause any significant gross changes in the developing embryos except mild haemorrhages. Endosulfan treatment did not produce any significant reduction in the weight of the embryos as well as the weight of the lymphoid organs.

On histopathological examinations endosulfan was found to be nephrotoxic and hepatotoxic to the embryos. Varying degrees of degeneration and necrosis was evident in kidney and liver. Effect on the bursa, thymus and spleen were mild to moderate where in the degenerative changes predominated. Dose dependent increase in the involution process of the thymus as evidenced by increase in the Hassal's zone was observed. The lesions in the developing lymphoid organs reflected that endosulfan is toxic to the system and going to affect the immunocompetency by way of immunosuppression and thereby rendering the chicks susceptible to various diseases. The study indicated that the endosulfan treatment did not have any effect on the preformed maternal immunoglobulin level in the egg yolk.